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**LATVIJAS LAUKSAIMNIECĪBAS UN MEŽA ZINĀTŅU AKADEMIJA**  
LATVIAN ACADEMY OF AGRICULTURAL AND FORESTRY SCIENCES

# **AGRONOMIJAS VĒSTIS**

LATVIAN JOURNAL OF AGRONOMY

**Nr. 11/ No. 11**

**JELGAVA 2008**

## DEVELOPMENT OF PLANT BREEDING AND CROP MANAGEMENT IN TIME AND SPACE

Issue of the International Scientific Conference  
Priekuli, Latvia, 16-18 July 2008

*To mark 95th anniversary of State Priekuli Plant Breeding Institute and 50th  
anniversary of the Long-term Experiments in Priekuli*

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**Izdevums publicēts ar Latvijas Republikas Zemkopības ministrijas finansiālo atbalstu**  
**Publication of the issue has been financed by the Latvia Ministry of Agriculture**

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**ISSN 1691-3485**

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## PLANT BREEDING

### ESTIMATION OF HETEROSIS AND COMBINING ABILITY IN MAIZE (*Zea mays L.*) FOR EAR WEIGHT (EW) USING THE DIALLEL CROSSING METHOD

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#### Abstract

A diallel cross between inbred lines of maize (*Zea mays L.*) with medium maturity and an evaluation to estimate the genetic parameters for ear weight (EW) was carried out. The main objective of this study was to evaluate the ear weight of 10 inbred lines and their F<sub>1</sub> hybrids, based on a diallel cross (without reciprocals) General Combining Ability (GCA) and Specific Combining Ability (SCA). The components of the genetic variance, were calculated using Griffing's formula (1956), method 2, mathematic model I.  $X_{ij} = \mu + gi + gj + s_{ij} + e$ , for EW to detect the relative importance of additive and non additive gene effects. Additive gene effects were more important than non additive since the ratio was 0.25 among GCA and SCA. The highest value for maximal EW was a heterozygote combination from the inbred lines L<sub>6</sub>xL<sub>10</sub> (xg = 376.2 g/ear), while the minimal average value obtained for the hybrid combination was L<sub>1</sub>xL<sub>10</sub> (240 g/ear). The variation between the largest and the smallest EW for the hybrid combination was 136.2 g or 45%. The grand experimental mean  $\mu$  of F<sub>1</sub> generation was 308.1 g/ear. The differences for the EW of F<sub>1</sub> generation were  $\pm 68.1$  g/ear or 23%, compared with the value  $\mu$  showing a high significance for EW. ANOVA's test for the combining ability of GCA and SCA effects were highly significant at the  $p \leq 0.05$  and  $p \leq 0.01$  level of probability, respectively.

**Key words:** Ear weight, combining ability – diallel analysis, maize inbred line.

#### Introduction

Many procedures have been used by plant breeders to an attempt to increase the ear weight of maize (Geadelmann and Peterson, 1980). The use of heterosis started in 1933 when in the USA approximately 1 % of the total farming acreage was planted with heterosis maize hybrids, while in 1953 the heterosis of the maize hybrids were expanded up to 96% (Sprague, 1962). The improvement of maize yields for ear weight depends on the knowledge of the type of the gene action involved in its inheritance and also the genetic control of related traits such as the capacity for production (Rezaei *et al.*, 2004). Also the choice of the most efficient breeding program depends on the said information (Liao 1989, Pal & Prodhon 1994). The effects of general Combining Abilities (GCA) and Specific Combining Abilities (SCA) are important indicators of potential value for inbred lines in hybrid combinations. Differences in GCA effects have been attributed to additive, the interaction of additive x additive, and the higher-order interactions of additive genetic effects in the base population, while differences in SCA effects have been attributed to non-additive genetic variance (Falconer, 1981). The concept of GCA and SCA has become increasingly important to plant breeders because of the widespread use of hybrid cultivars in many crops (Wilson *et al.*, 1978). The evaluation of crosses among inbred lines is an important step towards the development of hybrid varieties in maize (Hallauer, 1990). This process ideally should be through the evaluation of all possible crosses (diallel crosses), where the merits of each inbred line can be determined. A Diallel analysis provides good information on the genetic identity of genotypes especially on dominance-recessive relations and some other genetic interactions. Diallel crosses have been used in genetic research to determinate the inheritance of a trait among a set of genotypes and to identify superior parents for hybrid or cultivar development (Weikai Yan & Manjit Kang, 2003). The main objective of our study was to estimate the General combining ability (GCA) and Specific combining ability (SCA) among these maize inbred lines and, consequently, to identify superior single-cross hybrids (SCH) developed from them.

## Materials and methods

The plant materials used for crosses in this study were taken from 10 selected superior maize inbred lines coded as: L<sub>1</sub>, L<sub>2</sub>... L<sub>10</sub>, with medium maturity, originating from the Agriculture University of Tirana (AUT)- Albania (Table 1). All possible crosses among these inbred lines were made in a diallel crossing block. During a three year period the study investigated the adaptability of inbred lines to the specifics of this trait for agro - ecological conditions in Kosovo, especially in the area near Ferizaj (580 m a.s.l). After year four, ten (10) selected maize inbred lines were crossed using the diallel system (Griffing,1956), and in year five these genotypes were placed in experimental plots (EP) of hybrid combination (C) and a study on the General (GCA) and Specific (SCA) combining ability for Ear weight (EW) was conducted. Statistical analysis used the mathematical and statistical models "MSM" which involved randomized block design experiments (RBDE) with three replications, 45 hybrid combinations (C) x 3 replications (R) = 135 Experimental plots (EP). The experimental plot was 3 m long and 60 cm apart, with 30 cm plant to plant distance or 55000 plants per ha<sup>-1</sup>. The experimental plots were 5.4 m<sup>2</sup> per each replication x 3R = 16.20 m<sup>2</sup>, and the seeds were placed 3-5 cm deep and cultivated under intensive agro techniques, including the use of the fertilizer NPK (15:15:15) and UREA 46%. This number of plants per plot was used to cover the adjustment for plot yield. In order to determine the EW we measured the average of 10 ears or plants selected randomly from each plot (10 ear per plants x 3R =30 plants or in total 1350 plants). Genetic interpretations and analyses of similar experiments can be found in numerous papers such as Hayman, (1954), Griffing's (1956).

### Statistical analyses

The diallel analysis ,as described by Griffing's (1956) method 2, mathematic model I (fixed model) is a systematic method of evaluating populations or select groups of inbred lines for combining ability in hybrid combination:  $X_{ij}=\mu+gi+gj+s_{ij}+e$  ;

Where,  $X_{ij}$  = is the mean of  $i \times j^{th}$  genotypes,  $\mu$  = is the experimental grand mean,  $g_i$  and  $g_j$  = is the GCA effects of  $i^{th}$  female parent, effects of  $j^{th}$  male parent,  $S_{ij}$  = is the SCA effects specific to the hybrid of the  $i^{th}$  female line and the  $j^{th}$  male line , and  $e$  = is the experimental error.

The formula gives components of genetic variance and gene values for GCA and SCA mean

squares calculated below:  $g_i = \frac{1}{p} + 2(T_i + ii) - \frac{2}{p} GT$  and

$$S_{ij} = X_{ij} - \frac{1}{p+2} (T_i + ii) + \frac{2}{(p+1)x(p+2)} GT$$

Whereas Midparent heterosis (MPH) was calculated as:  $MPH = \frac{F_1 - MP}{MP} \times 100$

Where,  $F_1$  is the mean of the  $F_1$  hybrid performance and  $MP = \frac{P_1 + P_2}{2}$  , where  $P_1$  and  $P_2$  are the means of the inbred parents. Statistical analyses were calculated with the statistical program MSTAT-C version 2.10.

## Results and Discussion

The results of the research reported great hybrid combinations and showed a significant genotypic effect on EW. The average value of EW for all studied genotypes was 308.1 g/ear. This is a relatively high weight that can guarantee higher productivity (yield), as result of the heterosis of  $F_1$  generation and their heterotic structures are presented in Table 1. For the hybrid combination of inbred lines L<sub>1</sub>xL<sub>10</sub> the minimal average value was ( $X_g = 240$  g/ear), while the maximal value obtained for the hybrid combination L<sub>6</sub>xL<sub>10</sub> was ( $X_g = 376.2$  g/ear). The variation between extreme values was  $D = (L_6 \times L_{10}, x_g = 376.2 \text{ g}) - (L_1 \times L_{10}, x_g = 240) = 136.2 \text{ g}$  or 45%, and this difference is highly significant at  $p \leq 0.05$  and 0.01. Distinctions' between blocks (repetitions) did not have significant effects for the probability level of  $p \leq 0.05$  and 0.01. The coefficient of the variation (CV) of the total EW for all genotypes of  $F_1$  generation was 5.86%, while  $SE = \pm 16.8$ . The total variability of genotypes for extreme values was  $\pm 68$  g/ear, highly significant differences were observed among parents and the hybrid combination for EW.



Table 1. Ear Weight of parents (diagonal, underlined) and their F<sub>1</sub> hybrids (above diagonal)

Line	L <sub>1</sub>	L <sub>2</sub>	L <sub>3</sub>	L <sub>4</sub>	L <sub>5</sub>	L <sub>6</sub>	L <sub>7</sub>	L <sub>8</sub>	L <sub>9</sub>	L <sub>10</sub>	F <sub>1</sub> Mean
L <sub>1</sub>	<u>81.3</u>	305.8	324.1	310.0	365.1	353.0	251.0	356.3	334.2	240.1	315,5
L <sub>2</sub>		<u>95.3</u>	297.8	319.0	324.4	325.0	355.4	338.8	350.2	248.1	319,8
L <sub>3</sub>			<u>65.6</u>	276.3	307.4	244.8	308.1	329.0	310.0	294.3	295,7
L <sub>4</sub>				<u>74.1</u>	271.0	313.9	299.8	241.9	259.3	291.7	279,6
L <sub>5</sub>					<u>132.6</u>	321.0	310.4	326.0	252.4	249.6	291,9
L <sub>6</sub>						<u>44.5</u>	341.7	289.9	304.6	376.2	328,1
L <sub>7</sub>							<u>160</u>	362.3	346.7	344.1	351,0
L <sub>8</sub>								<u>78.5</u>	347.0	306.0	327,0
L <sub>9</sub>									<u>71.1</u>	299.3	309,3
L <sub>10</sub>										<u>113.1</u>	264,0
μ value											308.16

LSD  $p \leq 0.05 = 1.43$ ;  $p \leq 0.01 = 1.73$

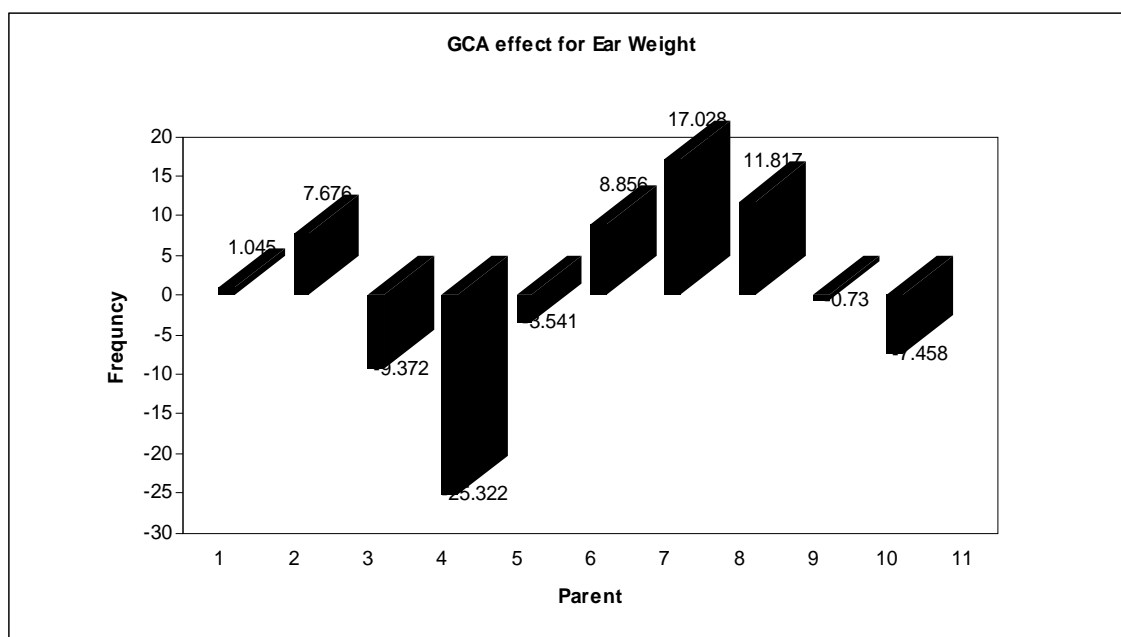
The difference between the mean of all F<sub>1</sub> hybrids and the mean of all parents (F<sub>1</sub>-MP) was + 216 g/ear. The highest and the lowest values of average heterosis were observed for ear weight at all genotypes. All hybrid combinations had positive heterosis, the highest value was 150% above the average of the parents value. The highest variability of EW values were obtained for hybrids L<sub>1</sub>xL<sub>4</sub> (xg = 245%) and L<sub>2</sub>xL<sub>10</sub> (xg = 77%), were statistically significant at  $p \leq 0.05$  and 0.01. Heterosis in EW is one of the commonest and most striking manifestations of hybrid vigour. The combining ability analyses of variance for EW were highly significant for differences observed for both GCA and SCA effects. A ratio of 0.25 for the variance components between GCA and SCA was observed. These values showed that additive gene effects were more important than non additive effects for EW. The are presented in Table 2.

Table 2. Estimated mean squares for GCA and SCA analyses for ear weight

Source	d.f	S.S	M.S	F-Value
GCA	9	49166.75	5462.9730	20.87**
SCA	45	981908.380	21820.1862	83.35**
E	108	28273.626	261.7928	

\*\*Significant at the  $\leq 0.01$  level of probability

The significance of GCA and their relatively high values were obtained in the inbred lines L<sub>7</sub> (+17.028), making it different from the inbred lines L<sub>6</sub> and L<sub>8</sub> and producing minimal differences compared with L<sub>7</sub>. The lowest GCA value was observed at L<sub>4</sub> (-25.32), and compared with the maximal values for GCA (L<sub>7</sub>), the variations were  $\pm 42.35$ . Crossing maize lines for GCA (gi-gj) created a variance of 14.54, while the average value of inbred lines (X<sub>ij</sub>) for F<sub>1</sub> generation was 87.26. Large proportion of the value F and differences among inbred lines for GCA were significant at  $p \leq 0.05$  and 0.01, and have a different intensity for heritage and variability (Figure.1). The SCA effects for the EW in each parent and parental combination are presented in Table 3.



LSD  $p \leq 0.05 = 12.35$ ;  $p \leq 0.01 = 16.96$ ; S.E (gi) = 6.54

Figure 1. Estimated of GCA effects for Ear Weight

Table 3. Estimated of SCA effects for Ear Weight in a diallel among 10 maize inbreds

Parent	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>	P <sub>4</sub>	P <sub>5</sub>	P <sub>6</sub>	P <sub>7</sub>	P <sub>8</sub>	P <sub>9</sub>	P <sub>10</sub>
P <sub>1</sub>	<u>-174.00</u>	21.16	56.51	58.36	91.42	67.18	-43.01	67.56	58.00	-29.43
P <sub>2</sub>	21.16	<u>-159.9</u>	23.61	60.76	44.35	32.52	54.75	43.42	67.31	-28.06
P <sub>3</sub>	56.51	23.61	<u>-141.8</u>	35.04	44.36	-30.59	24.56	50.67	44.22	35.25
P <sub>4</sub>	58.36	60.76	35.04	<u>-151.1</u>	23.98	54.45	32.14	-20.47	9.40	48.56
P <sub>5</sub>	91.65	44.35	44.36	23.98	<u>-136.3</u>	39.87	21.00	41.94	-19.30	-15.34
P <sub>6</sub>	67.18	32.52	-30.59	54.45	39.87	<u>-158.3</u>	39.93	-6.81	20.59	99.65
P <sub>7</sub>	-43.01	54.75	24.56	32.14	21.00	39.93	<u>-150.0</u>	57.51	54.52	58.58
P <sub>8</sub>	67.56	43.42	50.67	-20.47	41.94	-6.81	57.51	<u>-159.9</u>	59.86	26.06
P <sub>9</sub>	58.00	67.31	44.22	9.40	-19.30	20.59	54.52	59.86	<u>-163.12</u>	31.61
P <sub>10</sub>	-29.43	-28.06	35.25	48.56	-15.34	99.42	58.58	26.06	31.61	<u>-113.3</u>

LSD  $p \leq 0.05 = 41.34$ ;  $p \leq 0.01 = 54.49$ ; S.E.(sij) = 74.04

The results of the investigations for SCA also were highly significant at the  $p \leq 0.01$  level of probability. The highest specific combinations (SCA) were estimated for the hybrid combination L<sub>6</sub>xL<sub>10</sub> (+ 99.651), while the lowest value of SCA was obtained for the hybrid combination L<sub>1</sub>xL<sub>7</sub> (-43.018). For SCA value, the second hybrid combination was L<sub>1</sub>xL<sub>5</sub> (+ 91.423). The effect of SE (sij) for SCA of crossing parents was 74.04.

The study results show that both GCA and SCA effects are significant for ear weight trait, indicating that both additive and non-additive genetic actions were important combining of hybrids from the diallel crosses. Two factors are considered important for the evaluation of inbred lines in hybrid maize production - the characteristics of the line itself and the behaviour of the line in a particular hybrid combination (Malik *et al.*, 2004). As a basic principle (Spargue and Tatum, 1942) emphasised that SCA is more important than GCA among selected inbred lines. For the GCA, the parent lines L<sub>7</sub> (+17.028), L<sub>8</sub> (+11.81) and L<sub>6</sub> (+8.85), had the best GCA, while for SCA the highest positive effect was produced by the hybrid combination L<sub>6</sub>xL<sub>10</sub> (99.65). Satisfactory performances were also obtained from the combination L<sub>1</sub>xL<sub>5</sub> (91.42). This value showed variability between the investigative materials and productivity genotypes (Aliu, 2003). It was not possible to prove the rule that inbreds with good GCA usually had good SCA. Namely, the inbred L<sub>7</sub> and L<sub>8</sub> had the highest GCA for the investigated trait. On the other hand, the highest value of SCA was found for the hybrid L<sub>6</sub>xL<sub>10</sub>, but parental inbreds showed a very low SCA (8.85 for L<sub>6</sub>) or negative (- 7.45 for

L<sub>10</sub>). S.S. Sujiprihati *et al.*, 2001, interpreted, SCA as an indicator for the predominance of genes having dominance and epistatic effects while GCA as indicative for the predominance of genes having largely additive effects. However, EW was relatively more important than GCA among selected inbred lines. Additive gene effects were more important than non additive since the ratio in our results was 0.25 among GCA and SCA. The importance of additive and non-additive gene action was also reported by (Hansen *et al.*, 1977, Beck *et al.*, 1991, Alik., 1994, Sujiprihati *et al.*, 2001). These conclusions are in agreement with the results of this study involving selected inbred lines. The capacity of maize for the production of ear weight is different in our study and similar results for EW with minimal differences from those obtained by Misovic, (1964). The values reported were from 30-500 g/ear. Whereas dominant gene action would favour the production of hybrids, the additive gene action as a standard selection procedure would be effective in bringing advantageous changes to the investigated traits. Maize genotypes with lower or higher ear weight are well known as qualitative and productive genotypes. Many authors as (Santos I., Miranda G. V *et al.*, 2005) analysed some maize cultivars for EW and obtained results up to 184.5 g /ear which could be used as very good materials in a breeding program. Other results obtained by Radic (1980), for EW showed a value with oscillation from 212 till 390 g/ear, and compared to our study the results show a minimal difference from + 28.1 and -13.3 g/ear, respectively. The local studies carried out by (Fetahu, 2001), with 16 genotypes for EW in the agro ecological conditions of Kosovo present results for some different genotypes with the experimental average value  $\mu$  (255.17 g/ear) and compared with our results we have an increase of + 52.99 g /ear.

## Conclusions

The results of our research indicate significantly different combining abilities for EW. It was shown that the all hybrid combinations expressed a positive heterosis effect for EW in regard to their parents. The inbred line with the highest value for GCA was the maize line L<sub>7</sub>, L<sub>8</sub>, while for SCA was the combination L<sub>6</sub>xL<sub>10</sub>, L<sub>1</sub>xL<sub>5</sub>. The investigation suggests that some of the inbreds represent a highly valuable genetic material that could be successfully used for further breeding.

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## HETEROZES UN KOMBINATĪVĀS SPĒJAS IZVĒRTĒJUMS KUKURŪZAS VĀLĪTES SVARAM ( *ZEA MAYS L.* ), IZMANTOJOT DIALĒLISKO KRUSTOŠANU

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Pētījuma mērķis bija novērtēt kukurūzas 10 inbrēdo līniju (*Zea mays L.*) vispārīgo un specifisko kombinatīvo spēju. Lai veiktu novērtējumu, salīdzināts vāļītes masa inbrēdajām līnijām un to F<sub>1</sub> hibrīdiem, kas iegūti, izmantojot dialēlisko krustošanu (bez reciprokajām kombinācijām). Ģenētiskās mainības komponenti tika aprēķināti izmantojot Grifinga (Griffing's) formulu (1956)  $X_{ij} = \mu + g_i + g_j + s_{ij} + e$ , lai noteiktu aditīvo un neaditīvo gēnu efekta ietekmi uz vāļītes masu. Konstatēts, ka aditīvo gēnu efekts bija lielāks par neaditīvo gēnu efektu, jo attiecība starp vispārīgo un specifisko kombinatīvo spēju bija 0.25. Lielākais vāļītes masa iegūts no hibrīdās kombinācijas L<sub>6</sub>xL<sub>10</sub> (vāļītes svars vidēji 376.2 g), mazākais – kombinācijai L<sub>1</sub>xL<sub>10</sub> (vāļītes masa vidēji 240.0 g); izmēģinājumā vidēji F<sub>1</sub> paaudzē vāļītes masa bija 308.1 g. Salīdzinot ar vecākaugu līnijām, vāļītes masa F<sub>1</sub> hibrīdiem bija būtiski lielāka nekā vecākaugiem un tas vidēji izmēģinājumā bija +68.1 gramu katrā vāļītei. Ar varbūtību  $p \leq 0.01$  tika konstatēti būtiski vispārīgās un specifiskās kombinatīvās spējas efekti.

## CONSERVATION AND EVALUATION OF EX SITU AND IN VITRO COLLECTIONS OF ESTONIAN PLANT GENETIC RESOURCES

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### Abstract

The Estonian government has responded to the global efforts for conservation and sustainable use of biological diversity by ratifying international agreements and establishing the National Programme on Plant Genetic Resources for Food and Agriculture. The collection, identification and conservation of plant genetic material of Estonian origin as well as establishing the network are the essential activities of the National Programme. Since genetic resources provide the initial material for plant breeders and scientists, systematic detailed investigations and the improved use of genetic resources are required.

In this study evaluation of 13 oat and 59 potato accessions conserved in the Estonian ex situ and in vitro genebank was conducted. Descriptors for evaluation were selected from the Descriptor Lists developed by the working groups of the European Cooperative Programme for Plant Genetic Resources and promoted by Bioversity International. The results of the current study will be used in updating the databases of plant genetic resources and these data are applicable in plant breeding for further utilization of accessions.

**Key words:** genebank, plant breeding, oats, potato

### Introduction

Genebanks are dedicated to conserve plant genetic resources, which guarantee their utilization in the future (Maxted et al., 1997). According to the international commitment arising from the ratification of the Convention on Biological Diversity (Convention on ...), each country is responsible for conservation and sustainable use of plant genetic diversity as a local cultural and historical heritage to enhance the expediency of crop cultivation and ensure the sustainable development of society.

To realise these goals, the Estonian Government approved the National Programme for Plant Genetic Resources for Food and Agriculture (PGRFA) in 2002. The mandate of the programme is the collection, conservation, evaluation, characterization and documentation of plant genetic

material of agricultural crops of Estonian origin, thus providing a basis for the future use of the genetic resource by plant breeders and researchers (Annamaa and Kukk, 2005).

The responsibility to conserve the Estonian PGRFA has been taken by the following institutions: the Jõgeva Plant Breeding Institute (Jõgeva PBI) – long term ex situ seed collection; the Department of Plant Biotechnology EVIKA of the Estonian Research Institute of Agriculture (EVIKA) – meristem plants in vitro; the Polli Horticultural Research Centre of the Estonian University of Life Sciences – fruits and berries; the Botanical Garden of Tartu University – medicinal and aromatic plants, ornamentals; and the Department of Gene Technology of the Tallinn University of Technology – molecular and genetic characterization of accessions. About 4500 accessions, mainly of Estonian origin, have been collected and maintained by the relevant stakeholders. All accessions are documented in accordance with the internationally agreed passport descriptors (FAO/IPGRI Multi-Crop ...).

The International Treaty on PGRFA (International Treaty...) recognizes that the conservation, exploration, collection, characterization, evaluation and documentation of PGRFA are essential in meeting the goals for sustainable agricultural development for this and future generations. The Estonian Government, by approving the Treaty, has acknowledged the responsibilities and applied relevant finances for the second phase of the National Programme in the years of 2007–2013. During this period the main goal is to proceed with the characterization, evaluation and documentation of the maintained accessions. In the first place it is planned to focus on further investigation of crops whose regeneration is most urgent.

The evaluation and characterization of some crops such as oats, potato, wheat and forages has already been started during the first period of the National Programme. The varieties were characterized in field trials during which the identity of accessions was also checked in the field and in laboratories.

The aim of this study is to evaluate and characterize the oat accessions of Estonian origin conserved in the Gene Bank of the Jõgeva PBI and potato accessions conserved in vitro in EVIKA.

## Materials and Methods

Oats. The oat collection was formed on the basis of breeders working collections at the genebank of the Jõgeva Plant Breeding Institute. In total, there are 13 oat varieties (*Avena sativa* L.) of Estonian origin conserved in the genebank.. Eight oat varieties, the oldest of which was Kehra saagirikas bred in 1929, were repatriated from the Vavilov Research Institute of Plant Industry (VIR) (Kukk and Annamaa, 2005) Safety duplicates of the accessions are maintained at the Nordic Genetic Resources Center. Descriptions of all Estonian oat varieties are presented in the publications of the Jõgeva PBI (Tamm, 2003). The evaluation, characterization and regeneration of the genebank accessions are carried out in cooperation with the breeding departments of the Jõgeva PBI.

Field experiments were conducted on 13 oat accessions at the Jõgeva PBI in the 2006 and 2007 growing seasons. 5 m<sup>2</sup> plots were planted in two replications with the sowing rate of 600 seeds m<sup>2</sup>. The applied fertilizer level was N70P16K29 and a mixture of Lintur 70 WG (dicamba+triasulfuron) (120 g ha<sup>-1</sup>) and MCPA 750 (500 ml ha<sup>-1</sup>) was used for chemical weed control. The varieties Jaak and Villu were performed as standard varieties.

Oat accessions were characterized for 28 traits selected on the basis of biological and economical importance in Estonian conditions from the international oats descriptor list (Oats Descriptors, 1985). The accessions were visually assessed for 22 traits in the field (Table 1). Yield, grain moisture, 1000 grain weight, test weight and the percentage of husk for each accession were measured after harvesting. The protein content was determined by the Free and Open Source Software for Near Infrared Reflectance Spectroscopy (FOSSNIRS) system methods at the laboratory of the Jõgeva PBI.

Table 1. List of the descriptors of the oat collection observed in the field trial at the Jõgeva PBI during the growing season of 2006 and 2007.

1. Growth habit	9. Lemma colour	16. Lodging at immature stage
2. Plant height	10. Kernel covering	17. Lodging at mature stage
3. Nodes hairiness	11. Awedness	18. Crown rust
4. Hairiness of leaf sheath	12. Tillering	19. Stem rust
5. Hairiness of leaf margin	13. Days to heading	20. Powdery mildew
6. Shape of panicle	14. Days to harvest	21. Loose smut
7. Erectness of panicle	15. Number of fertile tillers	22. Spot blotch
8. Erectness of spikelets		

Potato. Active collections of potato genetic resources are conserved at the Jõgeva PBI (field collection) and EVIKA (meristem plants *in vitro*). In the field collection there is a risk of germplasm loss due to unfavourable climatic conditions or pests infections and pathogens, which can be minimised by introducing germplasm into tissue culture. In the research institutions involved in the conservation activities, preservation with microtubers is widely used (Kostrica, 1987; Kwiatowski *et al.*, 1988; Espinoza *et al.*, 1992; Dobranszki, 1997). In EVIKA a whole series of experiments were carried out with the aim to observe the influence of different culture medium components, growth conditions and other factors affecting plant regeneration, productivity and the prolongation of the sub-culturing interval. On the basis of these experiments, optimal preservation medium and long-term preservation conditions *in vitro* have been developed for many varieties. The microtubers are frozen for 1–1.5 years.

EVIKA has had the experience of preservation *in vitro* of potato varieties as well as breeding lines and local landraces for more than 20 years. All preserved accessions are disease-free and tested for virus infection many times. For the disease eradication the technology created in EVIKA is used (Kotkas and Rosenberg, 1999). The eradication system consists of 3 cycles: 1) the selection of the initial material, thermotherapy, the cultivation of meristem tips and testing for virus infection; 2) re-eradication and field-testing on varietal identity, quality and disease resistance; 3) renewal of the material.

Table 2. List of the evaluated descriptions of potato accessions in EVIKA in 2002–2004

1. Growth habit	20. Pigmentation of floral stalks	39. Sprout shape
2. Foliage cover	21. Pigmentation of cork ring	40. Sprout color
3. Plant height	22. Corolla size	41. Sprout pigmentation intensity
4. Stem thickness	23. Corolla color	42. Sprout pigmentation distribution
5. Stem number	24. Corolla color intensity	43. Color of rootlets
6. Wing size	25. Size of white tips	44. Sprout pubescence
7. Wing shape	26. Pigmentation of stigma	45. Maturity
8. Leaf size	27. Berry set	46. Foliage development
9. Leaf pubescence	28. Tuber shape	47. Tuberization
10. Leaf set on stem	29. Tuber shape uniformity	48. Number of tubers
11. Leaf intensity of color	30. Eye depth	49. Tuber size
12. Pigmentation of midribs	31. Heel end	50. Yield potential
13. Number of leaflets	32. Skin color	51. Grading
14. Terminal leaflet shape	33. Skin pigmentation intensity	52. Drought resistance
15. Lateral leaflet shape	34. Pattern of skin pigmentation	53. Drought susceptibility
16. Junction between leaflets	35. Distribution of skin pigmentation	54. General storage ability
17. Secondary leaflets	36. Skin texture	55. Dormancy
18. Degree of flowering	37. Flesh color	
19. Inflorescence size	38. Flesh pigmentation	

The field trial was conducted on 31 potato (*Solanum tuberosum* L.) varieties and 28 valuable breeding lines of Estonian origin in 2002–2004 (Table 2). Each year of the experiment, the plant material initially preserved and propagated as *in vitro* meristem plants was used. The pre-growth and acclimatization of meristem plants was carried out in the greenhouse. Adding to in the field

collection and cultivation was accomplished with the potato cultivation technology created in EVIKA. 20 plants per accession, 1180 plants in total, were planted into the first year field collection. Rooting, quality of plants, flowering, disease resistance and true-to-typeness were estimated during the vegetation period. 40 tubers per accessions were separated during the harvest for establishment in the field collections in 2002, 2003 and 2004.

Potato accessions were characterized for 44 botanical (tubers 11; sprouts 6; plant 27) and 9 agronomical traits in accordance with the accepted evaluation method of potato accessions and international potato descriptors (Descriptors for ..., 1977).

## Results and Discussion

Oats. The standards of oats descriptors (Oats Descriptors, 1985) and The International Union for the Protection of New Varieties of Plants (UPOV, 2000) were followed compiling the list of characteristics evaluated in the field trial at the Jõgeva PBI in a two-year experiment. The weather conditions of both trial years were exceptionally dry, the drought considerably affected the traits of the tested varieties.

The data of economic data of plant breeders interests in particular (Table 3) are investigated hereafter.

Table 3. The evaluation of the oat accessions at the Jõgeva PBI (the average of 2006 and 2007)

Accession number	Variety	Registr. year	Yield, kg ha <sup>-1</sup>	Plant Height cm	Growing period days	1000 g Weight G	Spot blotch points	Protein content g kg <sup>-1</sup>	Husk content g kg <sup>-1</sup>	Test weight g l <sup>-1</sup>
JPBI 4	Jaak	1995	3026	76	87	36.1	3	152	254	473
JPBI 13	Villu	1999	3015	68	89	32.8	3	145	245	496
JPBI 9	Kehra saagirikas	1929	2542	86	93	33.7	5	158	248	493
JPBI 7	Jõgeva roostekindlam	1930	2574	87	93	31.6	4	175	262	474
JPBI 10	Kehra varajane	1930	2638	87	90	32.5	3	156	257	477
JPBI 5	Jõgeva agu	1939	2336	79	93	32.9	4	171	247	454
JPBI 6	Jõgeva koidukaer	1939	2402	81	90	35.0	3	148	252	481
JPBI 8	Jõgeva seisukindlam	1939	2457	79	93	30.9	3	166	252	444
JPBI 3	Hämarik	1952	2653	87	92	33.5	4	156	276	495
JPBI 2	Ella	1976	2836	74	91	35.4	4	154	273	477
JPBI 12	Viker	1980	2732	84	92	33.8	4	159	261	489
JPBI 1	Alo	1986	2732	73	91	30.5	3	153	259	471
JPBI 11	Miku	1991	2906	68	88	31.0	3	139	249	515
LSD 0.05			182	4	2	1.7	0.5	9	10	9

Due to dry conditions there was no occurrence of the most widespread plant diseases in Estonia such as crown rust and black stem rust. Spot blotch was observed to a low to moderate level in both years, 3–5 points on a 9–point scale. Most varieties displayed almost equal levels of spot blotch infection, only Kehra saagirikas was more susceptible than the others.

The grain yield of the standard varieties Jaak and Villu exceeded the old varieties by about 7–30 %. The old varieties Jõgeva agu and Koidukaer showed the lowest yielding capacity.

In normal growing conditions the average 1000 grain weight of oats at the Jõgeva trials is 38 g (Tamm and Tamm, 2002) but in the dry trial years 2006 and 2007 it was significantly smaller. The varieties Jaak, Ella and Koidukaer were distinguished for large size of their kernel.

Standard variety Jaak is the tallest listed variety in Estonia but older varieties were even taller. Previous studies have shown that oat plants are usually about 20–30 cm taller in comparison to the 2006–2007 trial years which had dry conditions. The plant height of the older varieties exceeded by 6–19 cm the medium-height standard variety Villu. Older varieties are possibly more susceptible to lodging. No lodging however was observed in any of the cultivars over the trial period.

The duration of the vegetative period (days to harvest) is a very important trait in oat cultivation and is directly correlated with grain yield and its quality (Loskutov, 2005). The earliest variety was Jaak (87 days). All older varieties had a longer growing period than the standards.

The protein content was high in comparison to the results obtained by the Jõgeva PBI in field trials during the last decades. Some obsolete varieties (Jõgeva agu, Jõgeva roostekindlam) appeared to have high protein content compared to modern varieties.

The average husk content at the Jõgeva trials has been about 25 %. The Husk content of most of the older varieties was close to standard Jaak. Some older varieties exceeded the standards. There were no varieties characterised by low husk content.

The test weight of the majority of the obsolete varieties was equal or higher than that of the standard variety Jaak but lower compared to the standard variety of Villu.

The evaluation of the Estonian accessions will continue along with the evaluation of about 600 European oat accessions at the Jõgeva PBI within the framework of the project of the European Community programme “Avena genetic resources for quality in human consumption – AVEQ” in 2008–2010 (Avena genetic resources...). In addition to the descriptors characterized in 2006–2007, color of lemma, the emergence and the number of seeds per panicle will be observed in the field in six countries. Fat content and mycotoxin contamination will be analysed in laboratories.

Potato. 44 botanical characteristics and 9 agronomical characteristics of 59 Estonian potato accessions preserved the *in vitro* genebank in EVIKA were evaluated. The evaluation of potato cultivars based on phenotypic characteristics can be affected by environmental factors, therefore the evaluation was performed for three times. The average climatic conditions for a 3 year period were quite similar and every time the same persons were involved in the evaluation process. On account of this the results differed through the years only 1...2 points in scope in the case of plant height, degree of flowering, number of tubers and tuber size.

In the field collection evaluation were performed three times. The summarized data were analyzed and used to update the database. Also a catalogue with the definitions of the evaluated descriptors and character references per accessions was assembled.

All information obtained is available for potential potato genetic resource users in Estonia and abroad at the present on request, and also soon on the internet

The results of this study provide valuable information about the influences of long-term *in vitro* preservation on the genetic stability of potato meristem plants. The medium-term preservation of potato genetic resources as meristem plants *in vitro* does not affect the genetic stability of the genotypes.

*In vitro* preserved accessions can be used as an initial material for breeding, research, for the propagation of disease-free material for seed production and for the establishment of field collections.

## Conclusions

The conservation of the national heritage of plant genetic resources for food and agriculture is an obligation of every stakeholder confirmed by international agreements. Estonian PGR institutions have fulfilled their commitment in accordance with the International Treaty on PGRFA and the common guidelines of the European Cooperative Programme for Plant Genetic Resources (ECPGR). Passport descriptors of Estonian accessions were assessed and recorded in the national database during the first phase of the Estonian National PGR Programme in 2002–2006. The main objectives for the programme period 2007–2013 are the evaluation and characterization of maintained accessions according to the internationally agreed descriptors.

Relevant investigations were commenced on oat and potato accessions. The results of the current study are important for complementing the database of the Estonian PGR which in addition to the passport data and the evaluation and characterization data of potato and oats provides more detailed information about the accessions. Besides, the evaluation data of internationally agreed descriptors will be included in the European Crop Databases of the ECPGR and the European Plant Genetic Resources Search Catalogue (EURISCO), which makes the Estonian material more available for other institutions and at the same time it widens the genetic base of our local breeding opportunities.

The evaluation of the oat accessions of Estonian origin will continue during of 2008–2010 in the framework of the European Community project “Avena genetic resources for quality in human consumption – AVEQ”.



The follow-up project was carried out in EVIKA for the evaluation the next set of 60 potato accessions, including 26 landraces and 10 older varieties in 2005–2008.

### Acknowledgements

This study has been supported by the Estonian National Programme on Plant Genetic Resources.

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## IGAUNIJAS AUGU ĢENĒTISKO RESURSU *EX SITU* UN *IN VITRO* KOLEKCIJU SAGLABĀŠANA UN NOVĒRTĒŠANA

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Igaunijas valdība ir atsaukusies uz globālajiem bioloģiskās daudzveidības saglabāšanas un ilgtspējīgas izmantošanas centieniem, ratificējot starptautiskos līgumus un izveidojot nacionālo pārtikas un lauksaimniecības augu ģenētisko resursu programmu. Nozīmīgas programmas aktivitātes bija Igaunijas izcelsmes augu ģenētiskā materiāla savākšana, identificēšana un saglabāšana kā arī sistēmas izveidošana. Tā kā ģenētiskie resursi nodrošina izejmateriālu selekcionāriem un zinātniekiem, ir nepieciešami detalizēti pētījumi un jāpilnveido ģenētisko resursu izmantošana.

Šajā pētījumā veikta 13 auzu un 59 kartupeļu paraugu novērtēšana, kas tiek glabāti Igaunijas *ex situ* un *in vitro* gēnu bankā. Novērtēšanas deskriptori tika atlasīti no Eiropas Augu ģenētisko resursu kooperatīvās programmas darba grupu izveidotā un Biodiversity International atbalstītā deskriptoru

saraksta. Šā pētījuma rezultāti tiks izmantoti augu ģenētisko resursu datu bāzu papildināšanai un šie dati ir pielietojami tālākai paraugu izmantošanai augu selekcijā.

## NEW POTATO VARIETIES RESEARCH IN LITHUANIA

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### Abstract

Potato breeding and seed production in Lithuania is carried out at the Voke Branch of the Lithuanian Institute of Agriculture. Potato breeding work involved Lithuanian potato varieties, varieties from various collection and hybrids. Potato crosses were done at the autotetraploid level in the glasshouse and potato variety collection field. Up to two million hybrids were tested in the trial fields. The key objective was to select the varieties immune to wart disease, cyst nematodes, with high resistance to other diseases, with excellent agronomic and cooking qualities, suitable for the processing industry. As the result of breeding work five new varieties were produced: Venta, VB Rasa, VB Liepa, Goda and VB Aista. They all are immune to the worst potato disease - wart (*Synchtrium endobioticum* Schilb.). Most of them are resistant to a local potato type of nematodes (*Globodera rostochiensis* Woll.). Other advantages such as good yield, excellent cooking qualities, good taste or attractive shape were the main items in producing Lithuanian potato varieties as well. Potato seed production from meristem tissue at biotechnologic laboratory is carried out in the Voke branch of Lithuanian Institute of Agriculture. It is the centre for potato seed production in Lithuania.

**Key words:** potatoes, potato breeding, resistance to nematodes and diseases

### Introduction

For years potatoes have been a staple food crop in Lithuania. Potatoes were grown on every farm by everyone who had land for agricultural purposes. The main potato uses in Lithuania are for human consumption, livestock feeding and manufacturing.

Potatoes are usually bred using the hybridization method (Chauvin *et al.*, 2003). Parental plants which have perfect quality features such as high yielding, earliness, resistance to diseases and pests, dry matter content and can pass on these traits to their progeny are the desired ones. In the potato breeding process the most important are the varieties and hybrids which belong to or have progeny of nine systematic groups: *Comersoniana*, *Glabresantia*, *Acaulia*, and *Transa equaatorialia*, *Andigena*, *Tuberosa*, *Longipedicellata*, *Demissa* and *Pinnatisecta*. They give proper genetic diversity for the cultivated potato varieties (Lough *et al.*, 2001).

The most effective and most expensive way to fight nematodes is by breeding new varieties, resistant to nematodes. Potato varieties which have no resistance against nematodes die without yield production in soils with high number of nematodes. Resistant varieties in the infected fields decrease the number of nematodes in the soil (Ražukas, 2002). Resistant variety can decrease the number of the nematodes up to 70 %. Potato nematodes are quarantined in Lithuania. The import and export of infected tubers is strictly forbidden.

Another very important quarantined potato disease is wart disease (*Synchtrium endobioticum* Schilb.). The best way to fight wart disease is to grow resistant varieties. The growing of resistant varieties decreases wart infection in the field. The infection usually disappears after ten years. It is obligatory for all new potato varieties to have wart resistance for growers in the Republic of Lithuania.

In Lithuania and in all European countries it is quite important to secure a potato harvest in order to bring it to a summer market as soon as possible, when prices are higher (Bradshaw *et al.*, 2006). The developing Lithuanian potato industry also needs potatoes suitable for chips production, because the main crop variety after a long storage time. From the agrotechnic point of view potatoes are also greatly valued because of their short growing period. In crop rotation, the field

after potatoes can be sown with winter crops. As the result of the breeding work the new varieties Venta, VB Rasa, VB Liepa, Goda and VB Aista were bred.

The aim of many years of research was to breed new potato varieties, immune to wart disease, potato cyst nematodes and with other advantages - good resistance to diseases, resistant to mechanical injuries, with good agronomic and cookery qualities and suitability for the processing industry.

### Materials and Methods

Research on Lithuanian new potato varieties was carried out at the Voke Branch of the Lithuanian Institute of Agriculture during the period 1992-2007. The potato varieties were bred using the hybrid cross method. The variety Venta was obtained after crossing Priekulu visagrie x Pirmūnės. The variety VB Rasa – Cardinal x Viola. The variety VB Liepa - No 34/36 x Pirmūnės. The cultivars Goda – Ausonia x Franzi and VB Aista - No 263 x No 476-9.

For the crossing, tubers of parents varieties were planted in the greenhouse in the peat and organic manure mixture on the top of a brick. The first variety was a mother plant and the second one - a father plant. The hybrid berries were collected, planted in the peat pots next year and transplanted to the potato selection field after the spring freezing danger was over. The best potato clone was selected from many others. Selection of the clone resistant to late blight and other diseases in the field and during potato storage gave the expected results. The resistance to wart disease and potato cyst nematodes was tested at the Institute of Plant Protection in Byelorussia.

The varieties plots were established on sandy loam on carbonaceous fluvial-glacial gravel eluviated soil (IDp), according to FAO-UNESCO classification *Haplic Luvisols (LVh)*. All four varieties were tested and grown on soddy podzolic sandy loam soil in a crop rotation field of the breeding department of grasses. The trial field was fertilized with organic manure - 50 t ha<sup>-1</sup> and the mineral fertilizers 90 g kg<sup>-1</sup> N, 90 g kg<sup>-1</sup> P, 90 g kg<sup>-1</sup> K. Tubers were planted by hands into the rows. All plots of the field were fully randomized, the number of replications in different years was up to four. The size of the plots was also different, but each plant feeding plot always was 0.7 x 0.35 m<sup>2</sup>. In the first three years potatoes were harvested by hand, later with a potato digging machine. Tubers were stored in the underground potato storage at natural conditions: +1 to +2 °C and 80-90 % humidity in the winter season (Ražukas *et al.*, 2001).

#### Statistical data analysis

The results were analyzed statistically for randomized split-plot design and the Tukey test was used to verify the significance of differences at  $\alpha = 0.05$ . All data were subjected to ANOVA.

### Results and Discussion

Maincrop varieties produce the highest potato yield in Lithuania. There are donors with resistance to late blight in this group. The genetic material of early and late maturity groups is used for breeding maincrop maturity potato varieties. Special methods were used to prevent discrepancies in the flowering time.

Potato variety Venta (Priekulu visagrie x Pirmūnės). The variety Venta was bred at the Vokė Branch of the Lithuanian Institute of Agriculture. It has been the Lithuanian National Variety List since 1997. Venta matures very early. The tubers are round, with medium-deep eyes. Skin and flesh colour is light yellow. Cooking characteristics are perfect. There is no browning after peeling. The taste is excellent. The variety is the salad type. The starch content is to 14-16 %. The variety Venta is resistant to wart disease; it possesses good field resistance to the black leg and potato leafroll virus. Storage characters under controlled conditions are excellent.

Potato variety VB Rasa (Cardinal x Viola). The variety is late. The plants are compact, the flowers are violet. VB Rasa has high yielding and starch content characteristics, field resistance to common scab, immunity to wart disease and to the Ro1 patotype of golden nematodes.

Potato variety VB Liepa (No 34/36 x Pirmūnės). The variety is early. The plants are compact, flowers are violet. Tubers are round, medium in size, with shallow eyes. Flesh and skin colours is light yellow. Sugar content is low. No browning after peeling and cooking. Starch content is to 20 %. Resistant to potato cyst nematode. Tubers are fully resistant to late blight.

Potato variety Goda (Ausonia x Franzi). The potato variety Goda was bred at the Vokė Branch of the Lithuanian Institute of Agriculture. It has in the Lithuanian potato list since 2001. The variety is early. Tubers are oval round, medium in size, with shallow eyes. Flesh and skin colour is light yellow. The sugar content is low. No browbibg after peeling and cooking. Suitable for the chips industry. Starch content is to 16-17 %. The variety is resistant to potato cyst nematode Ro<sub>1</sub>, and wart disease. Foliage is fairly resistant and tubers fully resistant to late blight and to tuber blight. Storage is good.

Potato variety VB Aista (No 263 x No 476-9). The variety was bred at the Vokė Branch of the Lithuanian Institute of Agriculture. VB Aista has been on the Lithuanian potato list since 1999. The bush is stretched out, the stems are tall. The flowers are white, of medium size. The variety flowers abundantly and for a long time. The tubers are big, yellowish, flat round with shallow eyes. The output of commercial tubers is 90 %. Starch content is high, to 21 %. The variety is good for the starch industry. The potatoes are palatable. The boiled potatoes do not get dark. The variety is resistant to wart disease, potato cyst nematode Ro<sub>1</sub>, late blight. The variety is resistant to drought, can be grown in all kinds of soil using proper cultivation techniques. The variety is resistant to mechanical injuries while harvesting; is of good storage quality.

Potato varieties Venta, VB Rasa, VB Liepa, Goda and VB Aista main features description is presented in Table 1.

Table 1. The main feature varieties of Lithuanian potato

Characters	Potato varieties				
	Venta	VB Rasa	VB Liepa	Goda	VB Aista
Parantage	Priekulu visagrie x Pirmūnēs	Cardinal x Viola	No 34/36 x Pirmūnēs	Ausonia x Franzi	No 263 x No 476-9
Lightsprout: anthocyanin coloration of base <sup>1</sup>	2	1	2	2	1
Plant: frequency of flowers <sup>2</sup>	3	7	7	3	7
Flower corolla: color of inner side <sup>3</sup>	2	2	2	1	1
Tuber: shape <sup>4</sup>	1	3	1	1	1
Tuber: color of skin <sup>5</sup>	1	2	1	1	1
Tuber: color of flesh <sup>6</sup>	3	4	3	4	1
Plant: time of maturity <sup>7</sup>	1	7	3	3	9
Cooking type <sup>8</sup>	A	BC	BC	BC	BC
Resistance to leaf blight <sup>9</sup>	4	8	4	7	8
Resistance to tuber blight <sup>9</sup>	8	9	9	9	9
Resistance to nematode <sup>10</sup>	4	r	r	r	r

<sup>1</sup>anthocyanin coloration of base: 1 – red-violet and 2 – blue-violet; <sup>2</sup>frequency of flowers: 3 – low and 7 – high; <sup>3</sup>color of inner side: 1 – white and 2 – red-violet; <sup>4</sup>shape of tubers: 1 – round and 3 – oval; <sup>5</sup>color of skin: 1 – yellow and 2 – red; <sup>6</sup>color of flesh: 1 – white, 3 – light yellow and 4 – yellow; <sup>7</sup>maturity: 1 – very early, 3 – early, 7 – late and 9 – very late; <sup>8</sup>cooking type: A – firm, B – fairly firm and C – floury; <sup>9</sup>resistance to blight: 9 – very good, 8 – good, 7 – rather good and 5 – rather susceptible; <sup>10</sup>resistance to nematode: r – resistant and 4 – susceptible.

Concentrated attention is given to the disease and pest resistance of the new varieties. Such diseases as wart, nematodes and viruses can be eliminated partially or fully by producing new potato varieties using genetic material which has high resistance or are immune to one or another disease and pest (Asakaviciute *et al.*, 2006).

In Lithuania the most harmful potato disease is late blight (*Phytophthora infestans*). The disease decreases the plant leaf assimilation area and destroys potato foliage at potato tuberization. Virus infection affects the potato yield and during storage period causes different rots. Comparatively late maturity potato varieties such as VB Aista exhibited the highest resistance to late blight

At the present time there are eight potato varieties bred at the Vokė Branch of the Lithuanian Institute of Agriculture in the Lithuanian potato list. Mean quality data is presented in table 2.

Table 2. Lithuanian new potato varieties main quality points in competitive trials (Trakų Vokė, 2002–2007)

Potato varieties	Vegetation	Yield, t ha <sup>-1</sup>	Starch, g kg <sup>-1</sup>	Dry matter, g kg <sup>-1</sup>	Taste, points <sup>1</sup>
Venta	very early	27.9 ± 0.88	150.1 ± 6.71	202.4 ± 7.46	8.0 ± 0.09
VB Rasa	Late	24.6 ± 0.74	198.8 ± 8.60	236.6 ± 7.82	7.0 ± 0.04
VB Liepa	Early	29.3 ± 0.92	185.3 ± 7.44	224.1 ± 5.73	8.1 ± 0.07
Goda	Early	35.2 ± 1.21	170.4 ± 6.19	210.7 ± 4.91	8.6 ± 0.08
VB Aista	very late	28.4 ± 0.84	203.6 ± 9.75	247.3 ± 8.40	7.2 ± 0.05
<i>LSD<sub>05</sub></i>		0.861	6.068	7.548	0.143

<sup>1</sup> taste point -.0 (bad) – 9 (good)

Last five years of testing in the competitive potato comparison trials show that the lowest potato yield was from the VB Rasa variety. The mean data show that yields have reached 24.6 tones per hectare, the when longer maturity potato varieties – Goda – produced over 30 tones per hectare. The highest mean potato yield was from the potato variety Goda. It has reached 35,2 tones per hectare seed potatoe yield. The potato quality data show that the potato starch depended on the potato variety's. So the highest starch amount was produced by the special potato variety for the starch industry – VB Aista. It was over 20 percent, when shorter maturity potato varieties had a starch amount up to 18 percent. The best taste over 8 points belonged to the main table potato variety – Goda.

## Conclusions

The potato varieties Venta, VB Rasa, VB Liepa, Goda and VB Aista were bred at the Vokė Branch of the Lithuanian Institute of Agriculture. Varieties give good quality yield not only in the big farms but also in small potato garden plots.

All varieties are immune to the main quarantine object in Lithuania – wart disease. The potato varieties Goda, VB Liepa, VB Rasa and VB Aista are immune to the potato cyst nematode *Globodera rostochiensis* R<sub>01</sub> patotype. All varieties have good field resistance against the most widely spread diseases – black leg, viruses, common scab, rizoctonia and etc. Their foliage have a fair resistance and tubers a good resistance to late blight. Storage characteristics under controlled conditions are good.

The Lithuanian potato breeding program's main research object was and in the near future will be early and maincrop, immune to wart and nematodes potato selection. Due to their high resistance to disease and pests all Lithuanian potato varieties are exhalent for growing in ecological farms.

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## JAUNU KARTUPEĻU ŠĶIRŅU PĒTĪJUMI LIETUVĀ

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Kartupeļu selekcija un sēklaudzēšana Lietuvā tiek veikta Lietuvas zemkopības institūta Vokes nodaļā. Kartupeļu selekcijas darbs aptver Lietuvas kartupeļu šķirnes, kolekcijas šķirnes un klonus. Kartupeļu krustošana tetraploīdā līmenī tiek veikta siltumnīcās un lauka kolekcijā. Vairāk kā divi miljoni hibrīdu (klonu) tiek izvērtēti izmēģinājumu laukā. Galvenais mērķis ir veidot jaunas kartupeļu šķirnes, kas ir izturīgas pret vēzi un nematodēm, kurām ir augsts izturības līmenis pret citām slimībām, izcilas agronomiskās un garšas īpašības, kā arī piemērotība pārstrādei. Selekcijas darba rezultātā izveidotas piecas jaunas kartupeļu šķirnes: Venta, VB Rasa, VB Liepa, Goda un VB Aista. Tās visas ir izturīgas pret bīstamāko kartupeļu slimību – kartupeļu vēzi (*Synchtrium endobioticum* Schilb), vairākas no tām ir izturīgas pret vietējo nematodes patotipu (*Globodera rostochiensis* Woll.). Citas pazīmes kā augsta raža, izcilas garšas īpašības, kā arī pievilcīga forma bija galvenie iemesli šo šķirņu atlasē.

Kartupeļu sēklaudzēšana ar meristēmu metodi tiek veikta Lietuvas zemkopības institūta Vokes nodaļas biotehnoloģiskajā laboratorijā. Tas ir kartupeļu sēklaudzēšanas centrs Lietuvā.

## APPLYING COLCHICINE AND ORYZALIN IN LILIUM L. POLYPLOIDISATION

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### Abstract

The genus *Lilium* (*Lilium* L.) is a vegetatively propagated bulbous plant – one of the economically most important of bulb flowers. To obtain new varieties with a wide range of colors and resistance to grey mold caused by fungi *Botrytis Micheli* ex Fr. a breeding program was carried out. The spreading of this fungus disease causes heavy losses as plants lose their general attractiveness. In lily breeding current activities are directed towards the development of disease resistant cultivars to avoid the use of chemicals to be economically sound and ecologically safe. The goal of this research was to investigate and to optimize polyploidy in the breeding of lilies. Several biotechnological methods were used to obtain new lily varieties. A crossing between different hybrid groups of lilies is not possible under natural conditions therefore embryo cultivation techniques are being developed to overcome incompatibility between plants and limiting factors after fertilisation. Mitotic and meiotic polyploidisations are applied and can result in fertile allopolyploids. The chromosome count of the varieties can be changed by treating bulb scales with a 0.1, 0.5 and 1 g kg<sup>-1</sup> colchicine solution and 0.05, 0.1 and 0.5 g kg<sup>-1</sup> oryzalin solution.

**Key words:** chromosomes, mitotic and meiotic polyploidisations

### Introduction

The genus *Lilium* L. includes approximately 100 species, subspecies and varieties of species distributed throughout the cold and temperate parts of the Northern Hemisphere (McRae, 1998). The overall appearance of all plants is controlled largely by their genes that are packaged in chromosomes. Each species has a fixed number of chromosomes in their cells, but the number may differ between species. Each cell of *Lilium* species has 24 chromosomes, or 12 pairs of different chromosomes ( $2n = 2x = 24$ ). These plants with their paired chromosomes are termed diploid, from the Greek word for 'double' (McRae, 1998). Polyploids have more chromosomes in every cell than others. The offspring of a tetraploid parent and a diploid parent is a triploid; this results from a failure of meiosis in one of the parents. With their 36 chromosomes, triploid lilies are difficult to cross with others. Tetraploid lilies have 48 chromosomes; this is double the normal number of diploids.

The reasons for using polyploidy in lily breeding are the larger flowers, the stronger stems and in interspecific hybridization the restoration of F1-sterility at the tetraploid level (Van Holsteijn,

1994; Van Tuyl *et al.*, 1990). Interspecific hybridisation and polyploidy are recognized as the most important sources of evolution and domestication of flowering plants. To overcome F1-sterility, mitotic and meiotic polyploidisations are applied and can result in fertile allopolyploids. A distinction was made between mitotic polyploidisation and meiotic polyploidisation. Mitotic polyploidisations possess one homologous chromosome set. Mitotic polyploidisation comprises all techniques in which artificial chromosome number doubling was accomplished by treating bulb material with colchicine. Meiotic polyploidisation often shows irregular chromosome division that results in two unreduced chromosome numbers (Van Tuyl *et al.*, 1990; Van Tuyl and Ki-Byung, 2003).

Polyploids are obtained through artificial chromosome doubling by treatment of vegetative tissue with spindle inhibitors such as colchicine (Blakeslee and Avery, 1937; Emsweller, 1988) or oryzalin. Colchicine has been used for doubling the number of chromosomes of many crop plants over a period of more than 50 years (Blakeslee and Avery, 1937). Colchicine is a natural alkaloid with an antimitotic activity, obtained from the plant *Colchicum autumnale* L. (Emsweller, 1988; Van Tuyl *et al.*, 1990). When colchicine is present in a cell that is undergoing mitotic division, the chromosomes split at all points except the centromere. The main action of the colchicine is to prevent the formation of a spindle so the anaphase movement of the chromosomes does not take place and the cell fails to divide. When the daughter chromosomes finally divide, they are all included in one cell and the chromosome number is doubled. To be effective colchicine must be present in the cell when the chromosomes divide. Colchicine is very harmful to humans and in some cases shows undesirable mutagenetic activity on plants (Van Tuyl *et al.*, 1990). In addition to colchicines, several other chemicals are also effective in doubling the chromosome number. One of the chemicals that also inhibits mitosis activity and is used for doubling the chromosome number in lilies is oryzalin. For doubling the chromosome number, oryzalin is used for other plants as well – such as: potatoes (Van Tuyl *et al.*, 1992; Verhoeven *et al.*, 1990), tobacco (Scree Ramulu *et al.*, 1991). The goal of this research was to investigate and to optimize polyploidy in the breeding of lilies.

### Materials and Methods

The polyploid forms were produced utilizing bulb scales of diploid lilies ( $2n = 2x = 24$ ). In the present study, scales from diploidal lily bulbs from 13 different genotypes were tested: Asiatic hybrids – ‘Arabeska’, ‘Baltais Lācis’, ‘Brushstroke’, ‘Evrīka’, ‘Lastočka’, ‘Lolly’, ‘Miss Alice’, ‘Nakts Tango’, ‘Saules Meita’, ‘Višenka’; Trumpet hybrid ‘Zemgale’ and the species *L. kesselringianum* Misch. and *L. monadelphum* Bieb. The chemicals colchicine and oryzalin were used in chromosome doubling. Bulb scales were treated with a 0.1, 0.5 and 1 g kg<sup>-1</sup> colchicine solution and a 0.05, 0.1 and 0.5 g kg<sup>-1</sup> oryzalin solution.

For chromosome number determination, the bulbs were kept in a washed river sand medium at 25 °C for 3 weeks – until clean and white root tips had developed. Prior to chromosome counting, these bulbs were kept for 24 hours at 4 °C. After this treatment, undamaged healthy root tips were cut off – about 5 to 7 mm long, and washed under running water. Because the process of cell division can be stopped by colchicine before chromosomes multiply, all dividing cells are allowed to proceed up to this stage. The cut root tips were put into 50 ml beakers filled with 0.7 g kg<sup>-1</sup> colchicine solution and kept for 2 hours; then washed three times in running water and transferred to a modified Clarke's Fluid (750 g kg<sup>-1</sup> ethyl alcohol, 250 g kg<sup>-1</sup> ethanol glacial acetic acid) for 30 min at 20 °C. The acetic acid effect was neutralized by keeping the tips of the rootlets for 45 minutes in distilled water and 24 hours in 700 g kg<sup>-1</sup> ethyl alcohol. For chromosome counting, the root tips were left for 48 hours in a colouring solution of 5 g kg<sup>-1</sup> carmine in 450 g kg<sup>-1</sup> propionic acid. The root tips were cut to about 1-2 mm and, in a drop of stain, mashed with a steel needle. The preparation was then covered. With a microscope, the chromosomes were counted and cells of five rootlets inspected for each genotype.

### Results and Discussion

The bulb scales were treated with a 0.1, 0.5 and 1 g kg<sup>-1</sup> colchicine solutions. The treatment with 0.1 g kg<sup>-1</sup> colchicine solution resulted in the production of 1.1 bulblets per scale on average (min - 0.1; max - 2.8) (Fig 1).

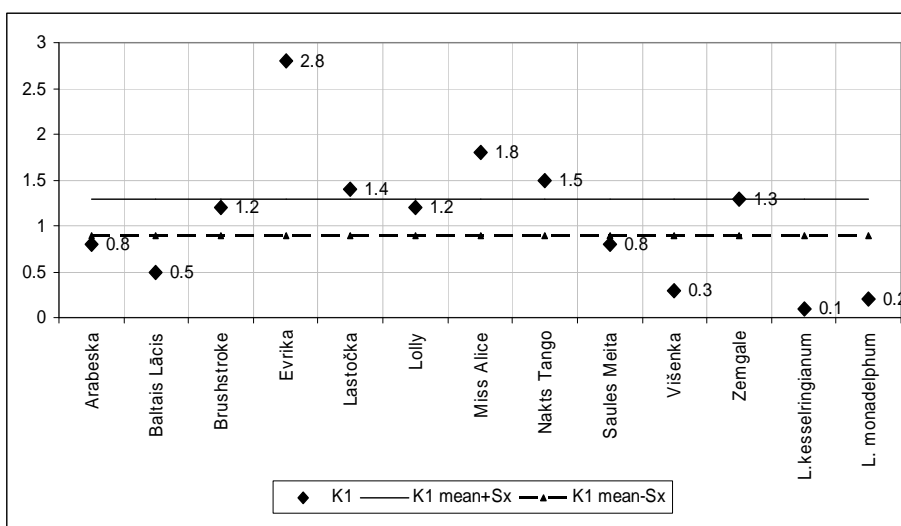


Figure 1. The results of the production of bulblets by treated bulb scales with 0.1 g kg<sup>-1</sup> colchicine  
Abbreviations: K1 – 0.1 g kg<sup>-1</sup> colchicine; Sx – Standard error

The treatment with a 0.5 g kg<sup>-1</sup> colchicine solution resulted in 0.97 bulblet on average (min - 0.1; max - 1.9) (Fig 2).

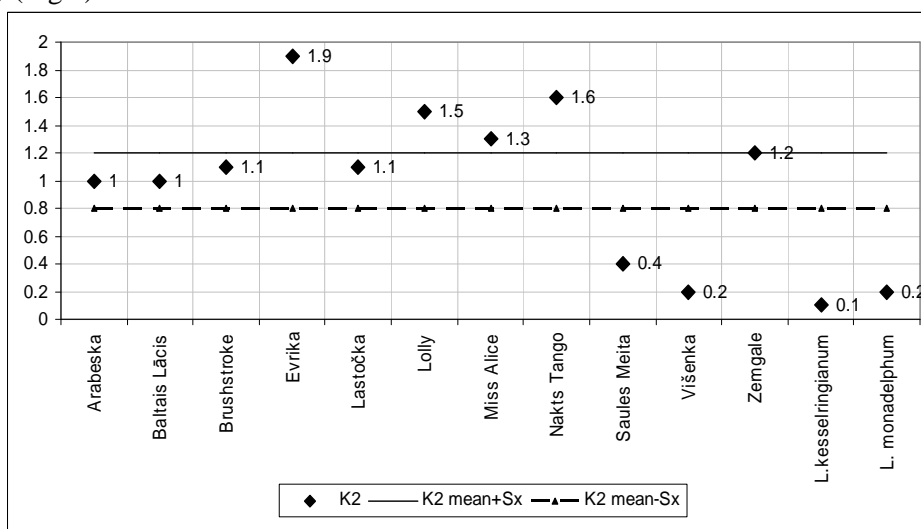


Figure 2. The results of the production of bulblets by treated bulb scales with 0.5 g kg<sup>-1</sup> colchicine  
Abbreviations: K2 – 0.5 g kg<sup>-1</sup> colchicine; Sx – Standard error

Bulb scales treated with 1 g kg<sup>-1</sup> colchicine solution produced 0.2 bulblet per scale on average (Fig 3). This concentration turned out to be toxic. The use of this concentration resulted in the production of 10 polyploid plants.



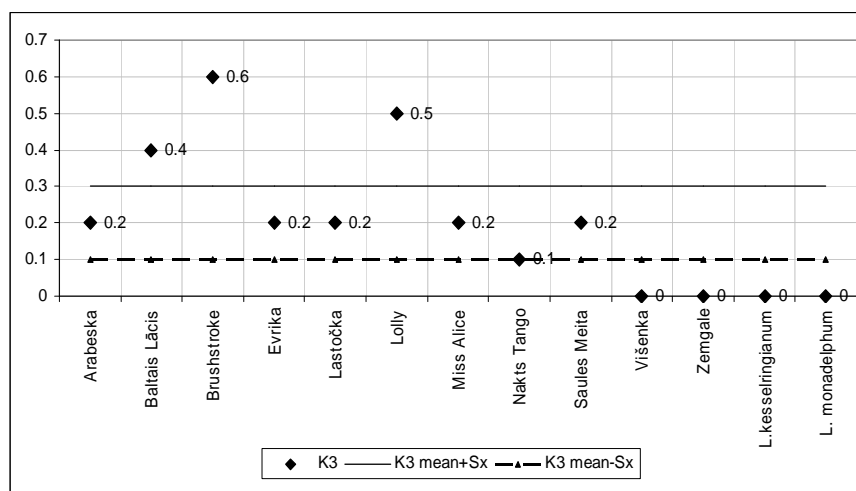


Figure 3. The results of the production of bulbets by treated bulb scales with 1 g kg<sup>-1</sup> colchicine  
Abbreviations: K3 – 1 g kg<sup>-1</sup> colchicine; Sx – Standard error

The cultivars that excelled with a greater amount of min and max bulbets were: ‘Evrika’ (1.9 and 2.8), ‘Miss Alice’ (1.3 and 1.8), ‘Nakts Tango’ (1.5 and 1.6), ‘Lolly’ (1.2 and 1.5). On average, less than 1 bulbet was obtained with ‘Višenka’ (0.2 and 0.3) and ‘Saules Meita’ (0.4 and 0.8). The species *L. monadelphum* produced 0.2 and 0.2 bulbets on average, and *L. kesselringianum* P. Miscz. - 0.1 and 0.1 bulbets on average. These results may be traced to the sensitivity of the species to concentrations of colchicine solution. Polyploid plants were not produced. Bulb scales were treated with oryzalin solution in concentrations of 0.05, 0.1 and 0.5 g kg<sup>-1</sup> (Fig 4; Fig 5 and Fig 6).

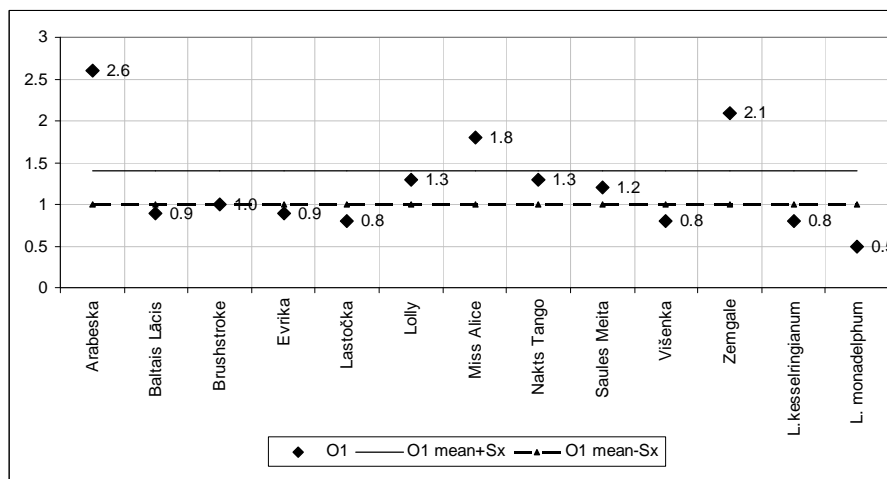


Figure 4. The results of the production of bulbets by treated bulb scales with 0.05 g kg<sup>-1</sup> oryzalin  
Abbreviations: O1 – 0.05 g kg<sup>-1</sup> oryzalin; Sx – Standard error

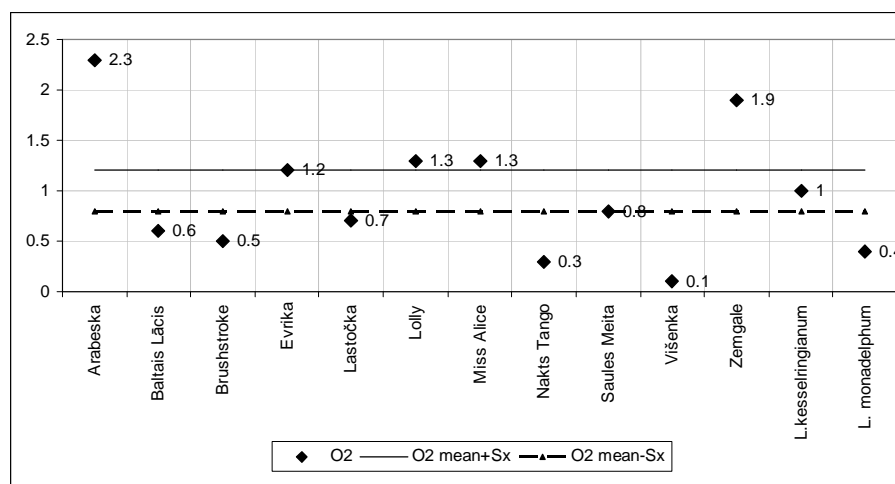


Figure 5. The results of the production of bulblets by treated bulb scales with 0.1 g kg<sup>-1</sup> oryzalin  
Abbreviations: O2 – 0.1 g kg<sup>-1</sup> oryzalin; Sx – Standard error

The obtained average yield of bulblets per scale was, respectively, 1.23 (min - 0.5, max - 2.6), 0.95 (min - 0.1, max - 2.3), and 0.42 (min 0.0, max 0.9). The use of 0.05 g kg<sup>-1</sup> oryzalin solution resulted in 2 polyploid plants, 0.1 g kg<sup>-1</sup> - in 13 polyploid plants, but with 0.5 g kg<sup>-1</sup> oryzalin solution no polyploids were produced. Bulblets more in count and greater in size were developed from outer scales of bulbs when compared to inner thinner scales.

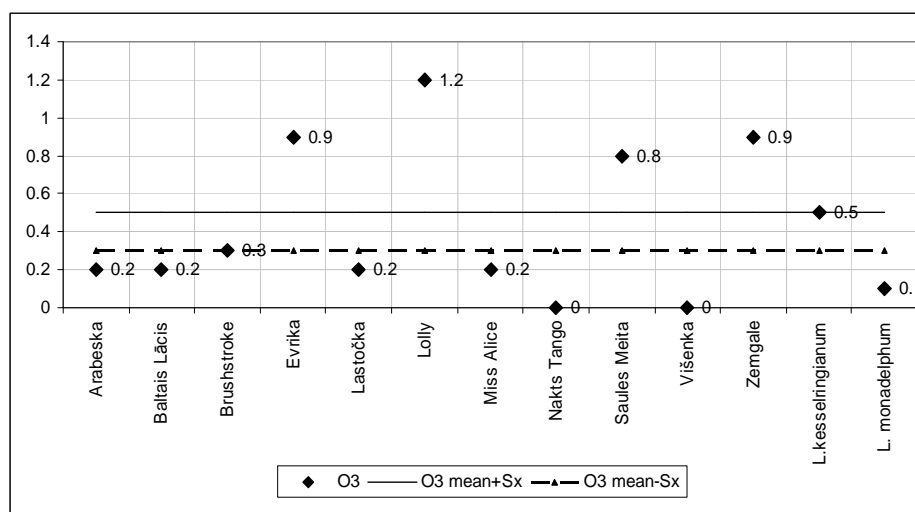


Figure 6. The results of the production of bulblets by treated bulb scales with 0.5 g kg<sup>-1</sup> oryzalin  
Abbreviations: O3 – 0.5 g kg<sup>-1</sup> oryzalin; Sx – Standard error

According to their morphological traits, selections of polyploidy plants were made and the degree of polyploidy was determined cytologically. Morphological differences between the diploid and tetraploid forms of the same clone have been studied in Asiatic hybrid lilies. Lengths of stems, lengths and widths of leaves, lengths of petals, numbers of leaves and flowers, data on flowering, degrees of leaf scorch were all recorded at flowering time (Okazaki and Hane, 2005). According to the findings, most tetraploid forms came into bloom later than diploids; in conclusion – higher ploidity correlates with delayed flowering.

In our study we found that, in comparison with diploids, the polyploid plants we produced had a larger flower diameter (+2 up to 2.5 cm), more extended plant height (+10 up to 20 cm), increased

flower count (+2), and bloomed 4-10 days later than diploids of the same variety. A visual estimation of the bulbs also indicated differences. When compared to diploids, the roots of polyploids were shorter, rather stout, stumpy, smaller in numbers, and bulb scales were wider, more swollen with the outer scales and curved in a 90 degree angle.

The polyploid forms raise genotypic variability in diploid genotypes; improve their general attractiveness; increase plasticity and resistance against diseases and unfavorable biotic and abiotic conditions. Okazaki and Hane, 2005 suggested that the production of true tetraploid Asiatic hybrid lilies via colchicine treatment is necessary in polyploid breeding. A full understanding of the agronomic traits of polyploid lilies requires the evaluation of the morphological and physiological differences among diploids, triploids and tetraploids.

## Conclusions

The scales of bulbs treated with 1 g kg<sup>-1</sup> colchicine and 0.1 g kg<sup>-1</sup> oryzaline solutions have successfully produced polyploid forms. The duration of exposure might be for 2, 4, 6 and 24 hours. Oryzalin inhibited plant cell division much more effectively than colchicine, and is applied successfully in doubling the number of chromosomes in lower concentrations (0.05, 0.1 and 0.5 g kg<sup>-1</sup>) than colchicine (1 g kg<sup>-1</sup>). In our research, 5.5 % polyploids were obtained from the total number of bulblets. The application of molecular, genomic and cytogenetic techniques can be of great help for fastening interspecific hybridisation programmes. Obtained polyploid forms were used in interspecific crossings to restore fertility.

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## KOLHICĪNA UN ORIZALĪNA PIELIETOŠANA LILIJU (LILIUM L.) POLIPLŪDIZĀCIJĀ

### Balode A.

Liliju ģints (*Lilium* L.) ir viena no ekonomiski nozīmīgākajām veģetatīvi pavairojamo sīpolaugu ģintīm. Lai iegūtu jaunas šķirnes ar vēlamām saimnieciskām īpašībām - plašu ziedu krāsu spektru, izturīgas pret pelēko puvi, kuru ierosina sēnes no *Botrytis Micheli* ex Fr. ģints, tiek veikta selekcija. Pelēkās puves infekcijas dēļ zaudējumus cieš liliju audzētāji, jo slimības rezultātā liliņas zaudē dekoratīvātāti. Mūsdienās selekcijas darbs ir vērsts uz to, lai veidotu pret slimībām izturīgas šķirnes, kuru audzēšanā nebūtu jāpielieto ķīmiskie augu aizsardzības līdzekļi, tā būtu ekonomiski izdevīga un ekoloģiski nekaitīga. Tā kā dažādu grupu liliju sugas savā starpā nekrustojas un, lai pārvarētu nesaderību, pēc apaugļošanās barjeru, izstrādā embriju kultivēšanas metodes. Poliploīdijas

pielietošana selekcijā arī paplašina mainības ietvarus, paaugstina augu plastiskumu un izturību pret nelabvēlīgiem biotiskiem un abiotiskiem apstākļiem. Pētījuma mērķis ir izpētīt un optimizēt poliploīdijas pielietošanas iespējas liliju selekcijā, apstrādājot liliju sīpolu zvīņlapas ar 0.1, 0.5 un 1 g kg<sup>-1</sup> kolhicīna šķīdumu un 0.05, 0.1 un 0.5 g kg<sup>-1</sup> orizalīna šķīdumu.

## GENETIC FINGERPRINTING OF LATVIAN RED CLOVER (*TRIFOLIUM PRATENSE* L.) VARIETIES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS: COMPARISONS OVER TIME AND SPACE

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### Abstract

We have established Simple Sequence Repeat (SSR) marker genetic fingerprinting protocols for red clover (*Trifolium pratense* L.) varieties found in the Latvian Gene Bank (LGB). As red clover is an obligate outcrosser, and the varieties are grown and renewed in the field without any particular isolation techniques, a high degree of intra-varietal heterogeneity is to be expected.

We analysed 7 diploid varieties, which were developed in three different breeding stations. We tested seeds from these varieties that were placed into the LGB in 1999 and 2000. In addition, we analysed a range of source material for one variety ('Priekuļi 66'). For this variety, we tested seeds that were repatriated from the VIR institute (placed into the VIR collection in 1982, seeds reproduced in 2005), the samples from the LGB (seed reproduction - 1997), and also plant material grown in the field this year (2007).

By analysing samples from various sources, we can examine the effect of space (varieties developed at different breeding stations), as well as time (repatriated seeds, LGB seeds, and current crop), in an obligate outcrossing crop species, where intra-varietal heterogeneity is high.

**Key words:** red clover, *Trifolium pratense* L., genetic fingerprinting, Simple Sequence Repeat, Latvian Gene Bank, plant genetic resources

### Introduction

Red clover is an important forage legume, widely grown in temperate regions and used in crop rotations. It is an obligate outcrosser, with a gametophytic self-incompatibility system (Taylor and Quesenberry 1996). Red clover is a diploid ( $2n = 2x = 14$ ) species, however artificial tetraploid varieties have been created in breeding programs. Generally breeding programs are based on mass selection, and therefore the varieties produced are heterogeneous with highly heterozygous individuals. Initial molecular analyses of this species were undertaken using dominant marker systems such as RAPDs and AFLPs (Ulloa *et al.*, 2003; Herrmann *et al.*, 2005). The development of Simple Sequence Repeat (SSR) markers for red clover has allowed the analysis of these highly heterozygous varieties using highly informative co-dominant markers (Kolliker *et al.*, 2006). At the Latvian Gene Bank, we are in the process of establishing protocols for genetic fingerprinting of all species in our collection. We have focused our efforts on the use of SSR markers for as many species as possible, due to their high information content (alleles per marker), co-dominant nature (which allows more sensitive detection of heterozygosity and variation within cultivars and lines), and their ease of use (provided appropriate SSR marker primers have been developed).

Our aims were to establish SSR fingerprinting protocols for the red clover varieties placed in the Latvian Gene Bank and to examine the inter- and intravarietal variation of Latvian clover varieties. Clover breeding has been undertaken in at least three breeding institutes in Latvia, and the Latvian Gene Bank holds seeds of accessions developed at all of these institutes. Currently, the main institute involved with clover breeding and maintaining the genetic resources is Skrīveri Breeding Institute, but varieties have also been developed at the Priekuli Breeding Institute and Stende. Prior to the establishment of the Latvian Gene Bank, some of these cultivars developed at these breeding

institutes were placed into the genetic resources collection at the Vavilov institute (VIR) in St. Petersburg, Russia. We examined one variety ('Priekuļi 66') in more detail in order to compare the SSR polymorphism of three separate collections of this variety. 'Priekuļi 66' was transferred into the VIR institute collection in 1982, and renewed there in 1988. This material was repatriated to Latvia in 2004, at which time the seed material was renewed. The 'Priekuļi 66' seeds were placed into the Latvian Gene Bank collection in 1997. We also took samples of this cultivar from the field collections at Priekuļi, where it is resown annually.

### Materials and Methods

Seed material was obtained from the Latvian Gene Bank collection. Seeds were germinated and genomic DNA was extracted from seedlings with the Genomic DNA Purification Kit K0512 (Fermentas, Lithuania). The red clover cultivars analysed are listed in Table 1. DNA from 24 individuals was extracted and analysed separately. In addition, seed material from the VIR institute was obtained and germinated. Plants from these germinated seeds were planted and seed material collected. These seed were germinated and DNA extracted from them. A total of 92 individuals derived from 34 repatriated seeds were analysed. For the field collections, leaf samples of 35 individuals were collected 2007, and the DNA extracted as above. To allow for a more detailed comparison with the VIR and field populations, 48 'Priekuļu 66' seeds from the gene bank collection were germinated and DNA extracted.

Table 1. Red clover cultivars analysed, breeding institute where each cultivar was developed, year of cultivar release and year of seed reproduction

Cultivar	Breeding institute	Year of cultivar release	Year of seed reproduction
Priekuļu 66 (Raunis)	Priekuļi	1968	
- Field			2007
- Gene Bank			1997
- VIR			2005
Stendes Agrais	Stende	1968	1994
Stendes Vēlais II	Stende	1951	1997
Dižstende	Stende	1999	1997
Agra	Skrīveri	1996	1997
Ārija	Skrīveri	1999	1999
Skrīveru Agrais	Skrīveri	1976	1997

Eight SSR markers were used to genotype these cultivars (TPSSR13, TPSSR17, TPSSR16, TPSSR34, TPSSR44 and TPSSR50) (Kolliker *et al.*, 2005). The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a fluorescent sequencer.

SSR locus amplification was carried out using the following PCR conditions: 95 °C for 3 min, 38 cycles of 95 °C for 30 sec, 55 °C – 30 sec, 72 °C – 30 sec; 72 °C - 10 min; in a total volume of reaction 20µl containing 50 ng template DNA, 1x PCR buffer, 2 mM MgCl<sub>2</sub>, 0.2 mM dNTP mix, 0.5 U *Taq* polymerase (*Fermentas*), 0.5 mM of forward (labelled) and reverse primers (*Applied Biosystems*). Amplification fragments were separated on an ABI Prism 3130xl Avant Genetic Analyzer (*Applied Biosystems*) and analyzed with GeneMapper 3.5. Population analyses were performed with GenAlEx 6 version (Peakall and Smouse, 2006), and dendrograms constructed using NTSYSpc2.1.

### Results and Discussion

The six SSR markers revealed a high level of genetic polymorphism, with 22-44 alleles detected per marker. Within populations, the majority of markers were in Hardy-Weinberg equilibrium, with only two populations having statistically significant departures ('Priekuļu 66' – Field – TPSSR16 and TPSSR17 and 'Priekuļu 66' – VIR – TPSSR44 and TPSSR50). Only three markers

significantly departed from equilibrium in more than one population (TPSSR16, TPSSR17 and TPSSR34).

The mean number of alleles found in each population ranged from 11.3 ('Ārija') to 19.2 'Priekuļi 66' - Gene Bank. The mean number of alleles per population with a frequency of over 5% ranged from 5.5-7.83. The mean number of effective alleles per population was similar between populations, ranging from 7.79-10.65. However, the mean number of unique alleles found per population was variable, ranging from 0.17 ('Stendes Vēlais II') to 2.33 ('Priekuļi 66' - VIR) (Table 2, Figure 1).

Table 2. Numbers of alleles (Na), alleles with a frequency over 5%, effective alleles (Ne), unique alleles, and expected heterozygosity (He) across populations

Number of alleles	Population									
	P66 Field	P66 Gene Bank	P66 VIR	Stendes Vēlais II	Stendes Agrais	Dižstende	Agra	Ārija	Skrīveru Agrais	
Na	17.67	19.17	15.00	13.33	15.83	12.33	12.67	11.33	14.83	
Na (freq. $\geq 5\%$ )	6.17	7.00	6.67	6.17	8.33	6.67	5.50	6.00	7.83	
Ne	10.10	10.65	9.90	7.79	10.01	7.91	9.29	8.09	10.52	
No. unique alleles	1.50	1.33	2.33	0.17	0.50	0.33	0.33	0.50	1.00	
He	0.84	0.85	0.87	0.84	0.85	0.82	0.84	0.85	0.88	

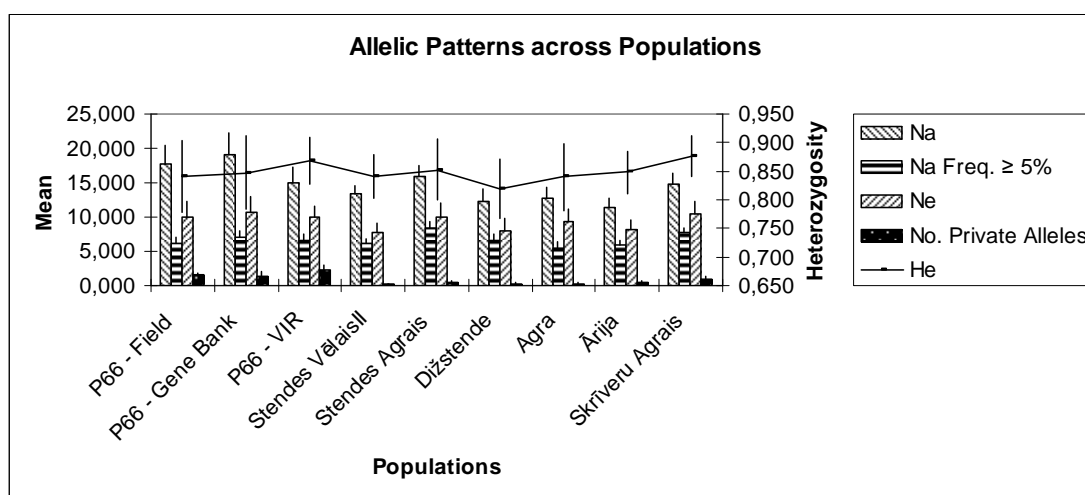


Figure 1. Allelic patterns across populations using 6 SSR markers (see also Table 2)

Pairwise  $F_{st}$  values revealed low population differentiation (values ranged from 0.006-0.043) (Table 3). AMOVA analysis revealed only 2% of the genetic polymorphism was found between populations ( $p=0.01$ ). Frequency based likelihood population assignment tests (Paetkau *et al*, 2004) also highlighted the low differentiation, with only 51% of individuals assigned to the correct population. The frequency of unique alleles found in the populations was under 0.1, with the exception of 2 alleles (tpSSR17/134bp / $f=0.136$  in 'Priekuļi 66' - VIR and tpSSR13/199bp/ $f=0.125$  in 'Ārija').

Table 3. Pairwise Fst values between cultivars and populations

Variety									
P66 - Field	0.000								
P66 - Gene Bank	0.006	0.000							
P66 - VIR	0.014	0.012	0.000						
Stendes Vēlais II	0.024	0.019	0.023	0.000					
Stendes Agrais	0.017	0.015	0.020	0.018	0.000				
Dižstende	0.032	0.026	0.028	0.030	0.020	0.000			
Agra	0.025	0.024	0.026	0.023	0.023	0.042	0.000		
Ārija	0.030	0.030	0.030	0.038	0.030	0.043	0.028	0.000	
Skrīveru Agrais	0.025	0.019	0.022	0.026	0.021	0.029	0.029	0.028	0.000

The cultivars were divided into populations according to the breeding institute where they were developed (Skrīveri, Stende or Priekuļi), and this enabled slightly better differentiation of these cultivars according to their provenance. AMOVA analysis revealed that the 2% variance found between populations was actually partitioned between breeding institutes. Population assignment calculations were also more successful, with 87% of individuals assigned to the correct breeding institute (Table 4). The dendrogram (constructed using Fst values and the Neighbour Joining clustering algorithm) confirms this division by provenance, with each of the cultivars developed in one particular breeding institute clustering together (Figure 2).

Table 4 - Summary of Population Assignment Outcomes (Number of individuals assigned to “self” or “other” population)

Pop	Self Pop	Other Pop
Stende	50	9
Skrīveri	33	8
Priekuļi	76	7
Total	159	24
Percent	87%	13%

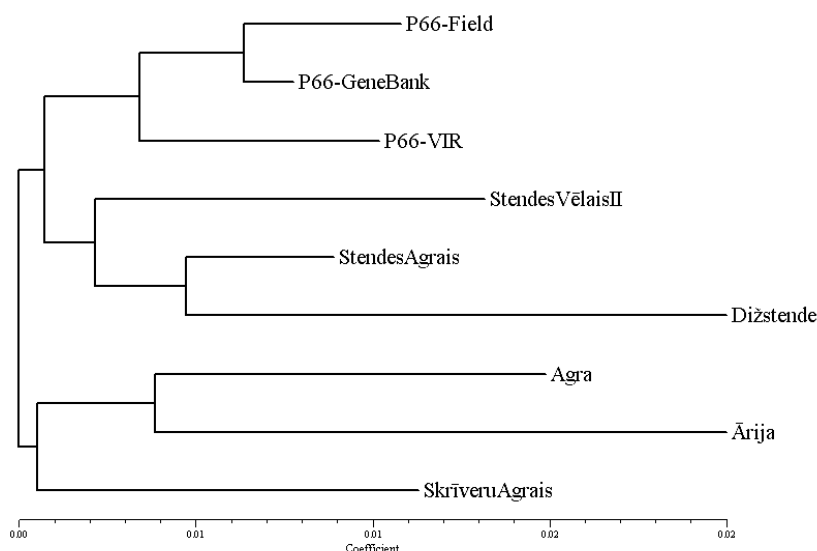


Figure 2 Dendrogram of red clover cultivars constructed using the Neighbour-Joining algorithm and Fst values

The SSR markers were highly polymorphic, confirming that these markers are useful for genetic studies in red clover. The majority of markers in the majority of populations did not deviate from Hardy-Weinberg equilibrium, indicating that there were no significant problems with null alleles, or the markers amplifying multiple loci.

A high level of polymorphism was revealed using these SSR markers, which is not surprising given the obligate outcrossing nature of red clover, and the mass selection breeding methods employed in this species. The number of alleles, particularly those over a frequency of 5% and the effective allele number was similar across all populations. However, the number of unique alleles differed, with the 'Priekuļu 66' – VIR population containing the most, with all 6 SSR markers identifying unique alleles in this population. This concentration of unique alleles in the repatriated population could indicate genetic drift occurring in the Latvian populations. Given that these cultivars are not reproduced in isolation, this would tend to spread the effect of genetic drift across these populations.  $F_{st}$  between the 'Priekuļu 66' – Field and Gene Bank populations was only 0.006, half of the  $F_{st}$  values between the Field and Gene Bank populations and the VIR population (0.014 and 0.012 respectively), which confirms this increased differentiation of the VIR population. These  $F_{st}$  values are approaching the values found between Latvian cultivars such as 'Priekuļu 66' – Field/Gene Bank populations and 'Stendes Agrais' (0.017 and 0.015 respectively). This indicates the effects of genetic drift and gene flow in these cultivars over the 25 year time span that these results survey.

The grouping of the cultivars into populations based on the breeding institute at which they developed yielded a much clearer division between them, as shown by the population assignment results. Using these 6 SSR markers, the probability of assigning a red clover individual to the correct breeding institute is 87%. By increasing the number of markers used for genetic fingerprinting, it should be possible to improve on this assignment probability, in particular if these markers are used in conjunction with phenotypic characteristics.

These results indicate that while the overall differentiation of Latvian red clover cultivars is low, the partition of genetic variance between breeding stations is much more marked. One obvious conclusion that can be drawn from these results is that when renewing the seed stocks to be placed into the Latvian Gene Bank, this should be done at the original breeding institute, rather than at a single location. The comparison of the genetic changes in the cultivar 'Priekuļu 66' demonstrates that the genetic composition of Latvian red clover cultivars is far from static. In order to maximise the genetic diversity of red clover accessions stored in the Latvian Gene Bank, it is necessary to carefully maintain and renew the seeds which have now been stored in the Gene Bank for approximately a decade. Furthermore, it would be desirable not only to renew seed stocks at the breeding institute of origin, but also to utilise appropriate isolation techniques to minimise gene flow into the Gene Bank material.

**Acknowledgements:** The Genetic Resource Centre and the Latvian Gene Bank are financed by the Latvian Ministry of Agriculture.

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## LATVIJAS SARKANĀ ĀBOLIŅA (*TRIFOLIUM PRATENSE* L.) ŠĶIRŅU ĢENĒTISKĀ PASPORTIZĀCIJA: VIETAS UN LAIKA IETEKME UZ ĢENĒTISKO DAUDZVEIDĪBU

**Bērziņa I., Zhuk A., Veinberga I., Rashal I. and Ruņģis D.**

Ģenētisko resursu centrā ir izstrādāta ģenētiskas pasportizācijas metodika Latvijas gēnu bankā (LGB) uzglabātām sarkanā āboliņa šķirnēm. Sarkanais āboliņš ir svešapputes augs un ņemot vērā, ka šķirnes ir audzētas un atjaunotas bez īpašām izolācijas metodēm, šķirnes robežās ir sagaidāma augsta ģenētiskā daudzveidība.

Izpētītas septiņas diploīdā sarkanā āboliņa šķirnes, kuras izveidotas trīs Latvijas selekcijas institūtos. Tika analizētas sēklas, kuras ievietotas LGB 1999.g. un 2000.g. Šķirnei 'Priekuļu 66' tika analizētas no VIR repatriētās sēklas (ievietotas VIR kolekcijā 1982.g.), Latvijas gēnu bankas sēklas (1999.g.), kā arī augi no Priekuļu lauka kolekcijas 2007. gadā.

Šāda dažādu avotu svešapputes augu ar augstu ģenētiskā daudzveidību analīze ļauj izpētīt ģeogrāfisko ietekmi uz ģenētisko daudzveidību (šķirnes izveidotas dažādos selekcijas institūtos), kā arī to izmaiņas laikā (repatriētās sēklas, LGB sēklas un lauku augi).

## GENETIC AND ENVIRONMENTAL EFFECT ON THE GRAIN QUALITY OF SPRING BARLEY

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### Abstract

The grain quality of spring barley (*Hordeum vulgare* L.) determines the further opportunities of their utilization. This study investigated the effects of genotype and environment on the grain quality of spring barley. Fifty two spring barley genotypes were used for this investigation. Test weight, 1000 grain weight and grain composition (starch, crude protein, crude fat,  $\beta$ -glucans, crude fibre, crude ash) were evaluated. Field experiments were carried out at the State Stende cereal breeding institute from 2004 to 2006. The results were analyzed to synthesize the relative proportion of the influence various factors ( $\eta$  %) such as variety or year as well as the influence of climatic conditions (mean air temperature and the amount of rainfall) during the period of grain filling. The analysis of variance suggested that the fluidity of grain quality indices were more strongly ( $p < 0.01$ ) affected by their genotype. While measuring the significance of each single factor, the influence of genotype factor was found to be over 70% in starch, crude protein,  $\beta$ -glucans, crude fibre, 1000 grain weight and test weight. A significant environmental influence on grain quality was found for all characteristics except  $\beta$ -glucan and test weight. This was attributed to the varied meteorological conditions during the years of investigation in the the first part of grain filling period.

**Key words:** spring barley, grain quality, analysis of variance, meteorological conditions.

### Introduction

Barley is used for a wide range of uses. It is an important crop used for feed for different livestock species in many areas of the world and also in Latvia.

The most commonly used characteristics to describe the quality of barley in breeding are the physical traits and chemical composition of the grain. The major chemical components of barley grain are starch, protein and  $\beta$ -glucans. The other constituents of barley include ash and fiber components. Fiber components include pentosans, cellulose, and lignin. They are important to measure because of their contribution toward the total dietary fiber. Ash represents the mineral component of barley. The amount of energy available to an animal from grain depends on the relative proportion of each chemical constituent (Shewry and Morell, 2001). Variation in these proportions is genetically controlled (Eagles *et al.*, 1995), but it is also influenced by environmental

factors (O' Brien, 1999; Paynter and Young, 2004; Helm, 1992). Barley with plumper grains and a higher test weight should have a greater percentage of starch or energy in the grain and should be lower in fibre (Shewry and Morell, 2001).

The different components of a barley grain are synthesized in sequence. The growth of cariopsis after anthesis can be divided into two main stages, cell division and grain filling (Hay and Porter, 2006). The duration of grain growth depends strongly on temperature, therefore it is one of the most important factors affecting grain quality (Pasarella, 2005). Another important factor influencing the duration of growth is the stage of cereals and the quality of the water supply. Water deficit influences grain filling through the reduction in the assimilate supply (Paynter and Young, 2004; Conzalet *et al.*, 2007).

It was assumed that the difference between varieties grown at the same location were due to the genetics of the plant. Plant breeders also discovered that the environment plays a big role in the resulting quality of the grain. If the main effect on the quality factor is due to the environment then breeding would have very little influence.

The objective of this study was to determine the influence of genotype and environment on the grain quality traits for covered, hullless, two-row and six-row barley grown in different growing seasons. Emphasis was placed the interpretation of the role of meteorological variables in the period of grain filling on grain quality differences over the years of the study.

### Materials and Methods

52 barley genotypes were chosen which represented a broad range of germplasm (two-row, six-row, covered, and hullless) of different origin (Table 1). Thirty-eight genotypes of covered spring barley, from which 27 with two-row and 10 with six-row ear types, and 15 hullless genotypes were used in this study. Only two-row hullless genotypes were included in this investigation.

Table 1. Spring barley genotypes used in the study

Barley type	n	Genotype, origin country
Two-row, covered	27	Ansis, Abava, Sencis, Kristaps, Rasa, Linga, Idumeja, Balga, Ruja, Gate, Malva, Klinta (Latvia); Hanka, Annabell, Danuta, Justina, Polygena (Germany); Austrian early, Landsorte Aus Tirol (Austria); Primus II (Sweden); Lysimax (Denmark); Hatvani 45/25 (Hungary); Cork, Century (Great Britain); Lechtaler (Portugal); Grimmet (Australia); 379 (Chile)
Six-row, covered	10	Druvis (Latvia); Colsess IV, July (Denmark); B90A, RNB-367 (Nepal); Zoapila, Puebla (Mexico); IV/192 (Macedonia); Valluno (Bolivia); Chosen (North Korea)
Two-row, hullless	15	L 302 (Latvia); KM 2084 (the Czech Republic); SW 1291 (Sweden); McGwire, Gainer, Merlin, Candle (Canada); X-4 (Lithuania); 10250 (Russia); Orzo Nudo di Altamura (Italy); 2474, Clho 7799 (Guatemala); C.P.I. 22817 (Russia); Sumire Mochi (Japan), Wanubet (USA)

The genotypes were grown at the State Stende Cereal Breeding Institute from 2004 to 2006. The soil at the site was sod-podzolic sandy loam, humus content – 12-15 mg kg<sup>-1</sup>, soil pH<sub>KCl</sub> – 6.0-6.7, precrop – potatoes, available for plants P – 88-94 mg kg<sup>-1</sup> and K – 103-122 mg kg<sup>-1</sup>. The size of the plot was 2 m<sup>2</sup>, 2 replicates, and seed rate - 400 seeds per m<sup>2</sup>. The plots were fertilized with N60 P15 K40 kg ha<sup>-1</sup>.

Prior to the analysis, a representative grain sample from both replications was ground in a Perten cyclone mill to pass a 0.8 mm screen. Crude protein content (CP) (N x 6.25) was determined by the Kjeldahl method (LVS 277).  $\alpha$ -glucans content (BGL) was analyzed enzymatically following the barley grain procedures of the commercial kits from *Megazyme* (Megazyme International Ireland Ltd.). Starch content (STR) (ISO 10520), crude fiber content (FBR) (ISO 5498), crude fat content (FAT) (ISO 6492) and crude ash content (ASH) (LVS 276:2000) were determined. All chemical analyses were reported on a dry matter basis. The dry matter content of the ground grains was determined by oven-drying at 130 °C for 2 h. 1000 grain weight (TGW) (LV ST ZM43-95) and test weight (TW) (LVS ISO 7971-2) were analyzed.

The growing season of 2006 had a lower amount of precipitation and a higher daily temperature in comparison with 2004 and 2005. The climatic conditions for the first part of the growing season

(April and May) of 2006 can be characterized as having a heightened temperature and a reduced amount of precipitation. The mean air temperature in June of 2004 and 2005 was below the long-term data (1.7 and 0.6 °C respectively), but in 2006 it was above the long term data (1.1 °C). The highest amount of precipitation was observed in June of 2004 (199% of the long-term data). June and July of 2005 as well as June, July and August of 2006 were characterized by a reduced amount of precipitation. July of 2004 and 2006 was cool (0.8 and 0.4 °C under the long-term data). The meteorological conditions of the month August during the years of investigation were comparatively warm (from 0.5 to 1.9 °C above the long-term data).

The heading and maturity date were determined. The grain filling period from heading to maturity was calculated. As the starchy endosperm of the major storage tissue differentiates during the first part of grain filling the periods from heading to maturity were divided into two similar parts – 20 to 25 day periods depending on the length of this period in order to determine the difference of the mean daily air temperature and the sum of rainfall during the grain filling period during the years of the investigation. The amount of rainfall and the mean daily air temperatures for each period were calculated (Table 2).

Table 2. Meteorological differences during the grain filling period of spring barley, 2004-2006

Variable	2004	2005	2006
Average heading date	1.07	27.06	28.06
Average maturity date	14.08	16.08	6.08
Average length of grain filling, days	45	51	40
Mean daily air temperature, °C			
Grain filling period	16.4a <sup>1</sup>	17.0a	16.3a
1st part of grain filling	15.0a	17.8b	19.4c
2nd part of grain filling	17.6a	16.2b	13.1c
Sum of precipitation during grain filling, mm			
Grain filling period	117.6	168.4	50.4
1st part of grain filling	79.5	31.30	5.1
2nd part of grain filling	38.1	137.1	45.3

<sup>1</sup>means in each comparison between years followed by different letters are significantly different at the  $p \leq 0.05$  level.

Two-way ANOVA was performed using the program *Microsoft Excel*. According to the analysis of variance the proportion influence of relative factors ( $\eta$  %) measuring the relative importance of each single factor (genotype, year, residual or interaction) was calculated. Comparisons were carried out using the t-test to estimate differences between the years.

### Results and Discussion

According to the analysis of variance for the three year data, a significant difference ( $p$ -value  $\leq 0.01$ ) between genotypes in all investigated grain quality traits was observed when all genotypes were included in the analysis (Table 3). Differences were found, if the analysis of variance was performed based on type of barley: covered, hullless, two-row and six row. For covered and hullless barley the genotype as a factor was also significant at the high level of probability ( $p \leq 0.01$ ). For six-row barley the genotype influence for crude protein, starch,  $\beta$ -glucans, crude fibre, 1000 grain weight and test weight was statistically significant ( $p \leq 0.01$ ), but for crude fat and crude ash content it was of no significance (Table 3). It means that there was no significant differences between six-row genotypes in both mentioned parameters.

Table 3. Analysis of variance for grain quality traits of different types of spring barley<sup>1</sup>

Source	CP	STR	FAT	BGL	ASH	FBR	TGW	TW
Proportion of factors influence (η%) and probability of F								
<i>Genotype</i>								
All genotypes	78.5** <sup>2</sup>	72.8**	67.0**	84.0**	52.1**	90.7**	80.6**	95.5**
Covered	63.2**	68.9**	57.4**	59.3**	33.4**	52.5**	76.9**	87.4**
Hulless	74.4**	73.1**	73.8**	79.5**	71.2**	67.1**	86.6**	84.7**
Two-row	49.5**	62.3**	62.6**	47.2*	23.2**	28.9	60.3**	80.9**
Six-row	75.7**	51.4* <sup>3</sup>	32.3	69.4**	41.4	67.0**	80.7**	65.1**
<i>Year</i>								
All genotypes	12.1**	6.4**	3.7**	0.4	24.6**	5.5**	8.9**	0.2
Covered	13.1**	3.2*	7.6**	3.3	39.4**	32.0**	9.8**	1.4*
Hulless	15.9**	6.6*	3.2	0.1	10.1**	17.2**	7.7**	2.2
Two-row	18.1**	13.5**	7.8**	3.2	59.0**	19.5**	19.5**	2.4*
Six-row	11.1**	14.1*	14.3	5.2	10.5	17.1**	5.9**	3.2
<i>Interraction</i>								
All genotypes	9.4	20.6	24.1	15.5	23.1	3.7	10.4	4.2
Covered	23.6	27.7	35.0	37.3	27.1	15.3	13.1	11.1
Hulless	9.6	20.1	22.9	20.3	18.6	15.2	5.6	13.0
Two-row	32.3	24.2	29.6	49.5	17.7	51.4	20.1	16.6
Six-row	13.1	34.4	53.3	25.2	48.1	15.7	12.3	31.5

<sup>1</sup>CP-crude protein, STR-starch, FAT-crude fat, BGL-β-glucan, ASH-crude ash, FBR-crude fibre, TGW-1000 grain weight, TW-test weight; <sup>2</sup> p</= 0.01; <sup>3</sup> p</= 0.05;

This parameter showed that starch (72,8 %), crude protein (78,5 %), β-glucans (84 %), crude fibre (90,7 %), 1000 grain weight (80,6 %) and test weight (95,5 %) were governed mainly by the genotypes (Table 3). The results showed that genotype as a factor had a lower influence on the grain quality traits of different types of barley. Only for hulless barley it was higher in starch, crude fat, crude ash and 1000 grain weight. This indicated the highest genotypic variability among these traits for this specific type of barley.

The influence of a specific year was significant (p</=0.01) for all grain quality traits except β-glucans and test weight when all genotypes were included in the analysis (Table 3). The influence of a year or growing season as a factor in the variance of grain quality traits was relatively small in comparison to the genotypic effect. The influence of density factors varied from 0.2% for the test weight to 12.1% for crude protein when all genotypes were included in the analysis. The relative value of a year as a factor in the final statistical dispersion increased when the quality traits were analyzed depending on the type of barley. The differences between growing seasons were not significant for hulless barley in crude fat, β-glucans and test weigh. For six-row barley it was not significant for crude fat, β-glucans, crude ash and test weight, but for two-row barley only for β-glucans.

The analysis of variances also revealed the interactions of genotype and environment for all measured characteristics (Table 4).

Table 4. Average values of grain quality traits of spring barley (2004-2006)

Grain quality indice	2004	2005	2006
Crude protein, g kg <sup>-1</sup>	137.3a <sup>1</sup>	132.2b	150.8c
Starch, g kg <sup>-1</sup>	619.4a	624.9a	606.8b
Crude fat, g kg <sup>-1</sup>	23.0a	24.6b	23.7a
β-glucans, g kg <sup>-1</sup>	42.7a	42.1a	43.2a
Crude ash, g kg <sup>-1</sup>	24.3a	21.6b	22.2c
Crude fibre, g kg <sup>-1</sup>	42.3a	43.8b	36.9c
1000 grain weight, g	45.5a	41.8b	44.4c
Test weight, g l <sup>-1</sup>	701.8a	695.6a	697.0a

<sup>1</sup> means in each comparison between years followed by different letters are significantly different at the p</=0.05 level.

The amount of interactions between the genotype and the year was relatively small for crude protein (9.4%), crude fibre (3.7%), 1000 grain weight (10.4%) and test weight (4.2%) if the analysis of variance was performed for all genotypes. For other traits it was from 15.5 to 24.1% of the total variance. The significant influence of the density factors was for two-row and six-row barley for all evaluated quality traits. The results showed that crude ash was a trait where genotype, year and interaction determined the variation of this trait at the same rate. This means that this trait depended more on the genotype reaction to different environments.

As the t-test indicated, the mean comparisons for grain crude protein, crude ash, crude fibre and 1000 grain weight was significant for all the years of the investigation (Table 4). Barley grown in 2004 had significantly higher crude ash, 1000 grain weight and test weight than barley grown in 2005 and 2006. Barley had the highest starch content and crude fibre content in the grain in 2005. In this year the barley genotypes formed significantly lower 1000 grain weight. During the growing season of 2006 barley had a significantly lower starch content and crude fibre content, however the barley grain had the highest crude protein content in comparison to other years. Grain test weight and  $\beta$ -glucans did not show significant differences through the test years.

This study was undertaken to determine whether the difference in grain quality measurements was due to the effects of the genotype (the variety) or the environment (meteorological variables) as well as the interaction between of these both factors. The genotypic factor was found to be the primary one that significantly effected grain quality. Differences in grain quality especially concerning starch and protein was also related to genetic differences in how each cultivar accumulated these components during grain development (Savin and Molina-Cano, 2002). The significant effect of genotypes on the variability of grain quality parameters could be primarily due to the difference between types of barley - covered, hulless, two-row and six-row. According to the earlier results (Bleidere and Grunte, 2007), when grain quality was compared between different types of barley, the grain of hulless barley had significantly higher mean values of crude protein, crude fat and significantly lower crude fibre and crude ash values than the covered ones. Six-row barley contained a significantly higher amount of crude protein, crude fibre, crude ash, but less starch than the two-row barley. Also Bach Knudsen *et al* (1987) examined the chemical composition of a range of barley grown during three years in Denmark. The analysis of variance identified highly significant differences between varieties concerning protein, starch and the total dietary fibre content.

The variation of grain quality of the same variety grown in different years is caused mainly by the difference in environmental conditions. In this study phenological and meteorological data were used to analyse the differences in grain quality parameters between the different years of the investigation. The actual process of grain formation and filling take place between the heading and maturity stages (Hay and Porter, 2006). The average heading and maturity dates for spring barley showed differences over the period examined (Table 1). The longest grain filling period in 2005 was associated with high amounts of rainfall during this period. In 2005 and 2006, the first part of the grain filling period took place under exceptionally hot conditions. Additionally, in 2006 this period was notably dry. M. Wallwork *et al.* (1998) indicated that if a short period of high temperature occurs at a certain point in the grain filling period, it may affect one or more components that are being synthesized concurrently and result in a different composition of the mature grain. The high temperatures and the stress of moisture can limit the amount of grain fill operating through the metabolism of starch in the grain. It is because the accumulation of starch is more sensitive to high temperatures than to the accumulation of nitrogen, which frequently determines increases in the grain nitrogen proportion and thus result in higher protein contents (Schelling *et al.*, 2003). The hot and dry period during the year of 2006 corresponded to the period of cell division in starchy endosperm. Such conditions shortened the length of this period thus influencing the accumulation of starch. Due to this reason the season of 2006 resulted in the lowest starch and the highest protein content (Table 1). These factors translated to the slight reduction of the feeding value of barley, as feed grains are used mainly as the source of energy.

The most likely factor contributing to the interaction of the effects on grain quality traits, especially for the grain weight and the test weight were attributed to the dissimilar resistance of genotypes to lodging (caused by increased plant height and excessive rain at the time of grain filling). During the second part of grain filling in 2005 (from July 25 to August 16) there were

particularly high amounts of precipitation that caused early lodging for part of the genotypes and consequently decreased the mean grain weight and test weight. This could be also the main reason for the significantly higher crude fibre content during this year compared to the other years of investigation.

### Conclusions

The analysis of variance suggested that all grain quality traits were more strongly ( $p < 0.01$ ) influenced by the genotype. The proportion of influencing factors ( $\eta$  %) showed starch, crude protein,  $\beta$ -glucans, crude fibre, 1000 grain weight and test weight to be governed mainly by the genotype. The relative magnitude of the genotype as a factor increased when the quality traits analyzed depended on the type of barley. The significant environmental influences for all characteristics except for  $\beta$ -glucans and test weight were attributed to the different meteorological conditions between the different years of investigation during the grain filling period. The factor that was most likely contributing to the interaction of grain quality traits, especially for grain weight and test weight, was attributed to the diverse resistance of genotypes to lodging. The use of genetic variation in endosperm composition and the relative contribution of genotype and environment illustrate the opportunities that exist to improve nutritional value by direct breeding efforts.

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## GENOTIPA UN VIDES IETEKME UZ VASARAS MIEŽU GRAUDU KVALITĀTI

**Bleidere M.**

Vasaras miežu (*Hordeum vulgare* L.) graudu kvalitāte būtiski nosaka to tālākās izmantošanas iespējas pārstrādē. Pētījuma mērķis bija noteikt genotipa un vides ietekmi uz miežu graudu kvalitāti. Pētījums veikts Valsts Stendes graudaugu selekcijas institūtā no 2004. līdz 2006. gadam. Piecdesmit diviem genotipiem novērtēja tilpummasu, 1000 graudu masu un graudu ķīmisko sastāvu (cieti, kopproteīnu, koptaustus, β-glikānus, kokšķiedru un koppelus). Analizēja faktoru (šķirne, gads) ietekmes īpatsvaru (η%), kā arī meteoroloģiskos apstākļus (vidējā gaisa temperatūra un nokrišņu summa) graudu veidošanās periodā. Dispersijas analīze liecina, ka visu novērtēto graudu kvalitātes rādītāju mainību būtiski ( $p \leq 0.01$ ) ietekmē genotips. Genotipa kā faktora ietekmes īpatsvars (η%) virs 70% bija cietei, kopproteīnam, β-glikāniem, kokšķiedrai, 1000 graudu masai un tilpummasai. Konstatēta būtiska gada ietekme uz graudu kvalitātes mainību visām pazīmēm, izņemot β-glikāniem un tilpummasai. To nosaka starp gadiem atšķirīgie meteoroloģiskie apstākļi graudu veidošanās periodā.

## THE EFFECT OF TILLER NUMBERS IN WHEAT HAPLOID PRODUCTION

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### Abstract

Winter wheat doubled haploid production is widely used not only for plant breeding acceleration but also in basic research, such as genetic mapping, haploid transformation and artificial seed production. It is essential to identify all factors which determine haploid system productivity. We have studied the effect of tiller numbers on embryo development and haploid regeneration in wheat x maize haploid production system. Four winter wheat F<sub>1</sub> hybrid lines were pollinated with the maize variety 'Golden Bantam' pollen. Embryo formation and haploid regeneration data were collected from the first five tillers in each plant. The data showed a clear tendency of reduction in embryo formation frequency on the successive tillers. There were 17.5% and only 12.4% embryos on average formed on the main and fourth tillers, respectively. However, two-way ANOVA analysis showed these differences to be insignificant ( $p > 0.05$ ) and only the genotype had a significant ( $p < 0.01$ ) effect on embryo formation. There was no clear effect by both genotype and tiller numbers on haploid regeneration frequency. The overall embryo formation and haploid regeneration frequency in this experiment was 14.6% (504 embryos) and 68.1% (367 haploids), respectively. The results obtained in this study indicate that as many as five tillers per plant can be used in wheat x maize crossing systems with no significant tiller effect on the efficiency of the haploid production system.

**Key words:** wheat x maize, haploid, tiller number.

### Introduction

Winter wheat doubled haploid production is widely used not only for plant breeding acceleration but also in basic research, such as genetic mapping, haploid transformation and artificial seed production. Several techniques exist for wheat haploid development. These include crossing with maize (Laurie and Bennet, 1988), anther culture (Last and Brettell, 1990) and isolated microspore culture (Gustafson *et al.*, 1995). Wheat x maize crossing is the system of choice in winter wheat breeding programs due to lower genotype dependency and albinism avoidance (Snape, 1998). However, wheat x maize crossing is a time consuming and costly technique. It requires manual application of maize pollen and subsequent embryo excision. Ways to determine the factors underlying the efficacy of the method are being searched for.

Various factors are related to the differences in wheat haploid formation after crossing with maize. Campbell *et al.*, (1998) determined that both temperature and light intensity influence embryo formation in the wheat x maize system. This effect was later associated with the abnormalities in

pollen tube growth at low light intensities (Campbell *et al.*, 2001). The maize genotype was also shown to have an effect on wheat haploid induction (Verma *et al.*, 1999). Several reports demonstrate the role of various hormone treatments on embryo survival (Matzk and Mahn, 1994, Ushiyama *et al.*, 2007). The effect of the spikelet position on embryo formation was shown by Martins-Lopes *et al.* (2001) where different spikelet positions (lower, middle and upper) gave highly significant differences in embryo formation. However these results contradict those reported by Bitsch *et al.* (1998), where they said that the time of pollination is not critical for the success of the crosses. Until now, only one report has shown the influence of the spike position on the plant in haploid induction in barley anther culture (Jacquard *et al.*, 2006).

In the present work we have studied the effect of tiller numbers on the plant in haploid embryo formation and regeneration after wheat x maize crossing.

### Materials and Methods

Four winter wheat F<sub>1</sub> hybrid lines (No. 601, 602, 603 and 604) were used for pollination with the pollen of the maize variety 'Golden Bantham'. Wheat hybrid genotypes were produced through various inter-varietal crossings at the Cereal Breeding Department of the Lithuanian Institute of Agriculture. Both wheat and maize plants were grown in a heated glasshouse with a temperature range of 15 -25 °C. Wheat plants were vernalized for 8 weeks at 3 °C in dim light. The first five tillers of each wheat plant were used for pollination. The first tiller to flower was denoted as the main tiller (MT) and the subsequent tillers were numbered from 1 to 4 (T1 - T4). Seven plants of each genotype were regarded as replications. Wheat florets were not emasculated but pollinated one day before anthesis. 23-28 florets were pollinated per spike. Hormone treatment was performed by submerging the whole wheat inflorescence in 50 mg l<sup>-1</sup> 2,4-D (Duchefa Biochemie B.V.) solution for 10 seconds 24 hours after pollination. Embryo rescue was made 17 days after pollination. Aseptically excised embryos were transferred into tubes with 5 ml B5 2/3 strength medium containing 3% sucrose and 0.7 % agar (all Duchefa Biochemie B.V.). Planted embryos were kept at 20 °C in the dark until germinated. Further growth was maintained at 24 °C with a 16 h day length.

The embryo formation frequency (EFF) was referred as the number of embryos formed per 100 florets pollinated and the haploid regeneration frequency (HRF) as the number of haploid plants regenerated per 100 embryos planted. Statistical analysis was performed as a two-way ANOVA with the software 'Selekcija'.

### Results and Discussion

A total of 3477 wheat florets were pollinated with maize pollen which resulted in 504 embryos. Embryos were obtained in all crossing combinations. Embryo formation frequency varied among the four wheat genotypes used. The highest EFF was recorded for the wheat line No. 604 where embryos formed in 17.6% of pollinated florets while line No. 602 had the EFF of only 9.9% (table1).

Table 1. The average values of embryo formation frequency (EFF) and haploid regeneration frequency (HRF) among wheat lines and tillers.

Tiller number	Wheat line								Mean	
	601		602		603		604		EFF, %	HRF, %
MT <sup>1</sup>	19.2	64.3	16.8	59.7	8.4	54.5	25.8	86.9	17.5	66.3
T1	16.3	49.5	6.5	85.7	20.0	80.1	17.2	69.1	15.0	71.1
T2	12.1	81.0	11.3	47.4	19.1	68.5	15.0	73.8	14.4	67.7
T3	17.5	78.8	5.6	50.0	13.4	72.6	17.9	67.7	13.6	67.3
T4	12.3	77.6	9.4	34.0	16.3	79.8	11.8	81.0	12.4	68.1
Mean	15.5	70.2	9.9	55.4	15.4	71.1	17.6	75.7	14.6	68.1

<sup>1</sup>MT – main tiller, T1, T2, T3, T4 – first, second, third and fourth tillers, respectively

ANOVA analysis confirmed the genotype effect on embryo formation to be significant (P < 0.01) (table2). The data showed a clear tendency of reduction in embryo formation frequency on the



successive tillers. There were 17.5, 15.0, 14.4, 13.6 and only 12.4% embryos on average formed on the main (MT), first (T1), second (T2), third (T3) and fourth (T4) tillers, respectively. However this tendency could not be proven by ANOVA analysis which showed the differences to be insignificant ( $P > 0.05$ ).

367 haploid plants were obtained from 504 embryos planted. The average haploid regeneration frequency varied from 55.4 to 75.7% for wheat lines No. 602 and 604, respectively (Table 1)

The same genotypes had the lowest and the highest embryo formation frequency, respectively. No obvious tendency was observed in HRF when compared with successive tillers. The highest HRF of 71.1% was recorded for T2 and the lowest HRF of 66.3% for MT. Neither the genotype nor the tiller number effect could be proven by ANOVA analysis. Both factors as well as their interaction had insignificant ( $P > 0.05$ ) differences among variables (Table 2).

Table 2. The results of ANOVA analysis for genotype, tiller number and their interaction

Factor	df	EFF	HRF
Genotype	3	5.28** <sup>1</sup>	1.96 n.s.
Tiller number	4	1.52 n.s.	0.05 n.s.
Genotype x tiller number	12	1.74 n.s.	1.44 n.s.

<sup>1</sup>\*\* $P < 0.01$ , n.s. – not significant ( $P > 0.05$ )

The wheat x maize crossing technique involves several key steps. These include: synchronization of wheat and maize flowering, manual wheat florets' pollination with maize pollen, embryo excision/culture *in vitro* and haploid treatment with colchicine. All these steps are labor intensive and raise the costs of the haploid production. Improvements are needed to increase the efficiency of the method.

The number of florets as potential haploid producers could be increased per plant by pollination (more florets per spike) or by using more spikes per plant. Previous studies have shown that different spikelet positions (lower, middle and upper) gave different success ratios for embryo initiation (Martins-Lopes *et al.*, 2001). However Bitsch *et al.* (1998) earlier reported that embryo initiation was found to be dispersed evenly all over the wheat spike. In a conventional wheat x maize crossing technique 25-30 florets are usually pollinated per spike. It would be difficult to increase this number due to the lag in flowering synchronization. However the number of the crossed spikes per plant could be easily increased by growing spaced plants but this would be feasible only if there is no considerable loss in method efficiency parameters on the subsequent tillers.

The results of our study indicate the tendency of influence of the tiller number on embryo formation. This tendency is supported by Jacquard *et al.* (2006) findings in barley anther culture. They have shown that the position of the tiller on the plate of tillering has an impact on both the number of regenerated plantlets and the percentage of green plants. The anther response decreased significantly when the donor spike originates from the main shoot or the fourth tiller. However our results could not be proven statistically. The tiller number effect on barley anther culture was explained by tiller competition for carbon assimilate or nitrogen compounds. Regular application of complex fertilizers to wheat plants could minimize the starvation effect in our experiment.

## Conclusions

The results obtained in this study indicate that as many as five tillers per plant can be used in wheat x maize crossing systems with no significant tiller effect on the efficiency of the haploid production system.

## Acknowledgements

We thank Dr. Vytautas Ruzgas for the supply of F<sub>1</sub> wheat hybrid lines used in this experiment and Lithuanian State Science and Studies Foundation for the financial support (Grant No. N-13/2007 'KVIETPOLIMER').

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## STIEBRU KĀRTAS SKAITA IETEKME UZ KVIEŠU HAPLOĪDU VEIDOŠANOS

### Brazauskas G.

Ziemas kviešu dubultotie haploīdi tiek plaši izmantoti ne tikai augu selekcijas procesa paātrināšanai, bet arī tādos fundamentālos pētījumos, kā, piemēram, ģenētiskā kartēšana, haploīdu transformācija un mākslīgo sēklu ražošana. Ļoti svarīgi ir noteikt visus faktoros, kas nosaka haploīdu iegūšanas efektivitāti. Izmēģinājumā pētīta dzinumu skaita ietekme uz embriju attīstību un haploīdu reģenerāciju iegūstot kviešu haploīdus ar kviešu x kukurūzas haploīdu iegūšanas shēmu. Četras ziemas kviešu F<sub>1</sub> hibrīdās līnijas tika apputeksnētas ar kukurūzas 'Golden Bantham' putekšņiem. Tika vērtēti embriju veidošanās un haploīdu reģenerācijas dati no katra auga pirmajām piecām vārpām. Iegūtie rezultāti parādīja tendenci, ka visvairāk embriju veidojās no galvenās vārvas, bet no pārējām to skaits secīgi samazinājās. No galvenās vārvas tika iegūti 17.5% embriju, bet no ceturtās – tikai 12.4%. Tomēr divu faktoru dispersijas analīze parādīja, ka starpība starp iegūto embriju skaitu no pirmās un sekojošām vārpām nav būtiska ( $p > 0.05$ ); embriju veidošanos būtiski ietekmēja tikai genotips ( $p < 0.01$ ). Netika konstatēta skaidra genotipa un stiebru skaita ietekme uz haploīdu reģenerācijas biežumu. Kopumā izmēģinājumā tika iegūti 14.6% (504) embriji un 68.1% (367) haploīdi. Izmēģinājuma rezultāti liecina, ka pirmās piecas vārvas no katra auga var tikt izmantotas kviešu x kukurūzas haploīdu ražošanas shēmā bez būtiskas dzinuma ietekmes uz iegūto haploīdu skaitu.

## DEVELOPMENT OF NEW FORAGE GRASS VARIETIES AT THE SKRIVERI RESEARCH INSTITUTE OF AGRICULTURE

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### Abstract

Grasses provide an inexpensive source of feed that is high in nutritional value for profitable milk as well as meat production. Permanent grasslands cover most of the agricultural land in Latvia. The Research Institute of Agriculture in Skriveri has successfully established new diploid and tetraploid forage grass varieties. Over the past few years one of the most important goals of the Department of Plant Breeding, has been the introduction of new perennial grass-plant varieties suitable for growing in Latvian soil, weather conditions and ensuring good dry matter and seed yields.

As a result of this work, five varieties of perennial grasses have been created, with all of them having been registered in the European common catalogue of varieties of agricultural plant species. These cultivars are: tetraploid perennial ryegrass 'Spidola' (4n), meadow fescue 'Patra' (4n), meadow fescue 'Silva' (2n), hybrid ryegrass 'Saikava' (4n), early-ripe timothy 'Teicis'. From 2007 through 2017 timothy 'Teicis' is included in the Lithuanian National List of Plant varieties. New varieties: late season timothy 'Varis', meadow fescue 'Vaira' (2n) and *Festulolium* 'Vizla' (4n) have passed distinctness, uniformity and stability (DUS) and satisfactory value for cultivation and use (CVU) testing in Poland and Latvia. In the future we plan expand our work on the creation of cocksfoot and meadow foxtail, all tall fescue varieties for forage and decorative use.

**Key words:** perennial grasses, plant breeding, varieties

### Introduction

Perennial grasses are important forage crops in Latvia and are cultivated in fields, meadows and pastures. With the development and use of new forage preparation technologies, grass breeding as well as research objectives are currently undergoing changes (Jansone, 1999). Grass growers are looking for grass varieties exhibiting high production and that are capable of withstanding multiple cuttings. The Research Institute of Agriculture, a unit of the Latvian University of Agriculture, is engaged in developing perennial grass varieties that see heavy utilization by the nation's forage industry. Before 1996 five grass varieties were bred by the Institute of Agriculture: tetraploid perennial ryegrass (*Lolium perenne*) 'Spīdola', tetraploid meadow fescue (*Festuca pratense*) 'Patra', diploid meadow fescue 'Silva', hybrid ryegrass (*Lolium x boucheanum Kunth*) 'Saikava', early season timothy (*Phleum pratense*) 'Teicis' (Bērziņš, *et al.*, 2006).

All introduced varieties have passed DUS and CVU testing and are included in the European common catalogue of varieties of agricultural plant species. In order to be added to a European National List, a variety must have passed DUS testing, with agricultural crops requiring VCU testing. For a grant of Plant Breeders' Rights to be awarded, a variety must be Distinct, Uniform and Stable.

The objective of our work is to develop and characterize perennial grass varieties suitable for industrial agriculture use in the conditions of Latvia. The purpose of this article is to review recent progress and to briefly describe the developed grass varieties.

### Materials and Methods

In order to perform the breeding, we established trial plots for different purposes at the Research Institute of Agriculture which is located in Skriveri. Plots for the growth of our grass collection, plots for hybridizations and trials of developed hybrids as well as plots for variety comparisons were established. For variety comparisons we used newly developed varieties, which were comprised of local and foreign grass varieties.

Meadow fescue and timothy variety trial plots were established in sod podzolic sandy loam soil (Luvic Phaeozem, WRB 1998), pH KCl 6.5, plant available P<sub>2</sub>O<sub>5</sub> 110 and K<sub>2</sub>O 204 mg kg<sup>-1</sup>

(Egner–Riehm), soil organic carbon 21 g kg<sup>-1</sup> (Tyurins' method). The size of the trial plots were 10 m<sup>2</sup> (1 x 10 m). The trial plots were fertilized with mineral phosphorus and potassium fertilizers 60 and 90 kg ha<sup>-1</sup> accordingly and nitrogen fertilizer 60 kg ha<sup>-1</sup> for each grass cut. Trial variants were arranged according to the standard method. In 2003, trial crop yields were obtained in four repetitions. The number of cuts throughout the vegetation period was dependent on the weather conditions; usually 2 to 3 cuts were made.

Timothy and meadow fescue during the 2004 – 2006 period was the dry matter yield, beginning of blossoming proportion in sward after 3-year use in autumn were determined.

Analysis of variance (ANOVA) was conducted using the GLM procedure of SAS (SAS Inst., 1990) at P=0.005 to test the effects of year, location, N treatment, and all interactions.

## Results and Discussion

So far, eight varieties of perennial grasses have been developed at the Research Institute of Agriculture. Five of them are listed in the Common EU Catalogue of Varieties of Agricultural Plant Species, with three still being in the test phase (Table 1).

Table 1. List of perennial grass varieties in the Catalogue of Plant Varieties

Variety denomination	UPOV, grant number	Acceptance date in the Latvian Catalogue of Plant Varieties, List “A”
Perennial ryegrass ‘Spidola’	1993	2001-2010
Meadow fescue ‘Patra’	1999	2001-2010
Hybrid ryegrass ‘Saikava’	1999	2003-2013
Timothy ‘Teicis’	2001	2002-2011
Meadow fescue ‘Silva’	2004	2005-2014
Timothy ‘Varis’	2007	Test
Meadow fescue ‘Vaira’	Test	Test
Festulolium ‘Vizla’	Test	Test

All the formerly established timothy varieties represent an early-ripening class. Currently there is a lack of late-ripe varieties for Latvian farmers. For this reason we started working on developing timothy varieties that are late-ripening. In 2005, we developed and delivered a late-ripening timothy variety ‘Varis’ for the DUS (distinctness, uniformity, stability) test trial in Poland. The variety was formed by interbreeding the timothy variety ‘Dolema’ (Germany) x ‘Priekuļu 2’ (Latvia) x ‘Teicis’ (Latvia). In 2007 we received a test certificate (UPOV Report on technical Examination (03.12.2007. COBORU Słupia Wielka) indicating that ‘Varis’ successfully passed the testing. Currently, in Latvia this variety is in the last year of CVU testing. ‘Varis’ was developed and tested by: P Berzins, S. Bumane, V. Stesele. Variety characterization according to UPOV is in Table 4.

At the beginning of vegetation, timothy ‘Varis’ develops slowly and flowers at the beginning of July, later than the varieties ‘Jumis’ and ‘Teicis’. It gives a good grass crop - 6.7 t ha<sup>-1</sup> of dry matter (Table 2), with seeds maturing at the end of August or the beginning of September. This late season variety exhibits abundant leaf coverage and forms nice sward.

Table 2. Competitive trials of timothy

Cultivars, Country Codes	DM yield, t ha <sup>-1</sup> , average 2 cuts				% from stand- dart	Beginning of blossoming
	2004	2005	2006	average, 3 years		
Jumis, standard LV	6.04	6.90	6.32	6.4	100	9.06.
Teicis, LV	6.34	7.28	6.69	6.8	105	5.06.
Varis, LV	6.31	7.11	6.74	6.7	105	15.06.
Žolis, LT	5.63	6.33	6.17	6.0	94	18.06.
Promisse, NL	6.29	7.15	6.75	6.7	105	10.06.
LSD <sub>0.05</sub>	0.32	0.31	0.29	0.24		

Timothy 'Varis' is suitable for very late season pasture cultivation in grass mixtures with other late season grasses as perennial ryegrass, creeping bent and late season varieties of red-clover. It is additionally suitable for growth in different soil types. In meadows timothy 'Varis' is useful for intensive culture where are 2 - 3 cuts during the vegetation season.

In 2007 the diploid meadow fescue variety 'Vaira' was forwarded to DUS and VCU test trials in Poland and Latvia. The new meadow fescue variety 'Vaira' is distinguished by higher dry matter yields - 6.1 t ha<sup>-1</sup> (Table 3) as compared to the standard variety 'Arita'. This meadow fescue variety stays longer in the sward, even longer than variety 'Silva'. 'Vaira' is similar to 'Silva' in that it forms leaves five days earlier than the standard variety 'Arita'. In addition, it has larger tillers, their length being up to 85 cm.

Table 3. Competitive trials of meadow fescue

Cultivars, country codes	DM yield, t ha <sup>-1</sup> , average 2 cuts					Proportion in sward after 3-year use in fall, %
	2004	2005	2006	average, 3 years	% from standard	
Arita 2n, (standard) LV	6.2	5.6	4.0	5.1	100	60
Silva 2n, LV	6.9	6.5	4.3	5.6	108	70
Patra 4n, LV	6.7	6.0	4.0	5.2	102	55
Vaira 2n, LV	7.7	7.0	4.6	6.1	118	80
Arni 2n, EE	6.1	6.0	3.1	4.8	93	65
Sigmund 2n, SE	6.4	5.7	3.6	5.0	97	60
LSD <sub>0.05</sub>	0.34	0.32	0.27	0.24		

In 2008 the new tetraploid festulolium (*Festuca spp. X Lolium spp.*) variety 'Vizla' was delivered for DUS testing in Poland. Festulolium 'Vizla' is formed partially by fertile triploid fescue and a ryegrass hybrid, which is obtained by colchicine treatment and tetraploid festulolium selection. Its winter hardiness is average. This is a late season variety, inflorescence emerges around June 15. This variety has good resistance to leaf rust and leaf spot diseases. This grass variety is well suited for silage and hay applications. The grass quality is good and has high carbohydrate content. Festulolium 'Vizla' can be used for the formation of late season swards as a single culture or in mixtures with late season clovers and other grasses. It is good for silage or hay preparation. Growth is excellent in mineral soils. The festulolium cv. 'Vizla' was developed by P. Berzins, S. Bumane, V. Stesele.

Table 4. Description of timothy variety, 'Varis' according UPOV

UPOV No	National No	Characteristics	State of expression
1	1	Ploidy	hexaploid
2	2	Plant: speed of inflorescence emergence in the year of sowing	absent
	3	Plant: tendency to form inflorescences in the year of sowing	absent
5	4	Plant: growth habit in 2 <sup>nd</sup> year before elongation	medium
	5	Plant: growth habit at inflorescence emergence (in 2 <sup>nd</sup> year)	medium
	6	Plant: natural height in spring (in 2 <sup>nd</sup> year)	medium
4	7	Leaf: width (in 2 <sup>nd</sup> year before elongation)	medium
3	8	Leaf: color (in 2 <sup>nd</sup> year before elongation)	medium green
9	9	Stem: length of longest stem	medium
10	10	Stem: length of upper internode	short to medium
	11	Flag leaf: attitude of blade	sloping to horizontal
7	12	Flag leaf: length	medium
8	13	Flag leaf: width	medium
6	17	Plant: time of inflorescence emergence	late
	18	Speedy of inflorescence emergence	slow
	19	Plant: number of generative stems	many
	20	Plant: tendency to form inflorescences in second cut	absent

## Conclusions

Eight perennial grass varieties adapted to the conditions and the agricultural needs of the Republic of Latvia have been developed in the Skriveri Research Institute of Agriculture during last decade. Two new grass varieties, timothy 'Varis' and festulolium 'Vizla' will be tested for DUS and VCU by the State Plant Protection Service Department this year.

During 2004 - 2006 we tested and compared newest timothy and meadow fescue varieties for different use.

Timothy 'Varis' and meadow fescue 'Vaira' delivers stable grass crops in Latvian conditions.

The new festulolium variety 'Vizla' can be used as a late season grass crop.

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## JAUNĀKĀS STIEBRZĀĻU ŠĶIRNES ZEMKOPĪBAS ZINĀTNISKAJĀ INSTITŪTĀ SKRĪVEROS

**Bumane S., Berzins P.**

Zālāji ir viens no lētākajiem lopbarības veidiem ar augstu barības vērtību piena un gaļas ražošanā. Ilggadīgie zālāji aizņem lielāko daļu Latvijas lauksaimnieciski izmantojamās zemes.

LLU aģentūras Zemkopības zinātniskais institūts ir selekcionējis Latvijas augšņu un agroklimatiskajiem apstākļiem audzēšanai piemērotas jaunas diploīdas un tetraploīdas stiebrzāļu šķirnes, kas nodrošina labas sausnas un sēklu ražas.

Selekcijas darba rezultātā ir radītas 5 jaunas stiebrzāļu šķirnes, kas ir reģistrētas Kopējā Eiropas Savienības lauksaimniecības augu šķirņu katalogā. Šīs šķirnes ir: tetraploīdā ganību airene 'Spīdola' (4n), pļavas auzene 'Patra' (4n), pļavas auzene 'Silva' (2n), hibrīdā airene 'Saikava' (4n), agrais timotiņš 'Teicis'. Timotiņš 'Teicis' no 2007. gada līdz 2017. gadam ir iekļauts arī Lietuvas Nacionālajā Augu šķirņu sarakstā. Jaunā šķirne - vēlais timotiņš 'Varis' ir izgājis AVS testu Polijā un pēdējo gadu Latvijā atrodas SĪN testa pārbaudē. Pašlaik Polijā un Latvijā pārbaudes testus iziet mūsu jaunās šķirnes: pļavas auzene 'Vaira' (2n) un auzenairene 'Vizla' (4n).

Selekcijas darbs turpinās pie jaunas kamolzāles, pļavas lapsastes un niedru auzenes šķirņu veidošanas.

## 21<sup>ST</sup> CENTURY PLANT BREEDING: THE EVOLUTION OF DIRECTED EVOLUTION

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### Abstract

Plant breeding began, by default, with human cultivation of plants nearly 10,000 years ago and quickly led to crop domestication as a result of natural selection toward adaptation to the new, cultivated environment. Natural selection, augmented by human preferential harvesting and planting of specific phenotypes, resulted in the creation of many locally adapted, reasonably productive landraces. These landraces were later exploited through the isolation of homogeneous populations of pure lines as varieties; this was concurrent with increased mechanization in planting, harvesting and processing operations. The discovery of Mendel's work at the beginning of the 20<sup>th</sup> century resulted in the application of genetics to plant breeding and the use of hybridization as a plant breeding tool. Thus 'directed evolution' came into being. New discoveries in genetics through the past 100 years (quantitative genetics, polyploidy, induced mutations, male sterility, understanding of DNA and molecular biology, haploidy, genetic transformation) were rapidly

applied as plant breeding tools to make the development of new varieties more effective, efficient and economical. Breeders have an ever-increasing tool chest of technology at their disposal. The challenge in breeding is to determine which of this multitude of high tech bio-tools are the most appropriate to maximize 1short-term, accelerated evolution for specific objectives in defined populations with limited budgets. As the 21<sup>st</sup> Century unfolds, plant breeding has evolved into a very effective, directed, evolutionary process which sustains human civilization as we now know it.

**Key words:** genetics, recurrent selection, male sterility, RIPE

### **Introduction**

Evolution is geared toward improving the adaptation of a population to the environment in which it is growing and reproducing through 'survival of the fittest' (Darwin, 1859) ensuring that those individuals which are best adapted produce more progeny than those individuals which are less well adapted. As the environment changes, different traits, or expressions of a trait, will be favoured and the allelic composition of the population will change in response.

Plant breeding began, by default, with human cultivation of plants around 10,000 years ago and quickly led to changes toward better adaptation to the new, cultivated, human-managed environment as a result of natural selection. These changes were essentially an evolution toward crop domestication; changes based, in most cases, on the selection of existing characters, not the creation of new ones (Roots, 2007) and due initially to natural selection. The result was fully domesticated crops which formed the basis of the emergence of modern agrarian civilization. As knowledge of biology and genetics accumulated over time, intentional selection and eventually modern breeding evolved. The purpose of this paper is to outline the history of crop improvement and the application of basic biological principles leading to efficient and effective breeding systems capable of coping with the biological, environmental and economic challenges of the next century of human progress.

### **Evolution under Domestication**

Natural seed dormancy and seed dispersal mechanisms essential for survival in the wild populations disappeared quickly from the early cultivated plant populations. Most plants were harvested and seeds replanted on an annual cycle so only those plants which successfully produced seed the first season contributed seed to the next growing cycle. The plants whose seeds shattered before harvest were lost from the population that was used for planting of the next generation. Selection for higher seed production came about as a result of the most productive plants contributing a higher proportion of the seed to the next generation, with a tendency toward self-pollination in most domesticated crops. Increased seed size occurred when smaller seeds were lost through the winnowing operation and through conscious selection of the larger seeds for planting. Cultivation reduced weed competition and gave crop plants a better opportunity to perform to their full genetic potential.

The selection by early farmers for types that suited their purposes better and were not negatively related to adaptation and fitness led to the emergence of free-threshing (hulless) wheats and barleys. The spontaneous mutations for these traits would have been selected and isolated from the early cultivated hulled crops. Hulless seed was a major step toward improving energy, nutrition and ease of processing as the hull was easily removed during the threshing operation itself.

Most of the crop species which we presently cultivate went through this domestication process long before recorded history. This natural selection for adaptation to cultivation, augmented by human preferential harvesting and planting of specific desirable phenotypes, resulted in the creation of many fully domesticated, locally adapted, reasonably productive landraces in ancient times. Such landraces have been cultivated by most agrarian societies for many hundreds of generations. Very few new species of crops have been domesticated in modern times.

The landraces were somewhat diverse for traits that were not directly selected for by Nature nor of specific interest to the farmers (i.e. height, maturity, seed colour). As a result of naturally occurring mutation, and occasional outcrossing, landraces accumulated many diverse, 'neutral' morphological characteristics. These landraces, with their local adaptation and basic background diversity, were excellent raw material to introduce to new environments. Seeds of such landraces

accompanied most migrating human populations and eventually many crop species were spread around the world to regions where the wild ancestors would not have been adapted.

These land races were later exploited through the isolation of single-plant derived homozygous and homogeneous pure lines as varieties. This resulted in more uniform and higher performing varieties, and occurred concurrently with increased mechanization in the planting, harvesting and processing operations. Synchrony of emergence, tillering, flowering and ripening was desired so that crop management operations could be performed on the crop on a field basis. Plants were no longer considered individually, but rather as components of a uniform population that was managed as a unit.

### **Genetics Applied to Plant Breeding**

The discovery of Mendel's research at the beginning of the 20<sup>th</sup> century resulted in the application of basic genetic principles to plant improvement and the use of inter-varietal hybridization and selection in the segregating generations as a plant breeding technique. Thus 'directed evolution' came into being. Luther Burbank (1902) at one of the first international plant breeding conferences in New York City said "The fundamental principles of plant breeding are simple, and may be stated in few words; the practical application of these principles demands the highest and most refined efforts of which the mind of man is capable, and no line of mental effort promises more for the elevation, advancement, prosperity and happiness of the whole human race". With the understanding of the genetic basis of segregation and independent assortment and plant breeders, it became possible to predict the outcome of crosses and produce specific combinations of traits. Breeding emerged as a discipline which applied the knowledge of genetics and biological systems to maximize the short-term, accelerated evolution of defined populations for specific objectives with finite resources. In 1908, Hardy and Weinberg independently developed a mathematical method of describing the quantitative genetic dynamics of populations which met certain specific criteria, and their concepts were immediately embraced by animal breeders and many quantitative geneticists. Initially it was difficult to reconcile the differences in observed phenotypic phenomena between the Mendelian and the biometrical concepts. There was considerable support for having a completely set of different bases for genetics and the inheritance of traits in animals (quantitative) and plants (Mendelian). Eventually this was rectified when quantitative genetics was shown to be based on Mendelian behavior at each of many contributing loci which were not as easily classified due to a more complex genetic architecture, gene interactions, and environmental effects.

### **New Tools**

New discoveries in genetics and biology over the past 100 years were rapidly applied as improved plant breeding tools to make the development of new crop varieties more effective, efficient and economical. Variations on the Hardy-Weinberg equilibrium were used to explain and predict the changes in population structure as a result of altering the assumptions toward more realistic breeding approaches. The science of quantitative genetics was developed and rapidly embraced by animal breeders, and later by plant breeders, particularly those working with cross-pollinated crops such as maize.

The discovery of polyploidy and the ability to manipulate chromosome numbers using colchicine and other mitotic control mechanisms led to the development of autopolyploidy and allopolyploidy as breeding tools. This was particularly applicable to interspecific hybridization and stimulated the creation of new species such as triticale. The occurrence of haploidy in a number of species, and methods of inducing higher frequencies of haploids through interspecific hybridization, and eventually anther culture and microspore culture, has made this a common pure line development technique in the Brassicas and some cereals.

The basis of natural mutations and the ability to induce them artificially opened up the possibility of creating 'new' genes, and hence new traits. Both chemical mutagens and radiation from various sources were extensively used in many of the small grain breeding programs in the 1950's and 1960's, especially barley. A number of dwarfing, earliness, and disease resistance genes that are widespread in modern varieties resulted from these early efforts.

Genetic male sterility as a tool for efficiently crossing normally self-pollinated crops was discovered in barley in 1940 (Suneson, 1940) and later in wheat and many other crops. Most self-



pollinated species have been found to have naturally occurring male sterile systems (Gottschalk and Kaul, 1974). Although most genetic male sterilities are recessive, some dominant male steriles have also been found in wheat (Maan and Williams, 1984; Huang and Deng, 1988). Cytoplasmic male sterility is also wide-spread in the plant kingdom, but has seen more use as a commercial hybridization mechanism than as a breeding tool, *per se*. Chemical hybridizing agents (CHA) have been developed to eliminate male fertility in a treated line that is then used as the female parent in hybrid seed production. These have been used to explore the production of hybrid wheat cultivars and, to a limited extent, in breeding programs.

The understanding of the role of DNA in inheritance, with the subsequent emergence of molecular biology, has opened up an array of techniques for monitoring and manipulating the genetic material in plants and animals for genetic studies and breeding purposes. The development of a number of DNA-based marker systems associated with allelic variation has given rise to molecular marker systems used to track genes from parents to progeny in breeding programs and to transfer specific desired alleles into recipient backgrounds. The use of quantitative trait loci (QTL) associated with traits of economic importance in breeding is fairly well developed. The ultimate application of molecular biology is in the transformation of totally new DNA segments from one species (or as wide as another kingdom) into another, well beyond the boundaries of normal sexual compatibility. Totally artificial DNA sequences can be constructed and used in transformation events to give phenotypes that have never occurred in nature. Transformation can also be thought of as 'directed mutation'.

Tools such as environmental control to create artificial environments, offseason nurseries, inoculation with diseases and pests, precise electronic measurement of numerous physical and biochemical parameters, and the use of immensely expanded computer power for analysis and data 'massaging' have made it possible to handle far more complex information than was possible in the past.

Population construction designs such as biparental crosses, diallele and half-diallele series, North Carolina Designs I and II, and convergent, conical, and composite crosses are all employed in breeding programs. Methods such as backcross breeding, the pedigree, bulk and SSD systems, and use of haploids and recurrent selection procedures are commonly used to develop pure lines from crosses for evaluation as potential varieties. Several different statistical approaches such as RCBD, lattice, moving means, nearest neighbours, periodic checks, AMMI, GGE Biplots, spacial adjustment, and honeycomb designs are used to reduce non-genetic variation in evaluation nurseries. Many different combinations of these methods have given rise to new cultivars in various breeding programs around the world.

It is evident that breeders have an ever-increasing tool chest of technology at their disposal. The challenge in breeding is to determine which of this multitude of bio-tools and technologies are the most appropriate for their particular situation. As the 21<sup>st</sup> Century unfolds, plant breeding is evolving into a very effective, directed, evolutionary process that sustains human civilization as we now know it.

### **Tools vs Toys**

Ultimately, the application of the many new [bio]technologies must be robust enough to lead to improved cultivars in farmers' fields. To be successful, breeders must manipulate the genetic factors that they understand (and many that they do not), try to minimize (or fix) the epistatic and genotype by environment interaction effects that are unpredictable and difficult to evaluate, as well as understand the error that is always present in measuring metric traits in biological systems.

Sometimes the tool chest is overflowing and it is difficult to distinguish the tools from toys. It is challenging and rewarding to identify a new gene or marker and follow it through the generations, but it does not often lead to improved cultivars that are commercially successful. In fact, most of the traits of economic and agronomic value are quantitative in nature and are due to the cumulative effects of many, small, additive effects that cannot be individually detected, and therefore there are no QTLs for them. Perhaps we can have too much information and sometimes get caught up in generating information just because we can. Much of the molecular information generated may not be necessary, or even useful, for a breeder. Breeders can successfully manage far more complex genetic interactions via phenotypic selection among fixed lines than even the most recent molecular

approaches can explain. A breeder with a large, multi-national breeding and chemical company was recently overheard to say that they would have made much greater breeding progress if they hadn't been so 'distracted' by molecular biology. There can be too much information, and generally not enough relevant knowledge.

### **Breeding in the Future**

We are now facing a future of global warming, the foreseeable end of economically extractable oil, and concerns about the sustainability of healthy and safe food production in degraded soils in an increasingly polluted environment. How can we address these issues through breeding in the future? In a world with a rapidly changing climate, greater variation in the weather patterns, with more severe weather events, reduced farm inputs (due to environmental concerns), and less reliance on inputs because of economics, one must explore methods that are more likely to succeed. More population buffering, better crop adaptation, with better defense mechanisms and greater stress tolerance, as well as improved productivity and stability, improved nutritional quality coupled with reduced inputs are going to be necessary in the next generation of crop cultivars. We don't need more short-term quick fixes based on single genes, we really need breeding systems that can handle a large suite of genes influencing numerous traits simultaneously. Evolution, as we now understand the mechanics of the process, is a very effective, if not particularly efficient, mechanism for bringing about population genetic change in response to such selective forces.

### **Evolution and Breeding**

Evolution is a change in allele frequencies in a population over generations (time). There are a few basic factors that affect allele frequencies in a population over time. They are mutation and migration (gene flow) which bring new alleles into a population, and drift and selection which result in the loss of some alleles to the next generation. This loss can be random (drift) or directed by the selection process. Evolutionary response is a reaction to the environment that a population has experienced prior to reproduction and is influenced by genotype x environment interactions. The evolutionary process, as it relates to breeding, was studied extensively by Sewall Wright (1963). He explained that any evolutionary process requires the coupling of a random process to furnish the raw material of variation and a selective process to give it direction. Natural mutation coupled with phenotypic mass selection is the basic form of the evolutionary process, but adding the process of recombination, through the mechanism of sexual reproduction, to amplify the potential genotypic combinations can speed up the rate of evolutionary change enormously. He further elaborates that a predominantly self pollinating species can evolve rapidly with phenotypic selection among fixed lines, as long as there is sufficiently frequent crossing among selected lines to give recombination and new variation each generation. Cross pollinating species must be subdivided into small populations and given sufficient isolation to permit differentiation among subpopulations under the joint effects of random drift, inbreeding, and intragroup selection, as long as it is also coupled with intergroup selection. Both of the situations described by Wright (1963) for speeding up evolutionary change involve outcrossing/geneflow to promote recombination, and some form of isolation, either geographic or morphological, to promote inbreeding/fixation, followed by selection among reasonably uniform lines or sub-groups, and then further recombination among selected 'superior' individuals within/among subgroups. The cyclic systems described are essentially recurrent selection methods which can be applied to both self-pollinated and cross-pollinated crops.

These concepts were developed further by Wright's student, Jay Lush (1936) in his career in animal breeding at Iowa State University. He stated that the breeder's main tool is selection, but the choice of breeding/mating system can have a great impact on the effectiveness of those selections. The breeder needs to design a system that will increase the frequency of the desired gene combinations, and then effectively select among the resulting progeny those individuals with the highest number of desirable genes for further breeding. Lush (1936) elaborates that a breeder cannot hope for complete success and can hardly expect to put animals [or plants] together gene by gene, as a mechanic puts an automobile together. The general picture thus presented is a process of mild inbreeding to fix traits, alternating with outcrossing to recombine desirable genes and traits, both accompanied at all times by intense selection. There should be a balance between fixing and

maintaining desirable genotypes that have been created, and the evolutionary potential to create even better genotypes in the recombined progeny. Finding and maintaining this balance in the long term is a challenge for plant breeders.

### **Recurrent Selection**

Summed up simply, recurrent selection is the best breeding system to maximize evolutionary response through an alteration between random mating (ie. genetic chaos) which creates variation, and intense inbreeding to fix various combinations of alleles in the homozygous condition for evaluation and selection. To make such a system efficient, selection must identify the best genotypes based on superior phenotype and they should be recombined in sufficient numbers to generate significant variation for the next cycle. The cycle time should be as short as possible. Very rapid response in a breeding population is possible, but is difficult to maintain. Duvick (1982) described recurrent selection as a method that is based on the probability of accumulating desirable alleles via recombination to create new genotypes which should be combined with a pedigree system to fix the new combinations rapidly through selfing to give reproducible phenotypes for evaluation and selection. Such a combination of outcrossing integrated with accelerated generation advance by selfing and early generation evaluation and selection for crossing is exemplified by the RIPE system (Falk, 2002) in barley where the complete cycle from selection of parents through evaluation of progeny involves five generations over two years.

If the objective of breeding is to change a population as much as possible for a particular trait, then the Illinois long-term recurrent selection program in corn provides an outstanding model of what is possible to achieve (Dudley and Lambert, 2004). This project was initiated in 1896 by C.G. Hopkins and has continued annually for more than 100 years, with minor modifications in population management and analytical techniques. The original population of 'Burr's White' was analyzed for oil and protein with approximately the 20% highest and lowest ears for each trait being saved to produce the next generation. The individual sub-populations were designated as Illinois High Oil (IHO), Illinois Low Oil (ILO), Illinois High Protein (IHP), and Illinois Low Protein (ILP). Mass selection for each trait was initially practiced, but later revised to ear-to-row selection for yield and ear quality followed by evaluation and selection for the specific traits. Approximately 60 ears were analyzed in each sub-population in each year and 20% (12 ears) selected for the following year. This amounts to mild selection on a small population with repeated annual cycles over many years.

The progress in selection for high oil and high protein in this population has been amazing. After 70 generations of selection, Dudley *et al.* (1974) reported that the means of the high protein (IHP), low protein (ILP), high oil (IHO) and low oil (ILO) strains were 12, 8, 27 and 10 phenotypic standard deviation (SD) units beyond the mean of the original population. They estimated that approximately 6000 ears had been evaluated in each strain; an extreme of 3.8 SD units from the mean would have been expected in an original population of that size. They reported their results as a 'vivid demonstration of the effectiveness of mild selection and recombination'. These conclusions were further supported after 100 generations (Dudley and Lambert, 2004). The physiological limits of low oil and low protein are likely being approached in the respective lines, but there is no suggestion that the upper limits have been reached in generation 99 in the high lines with the high protein line (IHP) having 26.9% protein and the high oil line (IHO) having 22.4% oil. These four subpopulations, all derived from a single open-pollinated variety from 1896, now represent the extreme expression for oil and protein for the entire *Zea mays* species. The original Burr's White population had an average of 4.7% oil and 10.9% protein, while modern hybrids generally having 7-9% protein.

Since most of the traits of economic interest in a plant breeding program are characterized by polygenic inheritance (Huehn, 1996), it makes sense to adopt quantitative and population breeding methods as the core of the breeding methodology. Lush (1936) notes that for most quantitative traits, there are some genes (often only a few) with important effects plus an uncertain, but large, number of minor modifying factors. QTL analysis may detect the few genes with significant individual effects, but will not be able to detect the multitude of minor and modifier genes which, when combined, may have a greater effect on the trait of interest.

The ultimate breeding system will be a balance between the ability to produce new gene combinations, and the new phenotypes derived from them, and the preservation of existing, successful phenotypes. Allard (1965) concludes that highly successful colonizing species of plants appear to have evolved genetic systems in which appropriate compromises are made between the high recombinational potential of outbreeders and the stability of inbreeders.

Drift may be the dominant force in very small populations or in populations where intense selection results in few individuals contributing gametes to the next generation. Small populations, which include most breeding programs, are affected not only by drift (random loss of alleles) but also by selection *per se*; the relationship of population size to both drift and selection is quite clearly illustrated by Grant (1963). He concludes that evolution is more effective in smaller populations when the force of drift is added to that of selection.

Recurrent selection has been identified by Comstock (1996) as a very natural process in most long-term, open-ended breeding programs. Recurrent selection is common, in one form or another, in both animal and plant breeding and most programs exhibit some of the features of recurrent selection (McProud, 1979). Recurrent selection results in an increase in the frequency of alleles with positive effects on the traits being selected over time. Even though the probability of an individual containing all the desired alleles may be very small at the beginning, as the frequency of the alleles increase in the population through selection and recombination, the probability of an individual occurring which has all the desired alleles increases greatly. After a sufficient number of cycles, and a significant increase in the frequency of the desired alleles, the probability of the ideal genotype being produced is large enough that it is likely to occur in a population of moderate size (Comstock, 1996). Lyrene (2004), in an attempt to adapt temperate peaches and blueberries to the subtropical conditions of Florida, concluded that recurrent selection “is the only breeding method that can accomplish the wholesale re-organization of the physiology of the plant.”

#### **RIPE: a working model system**

The process of producing and selecting the best possible lines in the existing population, then bringing in new, desirable alleles to further enhance the population, and producing the best possible new combinations is the essence of an ‘open-concept’ population breeding approach. The Recurrent Introgression for Population Enrichment (RIPE) system described for barley (Falk, 2002) details the combining of a recurrent selection system and a structured introgression mechanism to continually upgrade the potential of the breeding population. The system is based on an adapted foundation population undergoing recurrent selection at a moderate intensity with frequent recombination. Continual introgression of new material into the Elite level allows new, desirable alleles to be incorporated without disrupting the background from population structure.

Since its inception, a number of high-yielding, agronomically acceptable lines have been produced; more than 30 of them have been supported for registration on the basis of merit as cultivars by the Ontario Cereal Crop Committee, as part of the official Canadian variety registration system. The variety OAC Kawartha is currently the most widely grown barley in Ontario and has been the highest yielding six-rowed variety in each of the past five years in the Ontario Performance trial system (equivalent to National List Trials). In 2007, in official trials, more than half of the 16 six-rowed barley varieties being evaluated in the major barley producing region were from the RIPE system. These lines include the top five lines for test weight, the top line for kernel weight, and the only six-rowed lines with leaf rust resistance. They also have good powdery mildew resistance, desirable plant height, appropriate maturity, and good lodging resistance. All of them have combinations of desirable agronomic traits and disease resistance coupled with high yield. The RIPE system is producing more than just germplasm; the lines derived directly from the Elite population are fully competitive and commercially desirable varieties. They are equal (or superior) to, lines coming out of conventional and doubled haploid breeding programs being used by other breeders for the same target environments. The crosses which gave rise to the most recent lines represent about four to six cycles of recurrent selection. Many of their progeny are already in replicated, multi-location yield trials.

Thus, it has been possible to combine significant improvements in yield with advances in seed quality traits and disease resistance using the RIPE system of recurrent selection and introgression in barley in a relatively short time. The Elite population now consists of mostly high yielding

materials with large, plump grains, good plant type, and high levels of resistance to the prevalent pathogens powdery mildew and leaf rust. An attempt has been made to keep the effective breeding population size in the range of 15-20 male parents each cycle to avoid significant loss of genetic diversity. The periodic introgression of new alleles from exotic sources should also help to maintain (and increase) variability for the selected traits, ensuring that progress continues. New introgressed alleles may be giving rise to greater expression of desirable traits than would have been possible in the original Elite population by recombination alone. Walsh (2004) concluded that the Illinois high protein and high oil long-term recurrent selection populations have likely had some increase in variation due to mutation, so some of their astounding progress is also likely due to the input of new, desirable alleles through mutation, implying that new variation, either from migration or mutation, may be necessary to account for the progress exhibited.

The RIPE system, where effective recurrent selection to increase the frequency of desirable alleles in an Elite population is combined with a rapid introgression of new alleles, comes very close to meeting Wright's (1963) ideal conditions for maximizing the 'enormous evolutionary potential' of a population in the short time frame of a breeding program. Frequent opportunity for recombination through crossing of selected lines with male steriles is coupled with effective selection for yield on F3-derived F4 lines in the target environment to identify lines which will be evaluated more extensively in the following years. More importantly, selected lines are immediately used as male parents in the next round of crossing. Although some of the lines may be discontinued later in the evaluation system, many of their desirable alleles are being re-circulated in the breeding population. The superior lines that eventually are released as cultivars will have progeny in yield trials by the time their superiority is confirmed. This system addresses McProud's (1979) concerns about low numbers of founding parents, few new introductions and long cycle times in modern breeding programs. The RIPE system, as it has evolved, is highly efficient and effective in developing new high-yielding cultivars which maintain and recombine the suite of genes necessary for adaptation, and incorporate improved agronomic performance and disease resistance through accelerated introgression. This is how evolution has always worked, and how evolution and breeding will continue to do so in the foreseeable future.

### Summary

Breeding high-performing crops for a more commercially competitive, and an increasingly volatile environment requires methods which can maximize the effective utilization of elite germplasm through rapid cycling recurrent selection approaches, and incorporate new, desirable alleles from the vastly under-utilized exotic germplasm collections currently stagnating around the world. The RIPE system appears to fulfill this mandate as an upgraded, updated, accelerated, and improved form of the old reliable evolution that Charles Darwin conceptualized so long ago.

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## AUGU SELEKCIJA 21. GADSIMTĀ: CILVĒKA VIRZĪTĀS EVOLŪCIJAS ATTĪSTĪBA

**Falk D. E.**

Augu selekcija sākās, cilvēkam uzsākot augu kultivēšanu gandrīz pirms 10000 gadiem un ātri noveda pie laukaugu domestikācijas dabiskās izlases ceļā, piemērojoties augšanai kultivētos apstākļos. Dabiskās izlases ceļā, to papildinot ar ražas vākšanu izlases veidā un tikai specifisku fenotipu tālāku pavairošanu, radās daudzas vietējiem apstākļiem piemērotas, pietiekoši ražīgas vietējās šķirnes jeb landrases. Tās vēlāk tika izmantotas viendabīgu tīru līniju populāciju izolēšanai, kas kļuva par šķirnēm. Tas notika vienlaicīgi ar sējas, ražas novākšanas un apstrādes mehanizācijas palielināšanos. Mendeļa darbu atklāšanas rezultātā 20. gadsimtā augu selekcijā sāka pielietot ģenētiku un hibridizāciju. Tādējādi sāka darboties „virzītā evolūcija”. Jauni atklājumi ģenētikā pēdējo 100 gadu laikā (kvantitatīvā ģenētika, poliploīdija, inducētās mutācijas, vīrišķā sterilitāte, sapratne par DNS un molekulāro bioloģiju, haploīdija, ģenētiskā transformācija) tika strauji izmantoti selekcijas procesā lai padarītu jaunu šķirņu veidošanu efektīvāku un ekonomiskāku. Selekcionāriem ir pieejams aizvien lielāks jaunu tehnoloģiju klāsts. Selekcionāru uzdevums ir noteikt, kuras no šīm daudzveidīgajām augsti attīstītajām tehnoloģiskajām iespējām ir visvairāk piemērotas, lai maksimizētu īslaicīgu, strauju evolūciju noteiktās populācijās ar specifiskiem mērķiem un limitētiem budžeta līdzekļiem. Iesākoties 21. gadsimtam, augu selekcija ir izveidojusies par ļoti efektīvu, virzītu evolucionāru procesu, kas atbalsta civilizāciju.

## VARIATIONS IN THE MORPHOLOGICAL CHARACTERISTICS OF WINTER WHEAT (*TRITICUM AESTIVUM* L.)

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### Abstract

Estonia joined the International Union for the Protection of New Varieties (UPOV) in 2000. For that reason the requirements for a new variety changed. The law of variety protection came into

force in Estonia in 1994. Determination of distinctness, uniformity and stability (DUS) by UPOV was started at the Jõgeva Plant Breeding Institute in 2002.

The Estonian variety Sani and 5 lines from the Lithuanian Institute of Agriculture were tested during the period of 2006–2007. 26 characteristics were tested by the UPOV methods. For this research 12 the most distinctive ones were selected.

The most uniform characteristic was ear color, where all the lines had white ear color without the variation within the line and the ear shape in profile, where no variation among the SaniC, Lia 00116A and Lia 00127 was found. The most uniform of this material were SaniC and Lia 00127, because the variation was absent in 3-4 traits, other lines varied almost in all the traits.

The biggest effect of the variety ( $R_v^2=73-80\%$ ) had width of flag leaf, frequency of plants with recurved flag leaves, ear density and length of scurs. The biggest effect of the year ( $R_y^2=10.6-33\%$ ) appeared on glaucosity of the sheath of flag leaf, hairiness of the upper node of culm and the length of scurs.

The best morphological characteristics for distinctness of the varieties are anthocyanin coloration of the auricles of flag leaf, the frequency of plants with recurved flag leaves, glaucosity of ear, hairiness of upper node of culm, the ear shape in profile and the length of scurs.

**Key words:** winter wheat, UPOV DUS test, morphological characteristics

## Introduction

In 1961, the International Convention for the Protection of New Varieties of Plants was concluded and the International Union for the Protection of New Varieties of Plants (UPOV) established (TC/36/9, 2000). During the last decade the requirements for a new variety have changed.

In Estonia the law of variety protection was accepted in 1994 and Estonia has belonged to the UPOV since 2000 (RT I 1994,23, 385). This required Estonia to investigate varieties and breeds in accordance with UPOV protocol. Traditional cereal crops breeding is a long-term process, the time for breeding is from 10 to 15 years. Newly bred varieties have to undergo statutory testing before being marketed. Part of this testing determines their distinctness, uniformity and stability (DUS test). A candidate variety will be considered distinct when at least one difference has been determined which is clear and consistent over two years of testing. A variety must be sufficiently uniform with regard to the particular features of the reproductive system. A variety is stable in its essential characteristics when it remains true to its description after repeated reproduction. In general, if a variety demonstrates uniformity then it will also be considered to be stable (Cole, 1998). All observations for the assessment of distinctness and stability should be made on 20 plants or parts of 20 plants. For the assessment of the uniformity of characteristics on the plot as a whole, the number of non – typical plants or parts of plants should not exceed 5 in 2000 (UPOV, 1994).

The department of homogeneity breeding was established at the Jõgeva Plant Breeding Institute in 2002. The main activity of the department is the investigation of morphological characteristics for distinctness, uniformity and stability testing. Homogeneity breeding begins in the ninth generation when the genetic characteristics are fixed. This process per one variety lasts for three years. As a result of this process the pure breeder's seed is passed over to further seed production. Worldwide there are very few scientific articles about homogeneity breeding. Ulvinen (1974, 1994) from Finland has written some articles about the morphological characteristics of cereals. Cole (1998) from Guelph University has researched the use of morphological and rapid markers for the distinguishing of canola (*Brassica napus* L.) varieties. The scientist Smart *et al.* (2004) have carried out research on bluestem and switchgrass morphological characteristics.

The purpose of this research was the estimating of morphological characteristics to find out the distinctness, uniformity and stability of perspective breeds of winter wheat and how climatic conditions and varieties influence them.

## Materials and Methods

The Jõgeva Plant Breeding Institute has cooperation with the Lithuanian Institute of Agriculture. The morphological characteristics of the winter wheat lines of the Lithuanian Institute of Agriculture and of the Estonian material were investigated in trials at the Jõgeva Plant Breeding Institute in 2006–2007. The trial was carried out in 9 m<sup>2</sup> plots in three replications. There were five

breeds and three lines from the variety Sani in the trial. Sani is an old variety and the homogeneity breeding and breeder's seed production of this variety has not been elaborated. Division into phenotypes was applied also to other tested breeds when it was needed because of variation within a breed. The breeds were divided by the results of the phenol test. The grains are placed into a 1% phenol solution for four hours where the grain color turns from no coloration to dark brown (UPOV, 1994). That test shows the uniformity of the grains.

The weather conditions were quite similar during the testing years. The vegetation periods of the both years could be characterized as ones of drought.

There are 26 characteristics included in the UPOV protocol (UPOV, 1994). For this study 12 of the most distinctive ones were selected. The following features were observed in the field: the anthocyanin coloration of auricles of flag leaf, the hairiness of auricles of flag leaf, the width of flag leaf, the frequency of plants with recurved flag leaves, the glaucosity of sheath of flag leaf, the glaucosity of ear, the glaucosity of neck of culm, the hairiness of upper node of culm, the ear shape, the ear density, the ear colour and the length of scurs. The estimation scale was from 1 to 9 points, where 1 was the weakest and 9 the strongest appearance of the feature. Observations were carried out during the certain plant growth stage. Twenty randomly selected plants were estimated. The UPOV protocol contains standard varieties (26) that are used for the comparison of the characteristics of the tested lines. Statistical analyses were performed using the Agrobase software package. The data was analysed by analyses of variance. The analyses of variance and the estimates of the components of variance (determination coefficient) due to growing year  $R_Y^2$  and variety  $R_V^2$  were calculated and were expressed as % of the total variance. Uniformity analyses of the homogeneity of the morphological characteristics were based on a coefficient of variation (CV) (Agrobase<sup>TM</sup>, 1999). In characteristics as the ear colour and ear shape in profile, the data is absent, because it is not possible to carry out statistical analysis for these characteristics.

## Results and Discussion

The anthocyanin colouration of auricles of flag leaf. The optimal time for estimation is during the heading stage. The anthocyanin coloration of auricles of flag leaf is a good characteristic for the identification of breeds and varieties, which depends on genetic derivation and weather conditions. This characteristic occurs much stronger in dry and sunny and less in cool and rainy summers (Ulvinen, 1974). The average coloration during the two years was weak (1.6 points). In 2006, the anthocyanin coloration of auricles of flag leaf varied between very weak (1) and weak (3). The breed Lia 00116B and Lia 0019 had the strongest coloration. In 2007, this characteristic varied between very weak (1) and medium (3.8), the strongest coloration again was found in the breed Lia 00116B. The coefficient of variation (CV) of breeds varied in the range of 0–72.7% (Table 1). There was no variation for this trait in SaniB, SaniC and Lia 00105. Other lines varied in a wide range: 35.0–72.7 %. The influence of variety had a significant effect on the occurrence of this characteristic (Table 2), but the biggest was an unknown or residual effect. The vegetation periods of 2006 and 2007 had similar weather conditions, for that reason the results of the two years were quite similar.

The hairiness of auricles of flag leaf. The optimal time for the estimation of the hairiness of auricles is during heading (Ulvinen, 1974). Wheat has hair of auricles of flag leaf in general, but sometimes the hair may be absent (Ulvinen, 1974). The average estimation of characteristics of two years was medium (4.5 points). In 2006, the characteristics varied between weak to strong (3–7 points), the breed Lia 00127 had the most hairiness and the least Lia 0019 and SaniA. In 2007, the variations ranged from very weak to strong (1.3–7 points), the strongest for the breed Lia 00127, and the weakest for SaniB and SaniC. CV varied between 0–73%, the smallest for the breed Lia 00116B and the biggest for the variety SaniC. The variation was absent in the breeds Lia 00127 and Lia 0019. The hairiness of auricles was mainly influenced by the variety. In this study the hairiness of auricles appeared in all the material, but the results of the two years were different. It turned out from the results, that in 2007 the occurrence of the characteristic was stronger than in 2006.

Width of flag leaf. Flag leaf of wheat is a major source of photosynthesis for the developing grain and its size contributes to interplant competition (Spagnoletti *et al.*, 1990). Most often the flag leaf width is narrow to medium. Comparing the width of the leaf of spring and winter wheat, the leaves of winter wheat are wider than ordinarily. The leaf width depends also on weather conditions like



many other characteristics, for that reason the results can be different in different years. The average width of flag leaf was between medium and strong (6.6 points). In 2006, the characteristic varied between medium (4) to wide (8); the breeds Lia 00116B and Lia 0029 having the widest leaves. In 2007, the points were in the range of 5–9 (medium to very wide), the widest in the breed Lia 00116A and the most narrow in the variety SaniA. The variation in the width of flag leaf of SaniC was absent, but the variation coefficient for other lines ranged from 9.0-23.8 %. The width of the flag leaf was mainly determined by the variety.

The frequency of plants with recurved flag leaves. The optimal time for estimation is during the heading stage, when the spikelets are out of the sheath. The frequency of plants with recurved flag leaves can be very small (leaves erect), small (1/4 curved), medium (1/2 curved), big (3/4 curved) and very big (UPOV, 1994). The average frequency of plants with recurved flag leaves was medium (5.1 points). In 2006, the share of recurved leaves was very small (1) to very big (9). The frequency of recurved leaves was very big in SaniC and small in Lia 00116A and Lia 00127. In 2007, the share of recurved leaves was very small (1) to big (8 points), big in SaniA, SaniB and SaniC and small in Lia 00127 and Lia 00105. CV was 0–73.0%. Lia 00127 was the most uniform, absolutely no variation was fixed. Lia 00116A, Lia 00105 and Lia 00116B were characterized by high variability (CV=59.0-73.0%). This characteristic was mainly affected by the variety. This is a good characteristic for distinctness, because among breeds and varieties both types of leaves occurred: erect and strongly curved. The results of the two years were similar and not influenced by weather conditions.

Glaucoisity. The glaucoisity of the sheath of the flag leaf, the ear and the neck of the culm were estimated. The observations were carried out during inflorescencing. Wheat varieties and breeds often have a grayish blue waxy aril that protects the plants from some in environmental factors. This is a good characteristic for distinctness. This characteristic occurs more strongly in dry and sunny summers. The two years average glaucoisity of the sheath of the flag leaf was medium (6.2 points). The points varied in range from medium to strong (6–8). In 2006, Lia 00116A, Lia 00127 and Lia 00105 had the strongest glaucoisity, the weakest SaniA and SaniC. In 2007, the points were in the range of weak until becoming strong (3–6.8). Lia 00116A had the strongest glaucoisity and the weakest Lia 0029. The coefficient of variation was 8.4–42.2%, the less varied were SaniB and Lia 00116B (both 8.4%). More variable was Lia 0029 (42.2%). The effect of year and variety to the characteristics was similar.

Table 1. The coefficient of variation (CV) (%) of the morphological characteristics of winter wheat material in 2006–2007

Breed, variety/ Characteristics	SaniA	SaniB	SaniC	Lia 00116A	Lia 00116B	Lia 0029	Lia 00127	Lia 0019	Lia 00105
Anthocyanin coloration of auricles of flag leaf	72.7	0.0	0.0	35.0	62.0	61.2	61.2	60.7	0.0
Hairiness of auricles of flag leaf	30.6	64.5	73.0	32.3	14.4	16.3	0.0	0.0	18.2
Width of flag leaf	23.8	23.3	0.0	12.0	15.8	11.1	11.2	15.2	9.0
Frequency of plants with recurved flag leaves	21.3	20.9	9.4	73.0	59.0	12.2	0.0	25.8	61.2
Glaucoisity of sheath of flag leaf	19.1	8.4	12.2	13.7	8.4	42.2	18.2	27.5	22.1
Glaucoisity of neck of culm	15.9	7.7	6.0	8.4	18.3	22.6	0.0	6.0	5.7
Glaucoisity of ear	27.4	7.9	23.8	15.9	7.7	32.3	7.3	87.7	9.6
Hairiness of upper node of culm	12.0	23.5	0.0	27.9	28.8	0.0	28.8	56.5	42.7
Ear density	0.0	18.2	0.0	12.2	24.5	22.3	15.5	18.8	15.6
Length of scurs	25.3	14.9	61.2	30.6	35.0	14.1	15.5	12.9	25.6

The two years average glaucosity of the neck of the culm was medium to strong (6.5 points). In 2006, the estimated points varied between medium and strong (5–7.3), the breed Lia 00105 had the strongest glaucosity and the weakest Lia 00116B. In 2007, the points were 4.8–7.3 (medium to strong), Lia 00116A had the strongest and the weakest Lia 0029. The coefficient of variation was 0–22.6% for this trait, Lia 00105 (5.7%) had the smallest and no variation was found in Lia 00127. The estimation of characteristics was affected by the year and the variety. The biggest factor was the unknown effect.

The average of glaucosity of the ear was 5.4 points (medium). In 2006, the points varied in the range of 3.0–8.3 (weak to almost very strong), Lia 00105 having the strongest glaucosity and SaniA the weakest. In 2007, the points were in the range of 1.0–7.3 (very weak to strong), Lia 00127 and Lia 00105 had the strongest and the weakest Lia 0019. The variability of the glaucosity of the ears within the lines was low to high (CV= 7.3 to 87.7%), Lia 00127 (7.3%) had the smallest, the highest Lia 0019 (87.7%). The appearance of the glaucosity of the ear was mainly affected by the variety.

It emerged during this study that the glaucosity of the sheath of the flag leaf and of the ear differed between years. In 2007 these characteristics were weaker than in 2006. The points of glaucosity of the neck of the culm were similar in these years. The occurrence of the glaucosity on the sheath of the flag leaf was influenced by weather conditions.

The hairiness of upper node of culm. The optimal time for estimation is during inflorescencing. The hairiness of the upper node of the culm is an important characteristic for the distinctness of varieties (Ulvinen, 1994). The average hairiness was 6.7 points (strong) in the years of research. In 2006, the points were in the range of 2.3–9.0 (weak to very strong), the variety SaniC had the strongest and the weakest Lia 0019. In 2007, the points were 3.0–9.0 (weak to very strong), Lia 0029, SaniA, SaniB, SaniC had the strongest, and the weakest Lia 0019. Lines SaniC and Lia 0029 were characterized with uniformity and stability in this trait and variations were absent. Other lines were more variable, they had a coefficient of variation of 12.0–56.5 %. The hairiness of the upper node of the culm was mostly affected by the variety. In comparison between the two years it was found that the estimated points were in the range of 2.3(3.0)–9.0 in both years, although by statistical analysis was found, that some influence of the year appeared.

Table 2. Analyses of the variance of winter wheat traits. Components of variation due to year ( $R_Y^2$ ),

Characteristics	Determination coefficient (%)		Residual (%)
	Year, $R_Y^2$	Variety, $R_V^2$	
Anthocyanin coloration of auricles of flag leaf	0.0	39.7**	60.3
Hairiness of auricles of flag leaf	6.6**	64.2***	29.2
Width of flag leaf	4.6**	73.1***	22.3
Frequency of plants with recurved flag leaves	1.2*	81.8***	17.1
Glaucosity of sheath of flag leaf	32.5***	27.9**	39.7
Glaucosity of neck of culm	4.5ns	44.6***	51.0
Glaucosity of ear	1.3ns	69.8***	28.9
Hairiness of upper node of culm	12.5***	64.7***	22.8
Ear density	1.3ns	79.0***	19.8
Length of scurs	10.6***	80.4***	9.0

variety ( $R_V^2$ ) and residual in percentage of the total sum of variance probability \*\*\*  $P \leq 0.001$ , \*\*  $0.001 < P \leq 0.01$ , \*  $0.01 < P \leq 0.05$ , ns=not significant

Ear shape. The ear shape can be tapering (1 points), parallel sided (2 points), semiclavate (3 points), clavate (4 points) and fusiform (5 points). The ear shape was estimated during full ripeness. Most of the material had the tapering ear shape. In 2006, Lia 0019 had parallel sided ears and only SaniC had the clavate ear shape. The lines SaniA and SaniB, Lia 00116A and Lia 00116B, Lia 0029, Lia 00127, Lia 00105 had a tapering ear shape. In 2007, SaniB and Lia 0019 had shapes between the tapering and parallel sided and SaniC had the clavate ear shape. The lines SaniA, Lia 00116A and Lia 00116B, Lia 0029, Lia 00127, Lia 00105 had a tapering ear shape. Lia 0019 had small differences between the years, but no variation within the line. Ear shape may be influenced

by weather in some years (Ulvinen, 1974). In this research no differences between the years in ear shapes were found, except for the lines Lia 0019 and SaniB.

**Ear density.** The characteristic was estimated from dough development to the full ripeness stage. Ear density depends on the length of rachis segments. It is expressed by the number of rachis segments or spikelets per 10 cm (Annus, 1974). The ear density is mostly medium in wheat in Nordic conditions, but the varieties may also have very lax or dense heads. In this study the density of ears was lax to medium. The average ear density was 4.5 points (medium). In 2006, the points were 3.7–7.0 (lax to dense), the most lax were the breeds Lia 00116B (3.7 points) and Lia 00127 (3.7 points), the most dense was the ear of the variety SaniC (7 points). The same results were obtained in 2007. The coefficient of variation was 0–24.5%. The variation was not observed in SaniA and SaniC. Variability on the level of 12.2–15.6 % was noted for 3 lines, but much higher (18.2–24.5%) for 4 lines. The ear density was most influenced by the variety. This characteristic depends mostly on genetic derivation but weather conditions may have a little influence on the occurrence of this characteristic (Ulvinen, 1974). The same was ascertained in our research (Table 2).

**Ear color.** The characteristic was estimated in full ripeness. The color of ear was estimated on a scale of 1–2 points, where 1 was white and 2 was a colored ear. The majority of the wheat varieties and lines have a white ear color (Ulvinen, 1994). It is a stable and firm characteristic for distinguishing the varieties and breeds. Generally, this characteristic is uniform and does not depend on the external factors. Among this material the ears of all the varieties and breeds were white. The ears of investigated material were white and variation between the breeds and varieties was absent.

**Length of scurs.** The length of scurs was estimated from the dough development to the full ripeness stage. Wheat varieties (breeds) can be divided by awns into two types: awned and awnless ones (Annus, 1974). All the varieties cultivated in Estonia and the varieties and breeds tested in this study have scurs, but no awns. The average length of the scurs was 3.8 points (short). In 2006, the points were 1.7–7.0 (very short to long). The breed Lia 0019 had the longest scurs and the shortest the variety SaniC. In 2007, the points ranged between 1.0 and 5.8 (very short to medium). The breed Lia 0019 had longest scurs and the shortest the variety SaniC. The coefficient of variation was in the range of 12.9–61.2%, the less varied was Lia 0019. The length of scurs was mainly determined by the variety. It turned out from the results of the two years that the weather had a significant effect on the length of the scurs.

## Conclusions

The morphological characteristics of breeds of winter wheat of the Lithuanian Institute of Agriculture and the material from Estonia were investigated in trials at the Jõgeva Plant Breeding Institute in 2006–2007.

The most uniform characteristic was the ear colour, where all the lines had white ear colour without the variation within the line and the ear shape in profile, where no variation among the SaniC, Lia 00116A and Lia 00127 was found. The most uniform of this material were SaniC and Lia 00127, because the variation was absent in 3–4 traits, other lines varied almost in all the traits. If a variety is uniform, then it is also stable.

In this research the most ununiform characteristics were the glaucosity of the sheath of the flag leaf, the glaucosity of the ear and the length of the scurs. All of the tested lines had a high level of variation. The most ununiform breeds were Lia 00116A and Lia 00116B, because all the twelve characteristics varied. The homogeneity breeding of these breeds should be continued.

The variability of all characteristics was affected by the variety. The biggest was the influence of the variety ( $R_v^2$  73–80%) on the width of flag leaf, the frequency of plants with recurved flag leaves, the ear density and the length of scurs. The biggest effect of the year ( $R_y^2$  10.6–33%) was on the glaucosity of the sheath of the flag leaf, the hairiness of the upper node of the culm and the length of scurs.

The best characteristics to distinguish the varieties were the anthocyanin coloration of the auricles of the flag leaf, the frequency of plants with recurved flag leaves, the glaucosity of the ear, the hairiness of the upper node of the culm, the ear shape in profile and the length of scurs. There appeared clear differences between the varieties within the morphological characteristics. Variation

was mostly determined by the variety but the climatic conditions had some impact. Quite good characteristics are also the glaucosity of the sheath of the flag leaf and the glaucosity of the neck of the culm.

SaniC and Lia 00127 turned out to be the most stable, uniform and distinct and continue as the perspective line for further breeding process and breeder's seed production.

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## ZIEMAS KVIEŠU (*TRITICUM AESTIVUM* L.) MORFOLOĢISKO PAZĪMJU DAUDZVEIDĪBA

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Igaunija pievienojās Starptautiskajai jaunu šķirņu aizsardzības savienībai (UPOV) 2000. gadā. Šī iemesla dēļ mainījās prasības jaunām šķirnēm. 1994. gadā Igaunijā stājās spēkā šķirņu aizsardzības likums. Atšķirīguma, izlīdzinātības un viendabīguma (AVS) noteikšana saskaņā ar UPOV tika uzsākta Jōgevas Augu selekcijas institūtā 2002. gadā.

Laika periodā no 2006. līdz 2007. gadam tika pārbaudīta igauņu šķirne 'Sani' un 5 līnijas no Lietuvas zemkopības institūta. Pēc UPOV metodikas tika noteiktas 26 pazīmes. Šim pētījumam tika izvēlētas 12 visvairāk atšķirīgās pazīmes.

Visizlīdzinātākā pazīme bija vārpu krāsa, visām līnijām bija baltas vārpas un līniju robežās dažādības nebija, kā arī vārpu forma profilā, kam netika konstatētas atšķirības starp 'SaniC', Lia 00116A un Lia 00127. Visizlīdzinātākie no šī materiāla bija 'SaniC' and Lia 00127, jo šiem paraugiem daudzveidība netika konstatēta pēc 3-4 pazīmēm; citas līnijas nebija izlīdzinātas gandrīz pēc visām pazīmēm.

Lielākā šķirnes ietekme ( $R_V^2=73-80\%$ ) bija uz karoglapas platumu, augu daudzumu ar noliektām karoglapām, vārpas blīvumam un akotveida smaiļu garums. Audzēšanas gada ietekme bija lielāka ( $R_Y^2=10.6-33\%$ ) uz karoglapas maksts vaska apsarmi, stiebra augšējā posma apmatojumu un akotveida smaiļu garumu.

Labākās morfoloģiskās pazīmes šķirņu atšķirīguma noteikšanai ir karoglapas austiņu antociāna krāsojums, augu daudzums ar noliektām karoglapām, vārpas vaska apsarme, stiebra augšējā posma apmatojums, vārpas forma profilā un akotveida smaiļu garums.

## EFFECT OF ORGANIC AND CONVENTIONAL PRODUCTION ON YIELD AND THE QUALITY OF SPRING CEREALS

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### Abstract

The situation in the agricultural sector has changed during the last years. The need to increase sustainability and protect the environment has become more relevant. Also organic farming is increasing in Estonia. The field trials were carried out at the Jõgeva Plant Breeding Institute to compare the grain yield and quality characteristics of spring wheat, barley and oat in organic and conventional conditions. Thirteen varieties of each cereal crop were tested during a three year period (2005-2007). The trial results showed that all the spring crops were able to produce comparatively high yields in organic conditions. The highest yielding in the organic trial was obtained by oat followed by barley and wheat. The yield decrease in spring wheat was the biggest (34%) in organic conditions compared to conventional conditions. Yield reduction was mostly the result of the shortage of plant nutrients. Weather conditions were more favourable for cereals in 2005. Drought caused a decrease in grain yield and quality in 2006 and 2007. The grain quality of barley and oats was similar in both cropping systems; wheat produced bigger kernels in the organic trial. Protein content in organic conditions decreased, wheat having the largest decrease

**Key words:** spring wheat, barley, oat, yield, quality, organic, conventional condition

### Introduction

Cereals cultivated in organic conditions should produce sufficient yields without the use of mineral fertilizers and plant protection chemicals (pesticides). At the same time organic cultivation is oriented to high quality production. Yield usually takes priority in non-organic cultivation but will often have a lower priority in organic farming, relative to quality. Also, organic farming management is complex. In contrast to conventional systems, organic farmers rely mostly on preventive and adaptive management.

In Europe, Lammerts van Bueren (2003), Wolfe (2002) and Vogt-Kaute (2001) have tested and summarized the results of organic trials - organic plant breeding and crop production yield and yield stability, the efficiency of nutrient uptake, adaptation to organic inputs, quality characteristics etc.

In Estonia the following topics have been discussed during recent years. Sepp and his colleagues (2006) have tested the influence of organic crop rotation on yield and the quality and weediness of wheat and barley. Changes in the earthworm community in experimental plots of conventional and organic trials have been studied by Ivask and her group (2007). The comparison of spring cereals and their varieties in conventional and organic systems have not been carried out in Estonian conditions. The preliminary results of our trials about the yield and quality of spring cereals were published in 2007 (Tamm *et al.*, 2007).

A series of field trials comparing organic and conventional systems of the production of spring wheat, barley and oats was established in 2005 at the Jõgeva Plant Breeding Institute in Estonia. The objective of the study was the assessment of the suitability of different spring cereal crops and their varieties to organic cultivation.

### Materials and Methods

The trials were conducted from 2005 to 2007 and included 13 spring wheat, barley and oat varieties. The following barley varieties were included: Anni, Elo, Viire, Leeni (Estonia), Tocada, Danuta, Barke, Annabell (Germany), Wikingett, Mette (Sweden), Zazjorski 85 (Belorussia), Inari (Finland) and new the Estonian breed 3280.14.1.4. The oat varieties Jaak, Villu (Estonia), Hecht, Revisor, Nelson, Jumbo, Freddy, Aragon (Germany), Vendela, Belinda, Birgitta, Freja (Sweden) and Celsia (The Netherlands) were used. From the spring wheat varieties the following were

selected Helle, Meri, Mooni (Finnish-Estonian collaboration), Vinjett, Tjalve, Zebra, SW Estrad (Sweden), Munk, Triso, Monsun (Germany), Manu, Mahti (Finland) and Baldus (The Netherlands). The experimental design was a randomized complete block with 4 replications. The organic and conventional trials were established on soddy-podzolic soil. The average P content was good, the level of K average, the organic matter content medium and the pH slightly acid. The precrop in the organic trial was red clover in 2005 and 2006 and was followed by buckwheat in 2007. Mechanical weed control by repeated harrowing was carried out after germination and in the 3-4 leaves stage. The precrops in the conventional trials were potato and rapeseed. The fertilizer level  $N_{70}P_{16}K_{29}$  was used for oats and  $N_{90}P_{20}K_{38}$  for barley and wheat in the conventional trial. Weeds were controlled by herbicides. A seeding rate of 500 (barley) and 600 (wheat, oat) germinating seeds per  $m^2$  was used. The yield, 1000 kernel weight, volume weight, protein content, the falling number for wheat and the husk content for oats was measured after harvest. The falling number was determined by the ICC standard method 107/1, oat grains were dehulled by hand and the hulls and groats weighed separately.

Weather conditions in all of the tested years were drier than average (Figure 1). The most favourable for yield formation were the weather conditions in 2005. The first half of the vegetation period was cool and early season precipitation favoured plant growth and development. Drought in July had some effect on plant growth but the yielding level was above average. The driest was the vegetation period in 2006, but the distribution of precipitation caused only small yield decrease compared to the previous year. Severe drought (only 10 mm during the 2 weeks before heading) and a higher than average temperature in June of 2007 resulted in significantly shorter plants and fewer spikelets (Keppart, 2008). Yield decrease was remarkable. Because of drier than average vegetation periods in all of the 3 years there was almost no lodging and comparatively low disease incidence. Data was analysed using the Agrobases computer package (Agrobases gen.II<sup>TM</sup>, 2004).

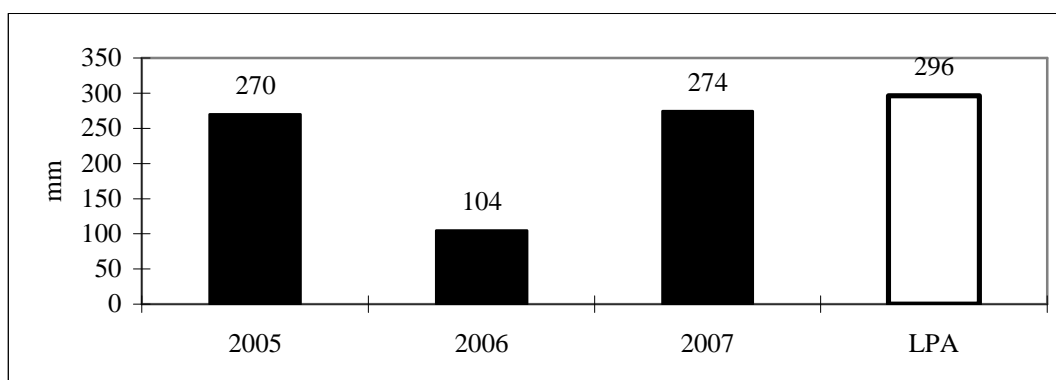


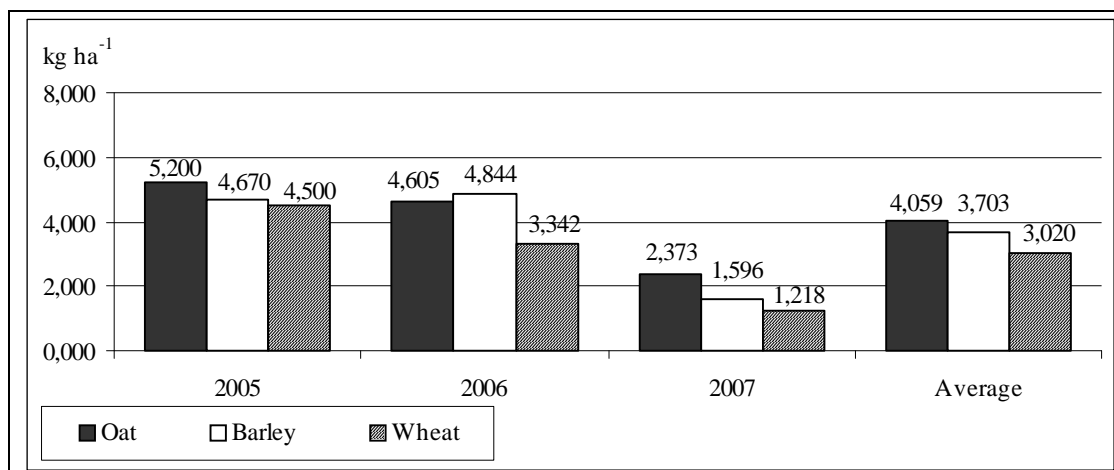
Figure 1. Precipitation (mm) of the vegetation period of 2005-2007 compared to LPA (long period average)

## Results and Discussion

**Grain yield.** In the first trial year the yielding capacity of all the spring cereals in the organic trial was high extending to  $4,500 \text{ kg ha}^{-1}$  (Figure 2). Yields of oats and barley were despite the drought on average level also in 2006 respectively by  $4,605$  and  $4,844 \text{ kg ha}^{-1}$ . Spring wheat suffered the most, producing only  $3,342 \text{ kg ha}^{-1}$ . Heavy soil crust during germination, early drought before heading and an unsuitable precrop (buckwheat) in 2007 decreased the yields the most.

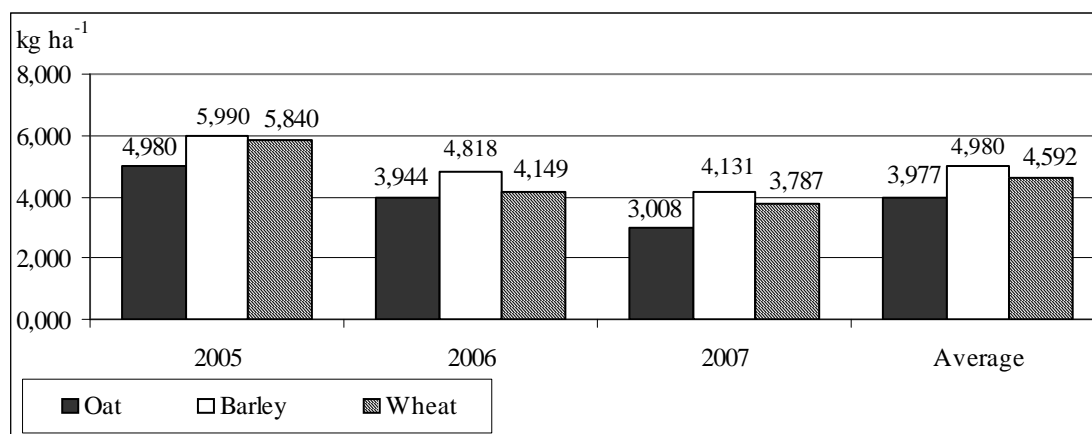
Similar to organic trials in conventional conditions the yield were the highest in 2005 and the lowest in 2007 (Figure 3). The grain yield of the oat varieties in conventional trials was lower than that of barley and wheat during all the trial years.

In the conventional trial barely yield the highest average ( $4,980 \text{ kg ha}^{-1}$ ) followed by spring wheat ( $4,592 \text{ kg ha}^{-1}$ ) and oat ( $3,977 \text{ kg ha}^{-1}$ ). Oats were less influenced by the two management systems and there was no significant difference in the average yield of the organic and conventional trials. In organic conditions the grain yield of barley constituted 74 % and wheat 66 % of the conventional yield.



(LSD<sub>0.05</sub> for 2005= 155, 2006=162, 2007=113 and average= 97 kg ha<sup>-1</sup>)

Figure 2. The grain yields of spring cereals in organic conditions in 2005–2007



(LSD<sub>0.05</sub> for 2005=182, 2006=147, 2007=138 and average=97 kg ha<sup>-1</sup>)

Figure 3. The grain yields of spring cereals in conventional conditions in 2005–2007

The most of the varieties ranked similarly by grain yield in both growing conditions. The correlations between the grain yields of oat, barley and wheat varieties in organic and conventional trials were significant (Table 1).

Table 1. The correlations between conventional and organic grain yield and quality characteristics of spring cereals

Characteristic	Oat	Barley	Wheat
Grain yield	0.76**	0.64*	0.80**
Thousand kernel weight	0.77**	0.93***	0.93***
Volume weight	0.85***	0.7*	0.71**
Protein content	0.76**	ns	0.88***
Husk content	0.81**	–	–
Falling number	–	–	0.7*

\* – significant for P<0.05; \*\* – significant for P<0.01 \*\*\* – significant for P<0.001; ns – not significant

The highest yielding of the three years average in the organic trial were the oat varieties Belinda (4,336 kg ha<sup>-1</sup>), Freddy (4,243 kg ha<sup>-1</sup>), Aragon (4,238 kg ha<sup>-1</sup>), Freja (4,147 kg ha<sup>-1</sup>), Villu (4,133 kg ha<sup>-1</sup>) and Jaak (4,082 kg ha<sup>-1</sup>); the barley varieties Annabell (4,164 kg ha<sup>-1</sup>), Tocada (3,942 kg

ha<sup>-1</sup>), Anni (3,845 kg ha<sup>-1</sup>), Barke (3,818 kg ha<sup>-1</sup>) and Viire (3,800 kg ha<sup>-1</sup>) and the spring wheat varieties Monsun (3,479 kg ha<sup>-1</sup>), Triso (3,305 kg ha<sup>-1</sup>), Zebra (3,153 kg ha<sup>-1</sup>), Vinjett (3,153 kg ha<sup>-1</sup>) and Munk (3,133 kg ha<sup>-1</sup>).

While comparing the yields of the two management systems the varieties of the different spring cereals formed quite clear groups (Figure 4).

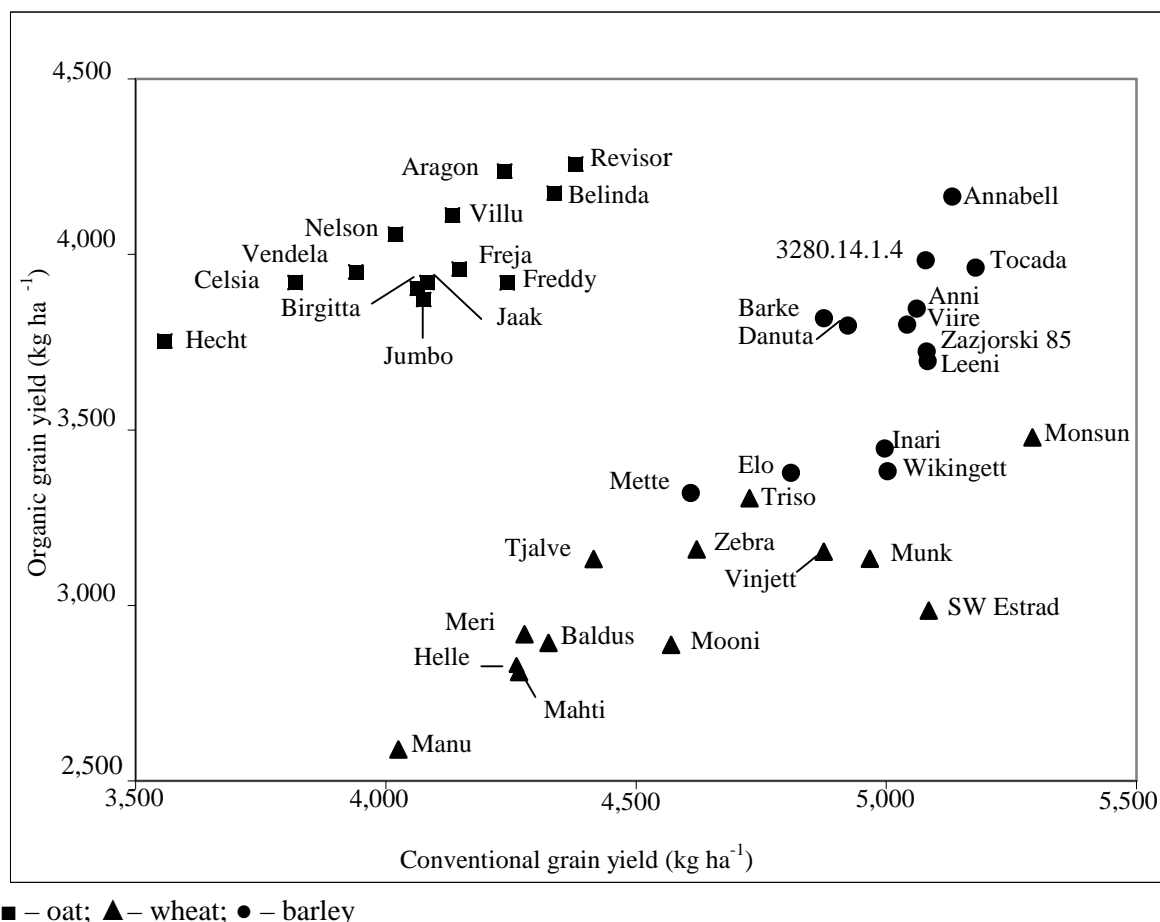


Figure 4. Comparison of organic and conventional grain yields of spring cereals ( $LSD_{0.05}=402$ )

The grain yield of the oat varieties in conventional management was lower on the average yield than the rest of the conventional trial and the reaction in the organic trial was the opposite – all the varieties outyielded the trial average. Most of the barley varieties had higher than average trial yields in conventional conditions and close to average or even a little above in the organic trials. Several spring wheat varieties produced higher than average yields in the conventional trial but all were lower than average in the organic trial. Under both management conditions increased grain yield was associated with late maturity. The correlation coefficient between the yield and the length of the growing period of wheat was 0.73 ( $P<0.01$ ) in the organic and 0.79 ( $P<0.01$ ) in the conventional trial. No significant correlation between the yield and the length of growing period of oats and barley was found.

Grain quality was higher in the same varieties in both management conditions. Significant positive correlations of quality characteristics between both management conditions were found except for barley protein content. No significant correlations were found between most of the tested traits in both systems. The only significant correlation was between yield and protein content of spring wheat in the organic and conventional trials, respectively  $-0.53$  ( $P<0.05$ ) and  $-0.82$  ( $P<0.01$ ). For oat and barley the correlation was not significant.

When comparing the management the conditions the quality characteristics of barley differed less than the others. For three years the average 1000 kernel weight was 46.6 g in both conditions.



Barley produced big kernels in organic conditions also in the low-yielding year 2007. Protein content was also similar – respectively 116 g kg<sup>-1</sup> in the organic and 120 g kg<sup>-1</sup> in the conventional trial. The results of volume weight followed the situation respectively by 684 and 685 g l<sup>-1</sup>.

In organic fertility management, oats also behaved similarly to barley – there were no significant differences. The tested years average 1000 kernel weight was 34.6 g in organic and 34.4 g in conventional trials and the same for every years. Husk content was also comparable by 262 and 265 g kg<sup>-1</sup> respectively. Average volume weight was somewhat lower in organic management by 470 g l<sup>-1</sup> compared to that of conventional management (503 g l<sup>-1</sup>). During the first two years, volume weight in the both management systems was equal. Significant differences in volume weight occurred in 2007, a year with an early drought, respectively 420 g l<sup>-1</sup> in organic and 512 g l<sup>-1</sup> in conventional trials. The average protein content was 120 g kg<sup>-1</sup> in organic and 129 g kg<sup>-1</sup> in conventional conditions. In 2005 and 2006 the content of protein was similar, but in 2007 significant differences occurred 117 in organic and 135 g kg<sup>-1</sup> in conventional trials.

Table 2. The grain quality characteristics of spring cereals in conventional and organic conditions

Characteristic	Conventional conditions				Organic conditions			
	Oat	Barley	Wheat	LSD <sub>0.05</sub>	Oat	Barley	Wheat	LSD <sub>0.05</sub>
Thousand kernel weight, g	34.4	46.6	34.2	1.8	34.6	46.6	36.5	1.9
Volume weight, g l <sup>-1</sup>	503	685	808	29	470	684	788	32
Protein content, g kg <sup>-1</sup>	129	120	151	10	120	116	127	10
Husk content, g kg <sup>-1</sup>	265	–	–	–	262	–	–	–
Falling number, s	–	–	296	–	–	–	285	–

Spring wheat reaction to different management systems was the most sensitive one. Average 1000 kernel weight was significantly bigger (36.5 g) in the organic trial compared to the conventional trial (34.2 g). Significant difference in volume weight was not measured. The biggest dissimilarity was found in protein content. It was significantly lower in the organic trial in all the tested years, being 127 g kg<sup>-1</sup> and 151 g kg<sup>-1</sup> respectively as the average. Spring wheat turned out to be the most sensitive to the shortage of nitrogen in the soil for the production of protein. Falling number values were not much influenced by the particular management system - 285 s in organic and 296 s in conventional trial.

During a year average oats showed the highest level of grain yield in organic conditions (4,059 kg ha<sup>-1</sup>), followed by barley (3,703 kg ha<sup>-1</sup>) and spring wheat (3,020 kg ha<sup>-1</sup>). It has also been a common experience in Norway that oats generally perform better than wheat with lower nutrient availability (Loes *et al.*, 2007). The grain yield of oat turned out to be 25% higher compared to that of wheat in organic trial. Nutrient requirements of oat are less than those of wheat (Forsberg and Reeves, 1995). Oats outyielded barley and wheat in organic trials in 2005 and 2007, but their yield was 5% lower than barley in 2006. This could be explained by the better tillering capacity of barley. The results of 3 years of trials show that some varieties were found to produce comparatively higher yields and better quality in organic conditions. To get the best possible yields on a given site, growers use varieties that are adapted to that particular environment and to nutrient levels, which fluctuate with the seasons (Lammerts van Bueren, 2003).

The decrease of the protein content of spring wheat by 16% was observed in organic trials compared to conventional trials. Previous studies have shown that the yield and protein content of wheat produced under organic conditions are often 20-40% lower than those achieved in conventional conditions (Taylor and Cormack, 2002; Mäder *et al.*, 2002). This may partially be due to on insufficient nitrogen supply during the later growth stages (Taylor and Cormarck, 2002). Cultivation of wheat in organic conditions is a challenge for a grower but has great value because of it can be utilized in multiple ways (Pedersen *et al.*, 2006).

## Conclusion

The results of the trials indicate that on fertile soil and after a suitable precrop (red clover in our trial) all the spring cereals produced comparatively high yields with good quality in organic conditions. Unfavourable weather conditions and an unsuitable precrop caused a significant yield

decrease. In terms of quality characteristics protein content and volume weight were the most influenced by the particular management system and the weather. In organic conditions the highest yielding was found in oats. In cultivating spring wheat high soil fertility is needed to produce a high yield with good quality.

### Acknowledgements

The project was supported within the state program "Agricultural applied research and development for 2004-2008" by the Estonian Ministry of Agriculture.

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## BIOĻĢISKĀS UN KONVENCIĀNĀLĀS AUDZĒŠANAS SISTĒMAS IETEKME UZ VASARĀJU GRAUDAUGU RAŽU UN KVALITĀTI

**Ingver A., Tamm I., Tamm Ü.**

Situācija lauksaimniecības sektorā pēdējo gadu laikā ir izmainījusies. Nozīmīgāka kļuvusi nepieciešamība palielināt ilgtspējību un aizsargāt vidi. Bioloģiskās lauksaimniecības platības Igaunijā palielinās.

Jogevas augu selekcijas institūtā tika iekārtoti lauka izmēģinājumi, lai salīdzinātu vasaras kviešu, miežu un auzu graudu ražu un kvalitātes pazīmes bioloģiskos un konvencionālos apstākļos. Katras graudaugu sugas 13 šķirnes tika pārbaudītas trīs gadu laikā (2005.-2007. g.). Izmēģinājumu rezultāti parādīja, ka visas vasarāju sugas bioloģiskos audzēšanas apstākļos spēja izveidot salīdzinoši augstas ražas. Ražīgākās bioloģiskajos izmēģinājumos bija auzas, tām sekoja mieži un kvieši. Lielākais ražas samazinājums (34%) bioloģiskos apstākļos salīdzinājumā ar konvencionālajiem apstākļiem bija vasaras kviešiem. Ražas samazinājums bija galvenokārt augu

barības vielu trūkuma dēļ. Laika apstākļi 2005. gadā bija graudaugiem labvēlīgāki. 2006. un 2007. gadā graudu ražas un kvalitātes samazināšanos izraisīja sausums. Miežu un auzu graudu kvalitāte abās audzēšanas sistēmās bija līdzīga; kviešiem bioloģiskos apstākļos veidojās lielāki graudi. Proteīna saturs bioloģiskos apstākļos samazinājās, lielākais samazinājums novērots kviešiem.

## THE NEW LITHUANIAN FIBRE FLAX VARIETY 'SNAIGIAI'

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### Abstract

Fibre flax (*Linum usitatissimum* L.) is the most important source of natural fibres for textile production in many countries where cotton is not grown. For 4 thousand years flax has been serving Lithuanians as raw material for clothing, food, medicine and other purposes. Linen (fabric from flax fibre) is highly hygroscopic, has high air permeability and heat conductivity, does not cause allergic reactions and is helpful in treating a number of allergic disorders. Because of exclusive value of this plant and interest of growers and processors, fibre flax has been bred in Lithuania since 1922. During this period eighteen fibre flax varieties have been developed.

The new fibre flax variety 'Snaigiai' (breeding line No. 2243-13) has been bred using the method of intervarietal hybridization. This breeding line was tested in the control nursery in 2001-2002 and in the preliminary variety testing trials in 2003. In the competitive variety trials 'Snaigiai' was tested in 2004-2005. It is a white flowering, moderately ripening, and lodging resistant variety. The seeds are brown, 1000 seed weight is 5.53 g. It exhibits high fibre quality and is suitable for textile production. 'Snaigiai' was bred by Dr. K. Bačelis. Since 2005 initial variety testing has been done by Dr. Z. Jankauskienė. Since 2007 the variety has been tested for DUS and VCU.

**Key words:** breeding, fibre flax, fibre quality, variety, yield.

### Introduction

*Linum* species belongs to the oldest arable crops. Even in ancient times fibre and oilseed were considered valuable agricultural products.

Flax (*Linum usitatissimum* L.) cultivation is considered a traditional branch of agriculture in Lithuania. For 4 thousand years flax has been serving Lithuanians as raw material for clothing, food, medicine and other purposes. For three centuries (XVII-XIX) flax was the main source of income for Lithuanian people. During all the periods of Lithuanian State development flax cultivation was one of the key branches of the crop production economy (Bražukienė, 2001).

Fibre flax breeding has been carried out in Lithuania since 1922 (Bačelis, 1998). Fibre flax breeding in Lithuania was started at the Breeding Station in Dotnuva, then continued at the Savitiškis Research Station, and since 1965 has been done at the Upytė Research Station of the Lithuanian Institute of Agriculture. The first flax breeder was Prof. D. Rudzinkas. At the beginning, flax varieties were bred by the method of individual selection. In 1932 synthetic flax breeding was initiated by crossing selected varieties. Since 1971 physical and chemical mutagens have been applied in flax breeding. From 1922 to 2005 eighteen fibre flax varieties were developed in our country (Bačelis, 2001).

In the first stages of breeding initial material for crossing must be evaluated. It has to be diverse and abundant and has to meet key requirements under local conditions (Rosenberg, 1995). The tested varieties or breeding lines, mutants that best meet the requirements are necessitated by for intervarietal hybridization with a view to developing novel breeding material.

The prime aim for fibre flax breeders is to increase fibre yield per hectare, but this characteristic has low heritability, and it is not easy to evaluate because it is largely influenced by the environment (Fouilloux, 1989). High fibre quality is one of the key requirements in fibre flax breeding for textile purposes (Хеллер и Рувльский, 2002). But the quality is yet less heritable than fibre productivity. Moreover, there seems to be negative genetic correlation between fibre

productivity and quality (Fouilloux, 1989). Furthermore, the new flax varieties should be resistant to lodging and diseases (Bačelis and Gruzdevienė, 2001; Trouve, 1996; Крылова *и др.*, 2002).

Therefore, it is not easy to breed fibre flax because in general, the important characteristics have poor heritability, are difficult to evaluate and need large quantity of seeds in field trials (Fouilloux, 1989; Keijzer and Metz, 1992).

Our aim is to develop novel high yielding fibre flax varieties, resistant to lodging, with a high fibre yield and quality, less susceptible to fungal diseases, with a moderately long vegetative growth period, well adapted to Lithuania's soil and climate conditions.

The hypothesis of the study is that the new variety will be high yielding, will have good fibre quality and will be less sensitive to lodging and diseases.

## Materials and Methods

The flax breeding was conducted on a Eutri-Endohypogleyic Cambisol (Buivydaitė et al., 2001). Flax was sown after winter wheat. Conventional cultivation practices were used. Fibre flax breeding was carried out according to the following scheme: 1) nurseries of initial material (collection, hybrids, mutants), 2) breeding nursery, 3) hybrid nursery, 4) selection nursery, 5) control nursery, 6) preliminary variety trials, 7) competitive variety trials. In the nursery of initial material the varieties and accessions were sown in plots of 0.2-1.0 m<sup>2</sup>, and in the breeding, selection and control nurseries – in the plots of 0.2-4.0 m<sup>2</sup>. In the control nursery flax was sown with 4-5 replications. Initial and competitive variety trials involved 3-4 replications. The size of a record plot was 11.2 and 16.0 m<sup>2</sup>, respectively. All field trials were conducted (with a few modifications) in compliance with the published methodology (Методические, 1978; Рогаш *и др.*, 1987).

In the competitive and initial variety trials the plots were sown by the sowing machine SNL-16 at a seed rate of 25 million seed per hectare, 10 cm space between rows. In the other nurseries the plots were sown manually at a seeds rate of 22 million seeds per ha. Insecticides were sprayed against flax flea beetles and herbicides were used to control weeds.

During the vegetative growth period phenological observations were done, lodging resistance and fungal diseases on the natural background were assessed (Лошакова *и др.*, 2002). Flax was pulled at the stage of early yellow ripeness, threshed by a MS thresher, the stems were retted in warm (33-37°C) water, the stems were scutched by a scutching tool SMT-200, fibre was hacked by combs number 9 and 13. The number of long fibre was determined in the laboratory, flexibility by the device G-2, the strength of fibre by the device DK-60 and thinness (divisibility) – following the special methodology by counting separate fibres in a fibre sample, the length of which is 1 cm, mass 10 mg. Long fibre rupture length (in km) was calculated using the formula (Методики, 1961):

$$RL = 0.1 * F + 0.2 * S + 0.013 * D + 2.1$$

Where: RL – Long fibre rupture length, km;

F – Long fibre flexibility, mm;

S – Long fibre strength, kg force;

D – Long fibre divisibility, units;

0.1; 0.2; 0.013 and 2.1 are constants.

Morphological analysis of plants was carried out. Stem, seed and fibre yield was evaluated using analysis of variance. For calculations we used the statistical software developed at the Lithuanian Institute of Agriculture (Tarakanovas and Raudonius, 2003).

Meteorological conditions during the period 1979-2005 were not favourable every year and had a marked effect on the yield. The years 1990, 1991, 1993, 1996, 1997 and 2000 were favourable for flax growing and a satisfactory seed and fibre yield was obtained in these years. In the years 1992, 1994 and 1999 hot and dry weather in June and July markedly a declining flax yield. In the year 1998 very abundant rainfall in July lodged the flax plants, which resulted in a marked deterioration of flax produce quality. In 2001 the weather conditions were adverse, especially in the second half of the growing season. Heavy rainfall lodged flax crops. The year 2002 was characterised by a shortage of moisture during the growing season. In 2003 because of the lack of rainfall in the first

half of the growing season, flax did not develop well, the end of the growing season was rainy and the flax stand was partially lodged.

## Results and Discussion

The new fibre flax variety 'Snaigiai' (breeding line No. 224313) has been bred at the Upytė Research Station of the Lithuanian Institute of Agriculture using the method of intervarietal hybridization. In 1979 the female variety 'T-10' (of Russian origin) was crossed with flax variety 'VNIL-6' (developed in Russia). After investigations on hybrids and selection nurseries, this breeding line was tested in the control nursery in 2001-2002 (Table 1).

Table 1. Some characteristics of the new fibre flax variety 'Snaigiai' (breeding line No. 2243-13) in the control nursery. Upytė, averaged data 2001-2002

Indices	'Snaigiai' (2243-13)	'Ariane' (standard)	'Snaigiai' (2243-13) compared to 'Ariane'
Stem yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.26)	4.75	4.63	102.6
Seed yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.05)	0.58	0.53	109.4
Long fibre yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.12)	0.65	0.61	106.6
Long fibre content, % (LSD <sub>05</sub> 2.5)	14.0	13.3	105.7
Long fibre flexibility, mm	41.9	37.4	112.0
Long fibre strength, kg F	14.9	11.4	130.8
Plant height, cm	88.3	74.3	118.9
Growing period, days	89.5	89.5	100.0
Resistance to lodging, points (9=not lodged)	8.6	8.1	105.6

In the initial variety trials 'Snaigiai' was tested in 2003, in the competitive variety trials in 2004-2005 (the results are presented in Table 2).

Table 2. Some agrobiological characteristics of the new fibre flax variety 'Snaigiai'. Upytė, competitive variety trials. Averaged data 2004-2005

Indices	'Snaigiai'	'Hermes' (standard)	'Belinka' (standard)	'Snaigiai' compared to 'Hermes'	'Snaigiai' compared to 'Belinka'
Stem yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.67)	6.30	5.85	-	107.7	-
Seed yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.11)	0.61	0.74	-	82.4	-
Long fibre yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.19)	1.65	1.57	-	105.1	-
Long fibre content, %	26.2	26.5	16.3	98.9	160.7
Long fibre flexibility, mm	46.6	38.1	42.5	122.3	109.7
Long fibre strength, kg F	13.9	15.6	14.1	89.1	98.6
Long fibre divisibility, units	283	248	246	114.1	115.5
Long fibre rupture length, km	13.2	12.3	12.4	107.3	106.5
Long fibre quality number	12.4	11.9	12.6	104.2	98.4
Plant height, cm	80.1	73.8	-	108.5	-
Technical stem length, cm	70.6	69.3	-	101.9	-
Number of capsules per plant	1.25	1.80	-	69.4	-
1000 seed weight, g	5.53	5.59	-	98.9	-
Growing period, days	89	91	-	97.8	-
Resistance to lodging, points (9=not lodged)	8.90	8.95	-	99.4	-
Disease incidence on stems, %	12.8	37.0	-	34.6	-

Since 2007 the variety has been tested for DUS and VCU. K. Bačelis is the author of 'Snaigiai'. Since 2005 competitive variety testing and initial seed multiplication have been carried out by Z. Jankauskienė. VCU results for the new fibre flax variety 'Snaigiai' in Pasvalys and Plungė State Variety Testing Stations in 2007 are presented in Tables 3 and 4. In the control nursery in 2001-2002 (Table 1), 'Snaigiai' produced slightly larger stem, seed and long fibre yield when compared to the standard variety 'Ariane'. The plants of 'Snaigiai' were taller than those of 'Ariane'. Flax

‘Snaigiai’ had slightly higher fibre content, more flexible and stronger fibre compared to ‘Ariane’ in the control nursery. The duration of the growing season for both varieties tested was the same. Average data of 2 years of competitive trials in 2004-2005 (Table 2) showed that the new variety ‘Snaigiai’ had taller plants, higher stem and long fibre yield, more flexible and thinner fibre (higher divisibility data), a higher quality number and rupture length, compared to the standard variety ‘Hermes’. Plants of ‘Snaigiai’ were more resistant to *Fusarium spp.* and *Colletotrichum lini*. Fibre quality of the variety ‘Belinka’ is known as the standard of good quality, thus for the evaluation of fibre quality in our trials the variety ‘Belinka’ was chosen as a reference (standard) variety. Compared to the data of ‘Belinka’ (quality standard), a flax of the new variety ‘Snaigiai’ had higher fibre content, more flexible fibre, higher fibre divisibility and rupture length. Investigations carried out at the LIA Upytė Research Station suggest that ‘Snaigiai’ is a white flowering, high fibre yielding, moderately late ripening, lodging resistant variety. Fibre quality is satisfactory and suitable for textile production. Seeds are brown, 1000 seed weight – 5.53 g. VCU testing results for the new fibre flax variety ‘Snaigiai’ at the Plungė State Variety Testing Station (western part of Lithuania, region with higher rainfall) in 2007 (Table 3) showed that flax of this variety had lower stem yield, but due to higher long fibre content, the long fibre yield was higher compared to that of the standard variety ‘Kastyčiai’.

Table 3. VCU testing results for the new fibre flax variety ‘Snaigiai’. (Plungė. VCU trials, 2007)

Indices	‘Snaigiai’	‘Kastyčiai’ (standard)	‘Snaigiai’ compared to ‘Kastyčiai’
Stem yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.30)	6.08	7.16	84.9
Long fibre yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.06)	1.16	1.08	106.9
Long fibre content, % (LSD <sub>05</sub> 2.37)	25.5	20.3	125.7
Long fibre flexibility, mm (LSD <sub>05</sub> 2.08)	31.9	35.5	89.8
Long fibre strength, kg F (LSD <sub>05</sub> 0.91)	13.5	12.7	105.7
Long fibre divisibility, units	194	181	107.6
Long fibre rupture length, km	10.54	10.50	99.7
Long fibre quality number (LSD <sub>05</sub> 0.53)	11.0	10.9	101.1
Plant height, cm	75	76	98.7
Growing period, days	91	89	102.2
Resistance to lodging, points (9=not lodged)	9.0	9.0	100.0

The fibre of ‘Snaigiai’ was less flexible than that of ‘Kastyčiai’, but that of the new variety was firmer (by 5.7 %) and finer (by 7.6 %). Long fibre rupture length, quality number, plant height, and resistance to lodging were very similar for both varieties tested.

Table 4. VCU testing results for the new fibre flax variety ‘Snaigiai’. (Pasvalys. VCU trials, 2007)

Indices	‘Snaigiai’	‘Kastyčiai’ (standard)	‘Snaigiai’ compared to ‘Kastyčiai’
Stem yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.75)	7.55	6.18	122.2
Long fibre yield, t ha <sup>-1</sup> (LSD <sub>05</sub> 0.09)	1.12	1.05	106.5
Long fibre content, % (LSD <sub>05</sub> 1.75)	20.9	23.9	87.6
Long fibre flexibility, mm (LSD <sub>05</sub> 1.67)	30.6	25.2	121.4
Long fibre strength, kg F (LSD <sub>05</sub> 1.36)	10.6	10.5	101.0
Long fibre divisibility, units	189	131	144.2
Long fibre rupture length, km	8.3	9.7	85.3
Long fibre quality number (LSD <sub>05</sub> 0.64)	11.5	9.8	117.9
Plant height, cm	75	71	105.6
Growing period, days	71	75	94.7
Resistance to lodging, points (9=not lodged)	9.0	9.0	100.0

The trials in the Pasvalys State Variety Testing Station (north-east part of Lithuania) in 2007 (Table 4) showed that the new fibre flax variety ‘Snaigiai’ had a higher stem yield (by 22.2 %), but the content of long fibre in the stems was lower compared to that of the standard variety ‘Kastyčiai’. The fibre of ‘Snaigiai’ was more flexible and thinner (higher divisibility data), and was evaluated under a higher quality number. The plants of ‘Snaigiai’ grew taller (by 4 cm), ripened 4 days earlier than the plants of the standard variety. Both varieties showed good results of lodging resistance.

The results in both State Variety Testing Stations could differ due to the different soil and climatic conditions. This confirms that flax yield and its quality are not stable and largely depend on the local conditions.

The new fibre flax variety 'Snaigiai' was found to be superior to different standard varieties.

## Conclusions

In the control nursery in 2001-2002 'Snaigiai' (breeding line No. 2243-13), produced a slightly longer stem, more seed and long fibre yield compared to the standard variety 'Ariane'. The plants of 'Snaigiai' were taller, had slightly higher fibre content and more flexible and stronger fibre. The duration of the growing period for 'Snaigiai' and 'Ariane' was the same.

In the competitive variety trials the new fibre flax variety 'Snaigiai' had taller plants, longer stem and long fibre yield, more flexible and thinner fibre (higher divisibility data), a higher quality number and rupture length compared to the standard variety 'Hermes'. The plants of 'Snaigiai' were more resistant to *Fusarium spp.* and *Colletotrichum lini*. When compared to 'Belinka' (quality standard), flax of the new variety 'Snaigiai' had higher fibre content, more flexible fibre, better fibre divisibility and rupture length.

VCU tests in 2007 showed that in the Plungė region the new fibre flax variety 'Snaigiai' had a higher long fibre content and yield, stronger and finer fibre, and in Pasvalys region taller plants, longer stem and long fibre yield, more flexible and thinner fibre (higher divisibility data), and a higher quality number compared to the standard variety 'Kastyčiai'.

'Snaigiai' (breeding line No. 2018-8) is a white flowering, high fibre yielding, moderately late ripening, lodging resistant variety. Fibre quality is satisfactory and suitable for textile production. The seeds are brown, 1000 seed weight is approximately 5.53 g.

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## JAUNA LIETUVAS ŠKIEDRAS LINU ŠKIRNE 'SNAIGAI'

**Jankauskienė Z., Bačelis K.**

Škiedras lini (*Linum usitatissimum* L.) ir nozīmīgākais dabīgo šķiedru nodrošinātājs tekstilrūpniecībā daudzās valstīs, kurās netiek audzēta kokvilna. Četrus tūkstošus gadu Lietuvā lini tika izmantoti apģērbam, pārtikai, medicīnai un citām vajadzībām. Lina audums (audums no linu šķidrām) ir izcili higroskopisks, tam piemīt gaisa caurlaidība un siltuma izolācija, tas neizraisa alerģiskas reakcijas un var tikt izmantots daudzu alerģisku traucējumu ārstēšanā. Tā kā augam piemīt izcila vērtība, kā arī balstoties uz audzētāju un pārstrādātāju ieinteresētību, šķiedras lini Lietuvā tiek selekcionēti kopš 1922. Astoņpadsmit šķiedras linu šķirnes ir izveidotas kopš tā laika. Jaunā šķiedras linu šķirne 'Snaigai' (līnija Nr. 2243-13) tika izveidota izmantojot starpšķirņu hibridizāciju. Selekcijas līnija tika pārbaudīta kontroles audzētavā 2001.-2002., iepriekšējā šķirņu pārbaudē – 2003.gadā. Salīdzinošajā šķirņu pārbaudē 'Snaigai' tika pārbaudīta 2004.-2005. Tā ir baltziedu, vidēji agrīna, veldres izturīga šķirne. Sēklas ir brūnas, 1000 sēklu svars ir 5.53 g. Šķiedras kvalitāte ir augsta un ir piemērota tekstilrūpniecībai. Dr. K. Bačelis ir izveidojis šķirni 'Snaigai'. Kopš 2005.gada šķirņu pārbaudi šķirnei veica Dr. Z. Jankauskienė. Kopš 2007. gada šķirne nodota AVS un SĪN pārbaudēm.

## THE PATH ANALYSIS OF YIELD TRAITS IN SUNFLOWER (*Helianthus annuus* L)

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### Abstract

Plant breeders have always tried to know that which characters contribute more in the seed yield that is a quantitative character influenced highly from environment and their relationships. Path coefficient analysis helps the breeders to explain the direct and indirect effects; hence it has been extensively used in breeding works by various researchers. The research covering yield performance and the path analysis of hybrids in the trials at the National Sunflower Research Project was conducted in Edirne province, where has 20% of the sunflower production in Turkey. The totals of 2932 sunflower hybrids were tested in 118 trials in this research. The 1000 seed weight gave the highest contribution to breeding for higher yield, and head diameter and plant height followed it respectively regarding to contribution to seed yield based on path and simple correlation analysis both in dry and rainy growing seasons.

**Key words:** sunflower, hybrid, seed yield, yield traits, path analysis.

### Introduction

Seed yield is a quantitative character, which is influenced more from climate and environmental factors in sunflower because of being controlled large number of genes. To increase the yield, the study of direct and indirect effects of yield components provides the basis for successful breeding program and hence the problem of yield increase can be more effectively tackled based on the performance of yield components and selection for closely related traits (Fehr, 1993). Head diameter, 1000 seed weight, plant height are valuable yield parameters to determine for yield improvement in the sunflower (Miller and Fick, 1997).

The use of simple correlation analysis could not fully explain the relationships among yield characteristics. Path coefficient analysis helps the breeder(s) to explain the direct and indirect effects for a more and complete determination of the impact of independent variable on dependent one among important yield traits (Singh and Chaudhary, 1979). Therefore, path coefficient analysis has extensively been used by many researchers (Kaya and Atakisi, 2003; Kaya *et al.*, 2003; Vidhyavathi, *et al.*, 2005; Göksoy and Turan, 2007). This research was conducted to determine the direct and indirect effects of yield traits on the sunflower yield by path analysis in conducted trials over many years in dry (1999-2001) and in rainy seasons (2002-05)) in Edirne, Turkey.



## Materials and Methods

The experimental hybrids developed by crossing female CMS and restorer lines and five control hybrids that have highest selling market share in Turkey and existed each year in the trials used in the research. The research was conducted in the Trakya Agricultural Research Institute fields in Edirne province which has the 20% of sunflower production proportion in Turkey between 1999 and 2005 as part of Turkish National Sunflower Research Project. The total of 2932 sunflower hybrids were tested in 118 trials in this project (635 hybrids in 26 yield trials in 1999, 650 hybrids in 23 trials in 2000, 457 hybrids in 17 trials in 2001, 365 hybrids in 15 trials in 2002, 176 hybrids in 8 trials in 2003, 295 hybrids in 13 yield trials in 2004 and 355 hybrids in 16 trials in 2005).

The experiments were conducted based on the Randomized Complete Block Design with three replicates. The TARPOGEN statistical package (Ozcan and Acikgoz, 1999) was used to analyze these relationships with detailed examination by path analysis (Singh and Chaudhary, 1979). The three rows plots were 6-m long with 70 x 35 cm plant spacing. The middle row was harvested and the border rows were discarded, and the plot size was 3.78 m<sup>2</sup> at harvest. Seed yield (SY) (kg ha<sup>-1</sup>), 1000 seed weight (TSW) (g), flowering (FP) and physiological maturity period (PM) (day), plant height (PH) and head diameter (HD) (cm), oil (OC) and husk content (HC) (%) were measured.

## Results and Discussion

Highly significant and positive correlations of SY with TSW were found in all years except in 2003. HD and PH were also significantly correlated with SY in the dry period (1999-2001). OC were also positive and significant in 2001 and 2004, both HC only in 2000 and 2001 (Table 1, 3, 5, 7, 9, 11, 13).

Table 1: Correlation values of sunflower hybrids in 1999.

	Seed Yield	1000 SW	Husk C.	Flw P.	P. Mat.	P. Height	Head Diam.
Seed Yield	1.000						
1000 S Weight	0.324**	1.000					
Husk Content	-0.014ns	0.105**	1.000				
F. period	-0.047ns	-0.101**	-0.035ns	1.000			
Phy. Maturity	0.073*	-0.019ns	-0.019ns	0.384**	1.000		
Plant Height	0.310**	0.302**	0.143**	0.138**	0.240**	1.000	
Head Diameter	0.355**	0.252**	-0.008ns	0.040ns	0.162**	0.244**	1.000

\*\* = Significant at 1 % level, \* = Significant at 5 % level, ns= Non Significant

FP and PM had generally non-significant relationships based on correlation analysis. FP was negatively correlated with SY in the dry seasons so it meant that earlier hybrids got higher yields. Since the simple correlation coefficients did not give clear information about the interrelationship between the causal and resultant variables, the correlation coefficient estimates were partitioned into direct and indirect effects to establish the intensity of effects of independent variables on dependent ones in path analysis. The path analysis of yield traits in the research is given in Table 2, 4, 6, 8, 10, 12 and 14.

Table 2: The path (p) coefficients and percentages (%) in seed yields of hybrids in 1999.

	1000 S W		Husk Content		Flower. period		Phy. Maturity		Plant Height		Head Diameter	
	P	%	P	%	P	%	P	%	P	%	p	%
TSW	<u>0.197</u>	<u>58.4</u>	0.021	17.3	-0.020	14.4	-0.004	2.8	0.060	17.1	0.050	13.8
HC	-0.007	2.0	<u>-0.065</u>	<u>54.1</u>	0.002	1.7	0.001	0.9	-0.009	2.7	0.001	0.1
FP	0.007	2.2	0.003	2.2	<u>-0.073</u>	<u>52.6</u>	-0.028	20.5	-0.010	2.9	-0.003	0.8
PM	0.000	0.1	0.000	0.2	0.005	3.6	<u>0.013</u>	<u>9.6</u>	0.003	0.9	0.002	0.6
PH	0.062	18.2	0.029	24.5	0.028	20.3	0.049	35.9	<u>0.204</u>	<u>58.5</u>	0.050	13.8
HD	0.064	19.1	-0.002	1.7	0.010	7.5	0.041	30.3	0.062	17.9	<u>0.256</u>	<u>70.9</u>

\*Bold lines direct effect of trait.

The 1000 seed weight had the highest direct effect amount (84.4% in 2005) on setting of yield and plant height followed by 82.5% in the 2002 research.

FP had a higher influence on seed yield due to the dry spring years in 1999-2001 than PM's ones. The direct effect of important yield traits such as TSW and PH was less in dry years so they affected positively seed yield utilizing over other traits.

Table 3: Correlation values of sunflower hybrids in 2000.

	S. Yield	Oil Cont.	1000 S W	Husk Con.	Flowering	Phy Mat.	Plant Hgt	Hd Diam.
Seed Yield	1.000							
Oil Content	0.017ns	1.000						
1000 S Weight	0.313**	-0.151**	1.000					
Husk Content	0.195**	-0.514**	0.234**	1.000				
Flow. period	0.068*	0.091**	0.013ns	0.009ns	1.000			
Phy. Maturity	0.061ns	0.073*	0.073*	0.078*	0.254**	1.000		
Plant Height	0.323**	0.094**	0.173**	0.094**	0.013ns	0.082**	1.000	
Head Diameter	-0.017ns	-0.016ns	0.043ns	-0.034ns	-0.056ns	0.109**	0.267**	1.000

Table 4: The path (p) coefficients and percentages (%) in seed yields of hybrids in 2000.

	Oil Cont.		1000 S W		Husk Con.		Flowering		Phy Mat.		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	P	%	P	%	P	%
OC	<u>0.105</u>	<u>40.9</u>	-0.016	4.5	-0.054	17.9	0.010	13.9	0.008	9.2	0.010	2.7	-0.002	0.9
TSW	-0.037	14.4	<u>0.246</u>	<u>69.7</u>	0.058	19.0	0.003	4.5	0.018	21.3	0.043	11.4	0.011	5.7
HC	-0.083	32.2	0.038	10.7	<u>0.162</u>	<u>53.3</u>	0.002	2.2	0.013	15.1	0.015	4.1	-0.005	2.9
FP	0.004	1.6	0.001	0.2	0.000	0.1	<u>0.045</u>	<u>66.1</u>	0.012	13.7	0.001	0.2	-0.003	1.4
PM	0.000	0.1	0.000	0.1	0.000	0.1	0.000	0.4	<u>-0.001</u>	<u>1.3</u>	0.000	0.1	0.000	0.1
PH	0.026	10.2	0.048	13.7	0.026	8.6	0.004	5.2	0.023	27.3	<u>0.280</u>	<u>75.1</u>	0.075	39.8
HD	0.002	0.6	-0.004	1.1	0.003	1.0	0.005	7.6	-0.010	12.1	-0.025	6.7	<u>-0.093</u>	<u>49.4</u>

Table 5: Correlation values of sunflower hybrids in 2001.

	S. Yield	Oil Cont.	1000 SW	Husk C.	Flowering	Phy Mat.	Plant Hgt	Hd Diam.
Seed Yield	1.000							
Oil Content	0.323**	1.000						
1000 S W	0.497**	0.311**	1.000					
Husk Cont	0.104*	-0.547**	0.165**	1.000				
F. period	-0.167**	0.034ns	-0.154**	-0.070ns	1.000			
Phy. Maturi.	0.078ns	0.082ns	0.016ns	-0.019ns	0.411**	1.000		
Plant Height	0.242**	0.227**	0.380**	0.131**	0.073ns	0.064ns	1.000	
Head Diam.	0.131**	0.160**	0.159**	0.115*	-0.116**	-0.005ns	0.225**	1.000

Table 6: The path (p) coefficients and percentages (%) in seed yields of hybrids in 2001.

	Oil Cont.		1000 S W		Husk Con.		Flowering		Phy Mat.		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	P	%	p	%	P	%
OC	<u>0.344</u>	<u>57.9</u>	0.107	21.2	0.188	38.4	0.012	4.0	0.028	12.7	0.078	28.1	0.055	30.3
TSW	0.100	16.8	<u>0.321</u>	<u>63.4</u>	0.053	10.8	-0.049	16.8	0.005	2.3	0.122	43.9	0.051	27.9
HC	-0.126	21.2	0.038	7.5	<u>0.230</u>	<u>46.9</u>	-0.016	5.4	-0.004	1.9	0.030	10.9	0.026	14.5
FP	-0.006	0.9	0.026	5.0	0.012	2.3	<u>-0.165</u>	<u>56.2</u>	-0.068	30.6	-0.012	4.4	0.019	10.5
PM	0.010	1.6	0.002	0.4	-0.002	0.4	0.048	16.1	<u>0.116</u>	<u>51.9</u>	0.007	2.7	-0.001	0.3
PH	0.005	0.9	0.009	1.7	0.003	0.6	0.002	0.6	0.001	0.7	<u>0.023</u>	<u>8.1</u>	0.005	2.8
HD	-0.004	0.7	-0.004	0.8	-0.003	0.6	0.003	1.0	0.000	0.1	-0.006	2.0	<u>-0.025</u>	<u>13.7</u>

Table 7: Correlation values of sunflower hybrids in 2002.

	S. Yield	Oil Cont.	1000 SW	Flowering	Phy Mat	Plant Height	Head Diam.
Seed Yield	1.000						
Oil Cont	0.080ns	1.000					
1000 S W	0.231**	-0.067ns	1.000				
F. period	0.004ns	0.093ns	-0.321**	1.000			
Phy. Mat.	0.134**	0.311**	-0.076ns	0.730**	1.000		
Plant Hght	0.297**	0.027ns	0.129**	0.084ns	0.168**	1.000	
Head Dim	0.099*	0.120*	0.226**	-0.030ns	0.085ns	0.118*	1.000

Table 8: The path (p) coefficients and percentages (%) in seed yields of hybrids in 2002.

	Oil Cont.		1000 SW		Flowering		Phy Mat		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	P	%	P	%
OC	<u>0.050</u>	<u>43.3</u>	-0.003	1.3	0.005	2.0	0.016	6.4	0.001	0.4	0.006	6.1
TSW	-0.013	11.1	<u>0.193</u>	<u>74.8</u>	-0.062	26.0	-0.015	6.1	0.025	8.1	0.044	44.1
FP	-0.005	4.4	0.018	6.8	<u>-0.055</u>	<u>23.1</u>	-0.040	16.5	-0.005	1.5	0.002	1.7
PM	0.040	34.7	-0.010	3.9	0.095	39.9	<u>0.130</u>	<u>53.4</u>	0.022	7.1	0.011	11.2
PH	0.007	5.8	0.033	12.7	0.021	8.9	0.042	17.4	<u>0.253</u>	<u>82.5</u>	0.030	30.3
HD	0.001	0.7	0.002	0.6	0.000	0.1	0.001	0.2	0.001	0.3	<u>0.007</u>	<u>6.7</u>

Table 9: Correlation values of sunflower hybrids in 2003.

	S. Yield	Oil Cont.	1000 SW	Flowering	Phy Mat	Plant Height	Head Diam.
Seed Yield	1.000						
Oil Content	0.037ns	1.000					
1000 S Wgt	0.055ns	-0.126ns	1.000				
Flw. Period	0.108ns	-0.059ns	-0.229**	1.000			
Phy. Matur.	0.157*	0.250**	-0.158*	0.670**	1.000		
Plant Hght	0.095ns	-0.233**	0.282**	0.078ns	-0.019ns	1.000	
Head Diam.	0.097ns	-0.105ns	0.148*	-0.012ns	-0.013ns	0.300**	1.000

The path coefficient analysis further indicated that the positive direct effect of OC was masked by the negative indirect effect of HC in the dry years, whereas there was positive and direct effect of some other characteristics including TSW, FP and PM in rainy seasons. The direct effect of FP was also masked mainly by PM especially in the rainy years, but the indirect effect of FP over PM was not higher like PM's.

Table 10: The path (p) coefficients and percentages (%) in the seed yields of hybrids in 2003.

	Oil Cont.		1000 SW		Flowering		Phy Mat		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	p	%	P	%
OC	<u>0.032</u>	<u>32.1</u>	-0.004	3.4	-0.002	1.4	0.008	4.5	-0.008	6.5	-0.003	3.1
TSW	-0.007	7.2	<u>0.057</u>	<u>48.3</u>	-0.013	9.4	-0.009	5.0	0.016	14.0	0.008	7.8
FP	-0.001	1.2	-0.005	4.0	<u>0.021</u>	<u>14.9</u>	0.014	7.8	0.002	1.4	0.000	0.2
PM	0.037	36.5	-0.023	19.5	0.098	70.2	<u>0.146</u>	<u>81.5</u>	-0.003	2.4	-0.002	1.8
PH	-0.015	15.0	0.018	15.4	0.005	3.6	-0.001	0.7	<u>0.065</u>	<u>56.1</u>	0.019	17.9
HD	-0.008	7.9	0.011	9.4	-0.001	0.7	-0.001	0.6	0.023	19.6	<u>0.075</u>	<u>69.3</u>

Table 11: Correlation values of sunflower hybrids in 2004.

	S. Yield	Oil Cont.	1000 SW	Flowering P.	Phy Mat.	Plant Height	Head Diam.
Seed Yield	1.000						
Oil Content	0.148*	1.000					
1000 S Wgt	0.349**	0.158**	1.000				
F. period	0.484**	0.149**	-0.224**	1.000			
Phy. Mat.	0.098ns	0.043ns	0.041ns	0.104ns	1.000		
Plant Height	0.081ns	0.280**	-0.168**	0.418**	-0.087ns	1.000	
Head Diam.	0.043ns	-0.040ns	-0.015ns	-0.027ns	-0.161**	0.113*	1.000

Table 12: The path (p) coefficients and percentages (%) in seed yields of hybrids in 2004.

	Oil Cont.		1000 SW		Flowering P.		Phy Maturity		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	p	%	P	%
OC	<u>0.014</u>	<u>6.4</u>	0.002	0.4	0.002	0.3	0.001	0.5	0.004	0.8	-0.001	0.5
TSW	0.075	33.7	<u>0.470</u>	<u>73.8</u>	-0.105	13.2	0.020	15.6	-0.079	16.5	-0.007	5.6
FP	0.095	43.0	-0.143	22.4	<u>0.638</u>	<u>79.8</u>	0.066	53.1	0.267	55.6	-0.018	14.0
PM	0.001	0.1	0.001	0.1	0.002	0.2	<u>0.015</u>	<u>11.7</u>	-0.001	0.3	-0.002	1.9
PH	-0.033	15.1	0.020	3.2	-0.050	6.2	0.010	8.3	<u>-0.119</u>	<u>24.9</u>	-0.014	10.8
HD	-0.003	1.5	-0.001	0.2	-0.002	0.3	-0.014	10.8	0.010	2.0	<u>0.084</u>	<u>67.2</u>

Table 13: Correlation values of sunflower hybrids in 2005.

	S. Yield	Oil Cont.	1000 SW	Flowering	Phy Mat	Plant Height	Head Diam.
Seed Yield	1,000						
Oil Cont	0,007ns	1,000					
1000 S W	0,290**	0,245**	1,000				
F. period	0,282**	0,003ns	0,032ns	1,000			
Phy. Mat.	0,438**	0,137**	0,021ns	0,362**	1,000		
Plant Hght	0,189**	0,027ns	0,096ns	0,242**	0,179**	1,000	
Head Dim	0,153**	-0,053ns	0,118*	0,022ns	0,123*	0,002ns	1,000

The data further indicated that the positive effect of TSW on seed yield was realized generally directly both in dry and rainy seasons and TSW has the highest contribution on forming of SY among yield components. TSW was utilized also from the positive and indirect effects mostly of PH. Plant height and head diameter were other contributing traits on the setting up of SY in the research. The positive direct effects of TSW, PH and HD established in this study supports the statements of Kaya and Atakisi (2003), Kaya *et al.* (2003), Vidhyavathi, *et al.* (2005), Göksoy and Turan (2007) that breeding for increased SY seems to the most effective method to get higher sunflower yields.

Table 14: The path (p) coefficients and percentages (%) in the seed yields of hybrids in 2005.

	Oil Cont.		1000 SW		Flowering		Phy Mat		Plant Height		Head Diam.	
	P	%	P	%	P	%	P	%	p	%	P	%
OC	<u>-0.116</u>	<u>47.2</u>	-0.028	8.2	0.000	0.1	-0.016	3.4	-0.003	1.6	0.006	4.0
TSW	0.072	29.1	<u>0.293</u>	<u>84.4</u>	0.009	3.3	0.006	1.3	0.028	14.4	0.034	22.5
FP	0.000	0.1	0.004	1.1	<u>0.115</u>	<u>40.8</u>	0.042	8.9	0.028	14.3	0.003	1.7
PM	0.053	21.5	0.008	2.3	0.140	49.5	<u>0.386</u>	<u>82.2</u>	0.069	35.3	0.048	31.1
PH	0.002	0.1	0.006	1.9	0.016	5.7	0.012	2.5	<u>0.067</u>	<u>34.2</u>	0.000	0.1
HD	-0.003	1.3	0.007	2.1	0.001	0.5	0.008	1.6	0.000	0.1	<u>0.062</u>	<u>40.5</u>

### Conclusion

By comparing the correlation coefficient values of six independent variables against the seed yield, significant differences became evident. TSW had a highly significant association with plant yield. By partitioning the mutual relationship among the independent variables into direct and indirect effects on yield, it became apparent that TSW, PH and HD were the main characteristics that

exhibited the highest direct effect on seed yield both in dry and rainy growing seasons. Therefore, both these traits seem to be good selection criteria to improve sunflower seed yield.

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## SAULESPUĶU (*Helianthus annuus* L) RAŽAS PAZĪMJU KORELĀCIJU ANALĪZE

**Kaya Y., Evcı G., Pekcan V., Gucer T., Durak S., Yilmaz M.I.**

Saulespuķu selekcionāriem jāzina, ka pazīmes, kuras galvenokārt nosaka sēklu ražu, ir kvantitatīvas un tās ietekmē vide, kā arī vides un pazīmes mijiedarbība. Korelācijas koeficientu analīze palīdz selekcionāram izskaidrot tiešās un netiešās ietekmes, tādejādi šī metode plaši tiek izmantota selekcijas darbā. Pētījums veikts, izmantojot ražas datus un korelāciju analīzes Nacionālā Saulespuķu pētījumu projekta izmēģinājumā iekļautajiem hibrīdiem. Projekts notiek Edirne provincē, kurā ražo 20 % no Turcijas saulespuķu produkcijas. Kopumā 2932 saulespuķu hibrīdi tika pētīti 118 izmēģinājumos šī pētījuma gaitā. Augstražīgu hibrīdu selekcijā pazīmei - 1000 graudu svars - bija būtiskākā nozīme. Ziedkopas diametrs un auga garums gan sausos, gan lietainos augšanas apstākļos bija nākamie nozīmīgākie, balstoties uz vienkāršām korelācijas analīzēm.

## MOLECULAR MARKER-BASED CHARACTERIZATION OF BARLEY POWDERY MILDEW *MLO* RESISTANCE LOCUS IN EUROPEAN VARIETIES AND BREEDING LINES

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## Abstract

Powdery mildew is an economically important barley disease, caused by a fungal pathogen *Blumeria (Erysiphe) graminis* f.sp. *hordei*. While the pathogen is relatively easily controlled by fungicides, it may represent a serious threat for barley produced in low input and organic agriculture. Alternatively, the disease can be controlled using resistance genes that are either specific for certain fungal pathotypes or confer resistance to a broad range of pathotypes. Naturally occurring and induced recessive mutations in the *Mlo* gene provide race nonspecific resistance that has been effective against almost all powdery mildew pathotypes. The *Mlo* gene has been cloned and several resistance alleles have been characterized at the DNA sequence level. Here we report the

identification of a novel *mlo* allele in a mutant in the cultivar Maja, which represents an amino acid Gly318 to Arg exchange. Available CAPS (Cleaved Amplified Polymorphic Sequence) and indel markers for *mlo-5*, *mlo-9* and *mlo-11* alleles were used to screen a selection of European barley varieties, as well as Latvian and foreign breeding lines for the presence of *mlo* resistance. The results of our study demonstrate the advantages of using molecular markers for the selection of mildew resistant hybrids.

**Key words:** barley, molecular marker, *Mlo* gene, Cleaved Amplified Polymorphic Sequences, CAPS, indel

## Introduction

The barley crop was the fourth largest in the world by production in 2005 (FAO, <http://www.fao.org>) with the end uses being mostly food, feed and malt production. The most important factors affecting barley production are abiotic stresses, such as drought, cold and soil salinity (Stanca, 2003), as well as biotic stresses, such as fungal pathogens (Weibull *et al.*, 2003). The control of pathogens is usually achieved by the application of fungicides during the growing season, but such practice has associated cost penalties as well as often unpredictable effects on wildlife biodiversity. Moreover, the use of pesticides is unacceptable in organic agriculture. The combination of these factors requires the development of barley varieties that combine high yield and defined quality parameters with natural disease resistance.

In barley, specific powdery mildew resistance is conferred by several loci of which the *Mla* locus is probably the best studied and the most important. The complex *Mla* locus is located on the short arm of the barley chromosome 1H and consists of several NBS-LRR type disease resistance genes providing resistance to different fungal pathotypes (Wei *et al.*, 1999). The locus has been completely sequenced from the susceptible cultivar Morex and the resistance alleles *Mla1* (Zhou *et al.*, 2001), *Mla6* (Halterman *et al.*, 2001), *Mla12* (Shen *et al.*, 2003) and *Mla13* (Halterman *et al.*, 2003) have been characterized (Mejlhede *et al.*, 2006). However, gene-for-gene disease resistance provided by *Mla* genes is relatively rapidly overcome by the evolution of pathogen avirulence factors (Weibull *et al.*, 2003), thus, race nonspecific resistance genes are desirable.

Naturally occurring and induced recessive mutations at the *Mlo* locus on the barley chromosome 4H confer race nonspecific resistance against most of the powdery mildew pathotypes (Jørgensen, 1992). The *Mlo* gene has been cloned, multiple resistance alleles have been characterized at the DNA sequence level and molecular markers are available for marker-assisted selection (Buschges *et al.*, 1997; Piffanelli *et al.*, 2004). *mlo*-mediated resistance comes with a yield penalty because of the necrotic lesions on plant leaves (Wolter *et al.*, 1993), therefore *mlo-11* allele is often used in plant breeding as it exhibits a residual production of a functional MLO protein (Piffanelli *et al.*, 2004). However, many varieties also possess the *mlo-5* and *mlo-9* alleles resulting from single nucleotide mutations in the *Mlo* gene (Buschges *et al.*, 1997).

CAPS (Cleaved Amplified Polymorphic Sequences) markers are PCR-amplified DNA regions that exhibit polymorphic DNA fragments after digestion with restriction endonucleases (Konieczny and Ausubel, 1993). In case of cloned genes, the CAPS markers can be designed based on the DNA sequence targeting specific DNA polymorphisms that alter restriction sites. CAPS markers are available for *mlo-5* and *mlo-9* alleles since mutations destroy the *EcoRV* and *HhaI* sites, respectively (Schulze-Lefert *et al.*, 1998). In addition to the CAPS marker, a SNaPSHOT assay has been reported for the *mlo-9* allele (Ovesna *et al.*, 2003). The naturally occurring *mlo-11* allele is caused by a complex sequence duplication of the *Mlo* locus, therefore detection of the *mlo-11* allele is based on the genotyping of closely linked loci (Piffanelli *et al.*, 2004; Tacconi *et al.*, 2006).

In this study we used molecular markers to characterize resistance alleles at the *Mlo* locus in a selection of European barley varieties and breeding lines, and we also sequenced a novel *mlo* allele. Molecular markers flanking the *Mlo* gene and targeting the induced mutations could be used to determine the presence and segregation of *mlo-5*, *mlo-9* and *mlo-11* alleles in hybrids.

## Materials and Methods

DNA from 44 European barley varieties and 15 Latvians breeding lines (Table 1) was extracted from a single barley plant using a modified procedure by (Edwards *et al.*, 1991). Plant tissue was collected either from a field-grown plant or a seedling germinated on Petri dish in the laboratory. Seeds were obtained from the State Priekuli Plant Breeding institute and State Stende Cereal Breeding institute. DNA extractions from barley hybrid lines were performed from a combined tissue sample originating from five plants. The Mildew resistant barley mutant line 792-9, previously characterized as allelic to *mlo* (I. Rashal, unpublished), was obtained from the Institute of Biology, University of Latvia. A single mutant plant was used for DNA extractions and sequence analysis.

PCR was performed in a 20 µl reaction consisting of ca. 50 ng DNA, 10 M primers, 1 x reaction buffer (Fermentas, Vilnius, Lithuania), 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTPs and 1 u of Hot Start *Taq* polymerase (Fermentas, Vilnius, Lithuania). PCR primers and conditions for *mlo-11* allele are described (Piffanelli *et al.*, 2004). Barley CAPS markers for *mlo-5* and *mlo-9* alleles are described (Schulze-Lefert *et al.*, 1998), except that the primers Y14573\_F02 (5'-CGCCAGCAAACCAGACACAC-3') and Y14573\_R01 (5'-TTCCATGAGGACGGACACGA-3') were used to amplify a 321 bp fragment. Digestion with enzymes *Hha*I and *Eco*R32I (Fermentas, Vilnius, Lithuania) was done according to manufacturer's recommendations. Restriction digests were analyzed on 1.5% w/v agarose gels (Fermentas, Vilnius, Lithuania).

Primers for PCR amplification and the sequencing of the *Mlo* coding region were from (Mejlhede *et al.*, 2006). Sequencing was done using BigDye 3.1. terminator mix (Applied Biosystems, Foster City, CA, USA) on an ABI3730 sequencer according to the manufacturer's recommendations. Base calling and the sequence assembly was done using the Staden software package (Staden, 1996). The sequence alignment of mutant and wild type *Mlo* genes was done with Clustal 1.83 software (Thompson *et al.*, 1997).

## Results and Discussion

CAPS markers for *mlo-5* and *mlo-9* alleles (Figure 1A for their location in *Mlo* gene) and an indel marker for the *mlo-11* allele (Piffanelli *et al.*, 2004) were used to screen a selection of European barley varieties and breeding lines (Table 1) for the presence of *mlo* resistance alleles. We identified one variety with the *mlo-5* allele, two varieties with *mlo-9* alleles and five varieties with the *mlo-11* allele (Table 1) based on DNA fragment sizes in agarose gels. In addition, two Latvian breeding lines appeared to carry *mlo-11*. The same markers were used to screen several barley hybrid lines (Figure 1B). Homozygous *mlo-9* and *mlo-11* alleles were detected in several hybrid lines, however, some hybrids showed a *mlo-9/Mlo* and *mlo-11/Mlo* restriction fragment pattern (Figure 1B, lanes 2 and 9, respectively). Because each hybrid line was represented by a pool of five plants, we could not distinguish between heterozygous plants and heterogeneous pools (Figure 1B), but it was a clear indication that the line was still segregating for *mlo* resistance. Registered Latvian barley varieties are known to be *Mlo* wild type, therefore introgression of *mlo* alleles in locally adapted germplasm could be beneficial for traditional and organic barley growers in Latvia. Application of the *mlo* molecular markers (Buschges *et al.*, 1997; Piffanelli *et al.*, 2004; Schulze-Lefert *et al.*, 1998) for barley breeding have the potential to facilitate the identification of *mlo* resistant progeny in crosses, in particular in the presence of other powdery mildew resistance genes, e.g., *Mla*.

Table 1. Molecular marker-based assessment of *mlo* alleles in a set of barley accessions

Accession	Country of origin	Length of a PCR product or restriction fragment in base pairs for each marker <sup>1</sup>			Detected allele <sup>2</sup>
		<i>mlo-5 Eco</i> 32I	<i>mlo-9 Hha</i> I	<i>mlo-11</i> (indel <sup>3</sup> )	
Alamo	CA	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Alexis	DE	321 bp	272, 49 bp	470 bp	<i>mlo-9</i>
Ametist	CZ	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Annabell	DE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Anni	EE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Ballistu	FR	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>

Barke	DE	321 bp	272, 49 bp	470 bp	<i>mlo-9</i>
Beatrice	FR	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Dziugiai	LT	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Effendi	NL	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Emir	NL	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Fager	NO	218, 103 bp	195, 77, 49 bp	470 bp	<i>mlo-5</i>
Fibar	CA	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Fink	DE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Fontana	FR	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
Forace	IT	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Fredrikson	US	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Freedom	CA	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Golf	UK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Hellana	AT	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Heris	CZ	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
Hily	SE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Hiproly	DK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Hockey	UK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Ivana	FI	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Jaspis	CZ	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Jet	ET	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Justina	DE	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
Karat	CZ	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Keti	DK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Klaxon	UK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Kompakt	SK	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Lawina	DE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Lysimax	DK	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
Magda	NL	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
Merlin	US	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Peggy	DE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Prosa	AT	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Rattan	CA	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Rupal	SE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Samson	CA	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Steffi	DE	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Wanubet	US	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
Washonubet	US	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
L-3118	LV	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
PR-2797	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3005	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3282	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3297	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3335	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3345	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3348	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3351	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3353	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3455	LV	321 bp	195, 77, 49 bp	530 bp	<i>mlo-11</i>
PR-3487	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3520	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3522	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>
PR-3524	LV	321 bp	195, 77, 49 bp	470 bp	<i>wt Mlo</i>

<sup>1</sup> Length of PCR amplicons and restriction fragments is predicted from the known sequence of the *Mlo* locus.

<sup>2</sup> *wt Mlo* – wild type *Mlo* allele.



<sup>3</sup> indel – PCR fragment length polymorphism caused by an insertion of transposable element (Piffanelli *et al.*, 2004).

While *mlo-5*, *mlo-9* and *mlo-11* alleles are mostly used in plant breeding (<http://www.crpmb.org/mlo/>), there is still a need for the characterization of additional mutant alleles both for the understanding of the *Mlo* function in plants and as a material for breeding. The complete coding sequence of the *Mlo* gene from the 792-9<sub>9</sub> mutant and the variety Maja was obtained and used to identify the cause of mutation. A single base exchange at position 2004 relative to the ATG codon was identified as the only difference between the mutant 792-9<sub>9</sub> and the wild type variety Maja (Figure 1A).

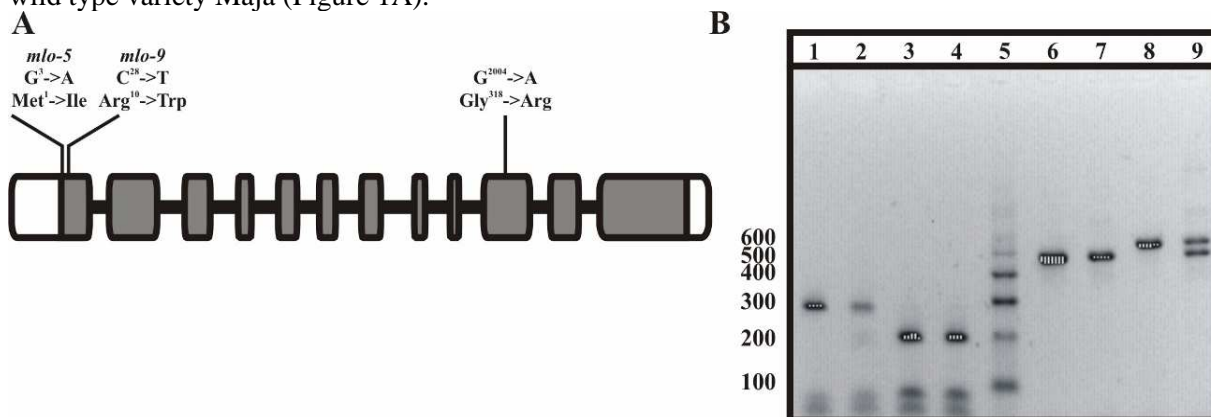


Figure 1. Molecular markers for the *mlo* alleles

**A** Structure of the *Mlo* gene with the *mlo-5* and *mlo-9* mutations indicated. A G to A transition in the 10<sup>th</sup> exon is a novel mutation identified in the variety Maja. Boxes represent exons; filled boxes represent coding regions, open boxes represent 5'- and 3'-untranslated regions. **B** Molecular marker-based detection of *mlo-9* and *mlo-11* alleles in barley hybrids. Lanes 1 – 4 – four hybrid lines analyzed with the *mlo-9* CAPS marker (Schulze-Lefert *et al.*, 1998); lane 5 – MW marker; lanes 6 – 9 – the same four hybrid lines analyzed with the *mlo-11* indel marker (Piffanelli *et al.*, 2004).

The mutation caused the amino acid Gly to Arg exchange at position 318 of the MLO protein, a position that has also been mutated in the *mlo-27* allele (Gly to Glu) (Piffanelli *et al.*, 2002). Thus, the predicted Gly to Arg exchange was likely to be responsible for the observed resistance phenotype and further confirmed the functional significance of this amino acid residue.

Powdery mildew is still one of the most damaging barley diseases in Europe regaining extensive use of pesticides. The exploitation of natural plant disease resistance provides a sustainable solution for pest control, however, breeding for disease resistance is often complicated because of difficulty of carrying out field phenotyping tests and the linkage drag of undesired traits with resistance genes. Molecular markers have the potential to facilitate plant breeding by the enabling one to carry out selection at seedling stage based on molecular marker genotypes and thus avoiding time- and labor-intensive field tests (Varshney *et al.*, 2005). Molecular markers also provide the opportunity to screen a large number of progeny to select for the rare recombinants that uncouple the resistance from closely linked deleterious alleles (Thomas *et al.*, 1998).

### Acknowledgements

The study was funded by the Latvian National programme in agrobiotechnology, the Latvian Council of Science project 07.2055 and University of Latvia grant ZP-59.

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## **MOLEKULĀRO MARKĪERU PIELIETOJUMS MILTRASAS IZTURĪBAS LOKUSA MLO RAKSTUROJUMAM EIROPAS MIEŽU ŠĶIRNĒS UN SELEKCIJAS LĪNIJĀS**

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Miltrasa ir plaši izplatīta miežu slimība, ko izraisa mikroskopiskā sēne *Blumeria (Erysiphe) graminis* f.sp. *hordei*. Slimību var kontrolēt izmantojot pesticīdus, taču to pielietošana paaugstina

ražošanas izmaksas un nav pieņemama bioloģiskajā lauksaimniecībā. Miežiem piemīt dabiskā izturība pret miltrasu, ko nosaka vairāki genoma rajoni, no kuriem svarīgākie ir *Mlo* un *Mla* lokusi. Dabiskas vai inducētas recesīvas mutācijas *Mlo* lokusā nodrošina plaša spektra izturību pret gandrīz visiem zināmajiem miltrasas patotipiem. *Mlo* gēns ir klonēts un vairākas mutācijas gēna DNS sekvencē, kas piešķir slimību izturību, ir zināmas. Mēs raksturojām jaunu *mlo* allēli mutantā, kas iegūts no šķirnes Maja, kurā notikusi aminoskābes Gly nomaiņa par Arg 318 pozīcijā. Lai raksturotu *mlo* miltrasas izturību Eiropas miežu šķirnēs, kā arī Latvijas un ārzemju selekcijas līnijās, tika izmantoti CAPS marķieri *mlo-5*, *mlo-9* un *mlo-11* allēlēm. Iegūtie rezultāti apstiprina molekulāro marķieru pielietojuma perspektīvu pret miltrasu izturīgu miežu hibrīdu selekcijā.

## A COMPARISON OF THE YIELD AND QUALITY TRAITS OF WINTER AND SPRING WHEAT

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### Abstract

Traditionally winter wheat is known by its higher yield potential and spring wheat by better baking quality. In this investigation we studied how yield and quality traits of spring and winter wheat differed at the Jõgeva PBI trials during 2004-2007. Yield and 1000 kernel weight of winter wheat exceeded spring wheat every year. Spring wheat had higher protein and gluten content and volume weight. There was no clear trend for the falling number and gluten index.

According to variance analyses, the value of yield and 1000 kernel weight were determined by the wheat type (spring or winter) but other characteristics were more affected by the weather conditions of a particular year. The effect of the weather conditions for the year was greater for yield, 1000 kernel weight, protein and gluten content, bread loaf volume and dough stability for the both types of wheat. For falling number the influence of the year was greater than that of the variety of spring wheat and the influence was reversed for winter wheat. Volume weight depended more on the weather for spring wheat and on the variety for winter wheat.

**Key words:** spring wheat, winter wheat, quality, yield

### Introduction

The climatic conditions in Estonia are suitable for cultivation of the both wheat types – spring and winter wheat. The acreage of wheat cultivation has enlarged from 78 to 102 thousand ha during the last 4 years (2004-2007). The acreage share of winter wheat is 1/3 smaller than that of spring wheat (but has a tendency to increase). Traditionally winter wheat is known for its higher yield potential and spring wheat for its better baking quality (Swenson, 2006; Baker and Townley-Smith, 1986). Yield and quality potential is largely determined by the variety, but the extent to which this potential is achieved depends upon factors such as seasonal weather conditions. Higher grain yields are usually associated with lower protein concentration (Terman *et al.*, 1969, Blackman and Payne, 1987). The protein is a primary quality component of cereal grains. The protein concentration is influenced by both environmental and genotypic factors that are difficult to separate (Fowler *et al.*, 1990). The protein content of wheat grains can vary from 6% up to as much as 25%, depending upon the growing conditions (Blackman and Payne, 1987). Terman *et al.* (1969) noted that protein content varied more widely among locations than among varieties at the growing location. Differences among cultivars tended to be greatest under optimum growth conditions (Terman, 1979). Protein content and protein quality have been also shown to be significant for baking quality (Johanson and Svensson, 1998). Fredericson *et al.* (1997, 1998) found that protein content was positively correlated with wet gluten content, farinogram dough stability and bread loaf volume. The great majority of wheat products are adversely affected by alfa-amylase. The activity of alfa-amylase can be described by the falling number test. High levels of alfa-amylase activity in the

grain may be due to naturally high endogenous levels of the enzyme, or to premature germination causing alfa-amylase to be synthesized de novo (Blackman and Payne, 1987).

Kernel weight, usually expressed in grams per 1000 kernels, is a function of kernel size and kernel density. Big wheat kernels usually have a higher ratio of endosperm to nonendosperm components. 1000 kernel weight can be used as a reliable guide to predict flour yield.

One of the most used criteria of wheat quality is volume weight. Volume weight is a good indication of the density and soundness of the wheat. Very low volume weight is normally associated, not with cultivar characteristics, but with sub-optimum growing and harvest conditions that cause shrinkage and shrivelling and subsequent loss of grade (Tipples, 1986).

The goal of this work was the comparison of yield and quality characteristics and variation the of these traits in spring and winter wheat; the comparison of the influence of wheat type and environment (growing year) on these traits; the comparison of the influence of genotype and environment separately on spring and winter wheat characteristics; to find out correlations between the various characteristics.

### Materials and Methods

Fifteen wheat varieties from the Estonian Variety List and the Jõgeva PBI collection trial of winter wheat (WW) and fourteen varieties of spring wheat (SW) were tested. The WW varieties were Ada, Bill, Ballad, Bjorke, Compliment, Gunbo, Korweta, Lars, Portal, Ramiro, Residence, Sani, Širvinta 1, Tarso and Urho and SW varieties Baldus, Helle, Mahti, Manu, Meri, Mooni, Munk, Satu, SW Estrad, Zebra, Tjalve, Trappe, Triso, Vinjett. Varieties were grown on 9 m<sup>2</sup> plots with three replications. The level of fertilizer was N 90 kg ha<sup>-1</sup>. Yield (Y), 1000 kernel weight (TKW) and volume weight (VW) was calculated as an average of three replications. Protein and gluten content the gluten index and falling number, dough stability time and bread volume was tested in one replication per variety by each year. Data about dough stability time and bread loaf volume were obtained from the years 2004-2006, other data from 2004-2007.

Protein content (PC) was determined by the Kjeldahl method. Wet gluten content (WGC) and gluten index (GI) were determined by the ICC standard method 137, 155 and 158 using the Glutomatic 2200 instrument. The falling number (FN) was determined by the ICC standard method 107/1. The farinogram test was conducted using the ICC standard method 115. By farinogram farinograph dough stability time (DST) was measured. Baking tests on 250g of flour according to the long fermentation process were carried out by the method of the Finnish State Granary (Suomen Valtion Viljavarasto Koeleivontamenetelmä, 1996). Bread volume was analysed by measuring the displacement of canola seeds.

Statistical analyses were performed using the Agrobases 4 software package. Data were analysed by the analyses of variance and for correlations the Spearman Rank Correlation was used. The analyses of variance and the estimates of the components of variance (determination coefficient) due to wheat type (spring and winter)  $R_T^2$ , environment (growing year)  $R_E^2$  and genotype  $R_G^2$  were calculated and was expressed as % of the total variance. The least significant differences ( $LSD_{0,05}$ ) among mean values were calculated. Stability analyses of genotypes and quality parameters were based on a coefficient of variation (CV).

### Results and Discussion

The grain yield of WW was higher compared to SW every year. The four years average was more than 2 t ha<sup>-1</sup> higher (Table 1). The variations of yield were similar. The highest yielding among SW and WW were respectively the varieties Trappe (5,601 kg ha<sup>-1</sup>) and Ballad (7,242 kg ha<sup>-1</sup>) (data not shown here). Y was more influenced by the wheat type than the growing year – the variation of this factor was 43.4 % from the total variation of yield (Table 2). The yield potential of autumn-sown cereal crops is considerably higher than that of spring-sown crops. A crop stand already established in spring is able to respond immediately to rising temperatures and increased of solar radiation; by contrast, since a spring crop cannot be sown until there are suitable soil conditions, part of the growing season is lost (Hay and Porter, 2004). Concerning the types; the Y of both types was significantly influenced by the climatic conditions of the year (Tables 3 and 4). The influence on the variety was bigger for SW, the effect of GxE for WW. The grain yield of a cereal crop can be split into three major components: ear population density, ear size and individual grain weight

(measured as TKW). The four years average TKW of WW was 9.4 g bigger than that of SW. The variation of kernel size was higher for SW. The biggest kernels among the SW varieties belonged to Triso, Zebra and Munk (35.4 g) and WW variety Širvinta 1(48.6 g). The mean grain weight is determined primarily by the quantity of assimilates available for transport to the ear between anthesis and maturity. This depends upon the green leaf area duration after anthesis and the photosynthetic activity of the ear. The period from anthesis to maturity was 37-56 days for WW and 41-49 days for SW during 2004-2007. The TKW of WW was bigger than that of SW even in the year when the grain filling period was shorter. Similarly to factors influencing Y, the wheat type had a bigger effect than the year also on kernel size - the variation of this factor was 49.5% from the total variation of TKW. For the kernel size of SW the influence of the year was more important ( $R_E^2 = 70.6$ ) than for WW where the effect of the two factors was distributed more equally ( $R_E^2 = 43.2$   $R_G^2 = 35.4$ ). The TKW of both types was positively correlated with VW (SW:  $r=0.78^{***}$ ; WW:  $r=0.22^{**}$ ; data of correlations are not shown in the tables).

Table 1. Average data of winter wheat and spring wheat varieties from 2004-2007

	Y, kg ha <sup>-1</sup>	TKW, g	VW, g l <sup>-1</sup>	PC, g kg <sup>-1</sup>	WGC, g kg <sup>-1</sup>	GI, %	FN, sec	LV <sup>a</sup> , cm <sup>3</sup>	DST <sup>a</sup> , min
SW	4,438	32.7	775	146	329	66	281	1,342	8.8
CV <sup>b</sup> (%)	20.2	15.1	4.9	12.9	17.1	29.3	34.7	8.2	63.0
WW	6,523	42.1	761	116	252	62	283	1,320	4.7
CV (%)	21.4	10.9	3.5	20.1	31	31.9	27.3	12.2	87.7
LSD <sub>0,05</sub>	167.8	0.55	3.9	2.7	0.94	4.6	18.7	30.8	1.2

WW – winter wheat, SW – spring wheat, Y – yield, TKW – thousand kernel weight, VW – volume weight, PC – protein content, WGC – wet gluten content, GI – gluten index, FN – falling number, LV – bread loaf volume, DST – dough stability time; <sup>a</sup> data of 2004-2006; <sup>b</sup> CV=coefficient of variation

 Table 2. Analyses of traits variance. Components of variation due to environment -year ( $R_E^2$ ), wheat type ( $R_T^2$ ), type by year ( $R_{TYE}^2$ ) and residuals in percentage of the total sum of square

Source of variation	Y	TKW	VW	PC	WGC	GI	FN	LV <sup>a</sup>	DST <sup>a</sup>
Environ.	6.1***	25.4***	20.2***	43.5***	42.1***	22.2***	36.8***	39.3***	46.0***
Type	43.4***	49.5***	4.5***	32.5***	24.4***	0.8ns	0.1ns	0.7ns	15.5***
Type by environ.	13.9***	3.4***	32.0***	13.0***	19.0***	20.3***	15.8***	24.8***	0.0
Residual	36.6	21.7	43.4	11.0	14.5	56.7	47.0	35.2	38.9
R <sup>2</sup>	0.63	0.78	0.57	0.89	0.86	0.43	0.53	0.65	0.61

ns=non-significant; \*\*\*, \*\*, \* significant at  $P < 0.001$ ; 0.01 and 0.05 respectively.

WW – winter wheat, SW – spring wheat, Y – yield, TKW – thousand kernel weight, VW – volume weight, PC – protein content, WGC – wet gluten content, GI – gluten index, FN – falling number, LV – bread loaf volume, DST – dough stability time; <sup>a</sup> data of 2004-2006.

 Table 3. Analyses of variance traits in spring wheat. Components of variation due to environment - year ( $R_E^2$ ), genotype ( $R_G^2$ ), genotype x year ( $R_{GXE}^2$ ) and residual in percentage of the total sum of square.

Source of variation	Y	TKW	VW	PC	WGC	GI	FN	LV <sup>b</sup>	DST <sup>b</sup>
Environ.	36.6***	70.6***	77.8***	74.7***	66.8***	41.9***	61.9***	40.4***	53.8***
Genotype	24.4***	21.2***	8.6***	20.6***	26.9***	45.6***	20.1**	24.7ns	24.0ns
Genotype by env.	14.1*	5.3***	7.9***	NA	NA	NA	NA	NA	NA
Residual	24.9	2.9	5.7	4.6	6.3	12.5	18.0	34.9	22.2
R <sup>2</sup>	0.75	0.97	0.94	0.95	0.94	0.87	0.82	0.65	0.78

ns=non-significant; \*\*\*, \*\*, \* significant at  $P < 0.001$ ; 0.01 and 0.05 respectively.

NA – data not available, Y – yield, TKW – thousand kernel weight, VW – volume weight, PC – protein content, WGC – wet gluten content, GI – gluten index, FN – falling number, LV – bread loaf volume, DST – dough stability time; <sup>a</sup> data of 2004-2006;

Table 4. Analyses of variance traits in winter wheat. Components of variation due to environment - year ( $R_E^2$ ), genotype ( $R_G^2$ ), genotype x year ( $R_{G \times E}^2$ ) and residual in percentage of the total sum of square.

Source of variation	Y	TKW	VW	PC	WGC	GI	FN	LV	DST
Environ.	34.9***	43.2***	13.6***	88.7***	87.2***	43.6***	39.6***	74.0***	54.1***
Genotype	6.2**	35.4***	58.7***	5.4**	6.7**	27.5**	40.4***	18.2***	24.8ns
Genotype by env.	37.9***	16.4***	15.0***	NA	NA	NA	NA	NA	NA
Residual	21.0	5.0	12.7	5.9	6.1	28.9	20.1	7.1	21.1
$R^2$	0.79	0.95	0.87	0.94	0.94	0.71	0.80	0.93	0.79

ns=non-significant; \*\*\*, \*\*, \* significant at  $P < 0.001$ ; 0.01 and 0.05 respectively.

NA – data not available, Y – yield, TKW – thousand kernel weight, VW – volume weight, PC – protein content, WGC – wet gluten content, GI – gluten index, FN – falling number, LV – bread loaf volume, DST – dough stability time; <sup>a</sup> data of 2004-2006

According to Chung (2003) there was no significant difference between the mean VW for WW and SW when these were cultivated under the same environmental conditions without effect of different. According to Monsalve-Conzales and Pomeranz, (1993) over-wintering increased the test weight when the tested wheat was facultative wheat which was sown in the winter and in the spring after verbalization. The average VW of SW was 14 g l<sup>-1</sup> higher in our study. The CV was higher for SW. The highest average VW were found in the SW varieties Satu (795 g l<sup>-1</sup>) and the WW variety Ada (801 g l<sup>-1</sup>). The wheat type factor explained only 4.5 % from total sum of square. The effect of genotype was low for SW ( $R_G^2 = 8.6^{***}$ ) but highest than any other factor for WW ( $R_G^2 = 58.7^{***}$ ).

The average amylolytic activity, which was measured as FN for both wheat types was similar (SW: 281 sec, WW: 283 sec). More variable were the SW varieties. The variation was greater in the year with a lower average FN (data not shown). The higher FN had SW variety Mooni (408 sec) and WW Tarso (401 sec). The effect of the climatic conditions was stronger than the influence of type. The effect of the climatic conditions on SW was larger. For WW the effect of year and genotype were similar.

Research has demonstrated that there is a strong positive correlation between protein content and bread volume, and that the baking quality of spring wheat is directly related to protein content and wet gluten (Hanell, 2004). In this investigation the average PC of SW was 30 g kg<sup>-1</sup> higher than WW. The highest protein content was found in the early SW variety Manu by 161 g kg<sup>-1</sup> and WW Ada by 126 g kg<sup>-1</sup>. The PC is positively correlated with WGC (Fredericson *et al*, 1997; 1998), which is strongly influenced by the growing environment (Grausgruber *et al*, 2000). The correlation between protein and gluten content was positive for both types of wheat and stronger for SW (SW:  $r = 0.96^{***}$ ; WW:  $r = 0.97^{***}$ ) in our investigation. The four years average WGC of SW was 329 and 252 g kg<sup>-1</sup> for WW. The variety Helle (SW) produced the highest average gluten content in the period of 2004-2007 (378 g kg<sup>-1</sup>) compared to the best from among the WW, the variety Širvinta 1 by 280 g kg<sup>-1</sup>. The cause of this kind of big difference between the two types can be explained by two extremely unfavourable years (2005 and 2007) for the accumulation of protein for WW. In 2005 the average gluten content of WW was only 142 g kg<sup>-1</sup> compared to 309 g kg<sup>-1</sup> for SW. Two years later the situation was as follows - 234 for WW and 331 g kg<sup>-1</sup> for SW. The most favourable year for protein and gluten concentration was 2006, when WW ranged between 281-380 g kg<sup>-1</sup> and SW 351-479 g kg<sup>-1</sup>. According to Johansson and Svensson (1988) the influence of the mean temperature and rainfall on protein content is clearer for spring wheat. In our investigation the influence of the environment was greater for WW -variation of PC and WGC was higher for WW varieties. For both protein and gluten content, the main influence was the climatic effect (PC:

$R_E^2 = 43.5^{***}$ ; WGC:  $R_E^2 = 42.1^{***}$ ) and of secondary importance was the influence of the wheat type. The effect of climatic conditions was greater than the genotypic impact for both types. The influence of genotype was especially low for WW.

PC and Y were inversely related. This trend is in accordance with other research (Grant *et al.*, 1985; Peltonen, 1992; Bly and Woodard, 2003). The  $r$  according to the Spearman Rank Correlation was  $-0.41^{***}$  for WW and  $-0.54^{***}$  for SW. The inverse relationship between yield and protein may be partly due to the effect of the dilution of N. As grain yield increases, a limited amount of protein is diluted within the greater mass of grain.

One measurement to express protein and gluten quality character is GI. Protein quality is much less affected by the environment and is mainly genetically controlled (Blackman and Payne, 1987). There was no significant difference of the average GI between WW and SW. The variation of this trait was also similar for two wheat types. There was no significant influence of wheat type. For SW the effect of the environment and genotype were similar ( $R_E^2 = 41.9^{***}$   $R_G^2 = 45.9^{***}$ ) but for WW the influence of the environment was greater than genotype ( $R_E^2 = 43.6^{***}$ ;  $R_G^2 = 27.5^{**}$ ).

There wasn't a significant difference between the LV of WW and SW. From the WW varieties Compliment had the highest average LV ( $1460 \text{ cm}^3$ ) of all the tested varieties. The second highest was the LV of the SW variety Meri ( $1440 \text{ cm}^3$ ). The CV was higher for WW. Over the years type interaction had a significant influence on the LV but no influence of type. The main factor influencing the LV of WW the years ( $R_E^2 = 74.7^{***}$ ). For SW the influence of the year was lower and the effect of the genotype even of no significant. Johansson and Svensson (1998) found that the correlation between PC and LV is not significant in SW material with large differences in protein quality. Other researches have demonstrated that there is a strong positive correlation between PC and bread volume, and that the baking quality of SW is directly related to PC and WGC (Hanell, 2004). Variations in LV resulted mainly from the quantitative effects of gluten proteins (Chung *et al.*, 2003). According to Peterson *et al.* (1998) for many baking parameters, variation attributed to environmental effects was of greater magnitude than for the genotype of WW and correlations of protein components with baking parameters were generally low. According to Wieser and Kieffer (1999) bread volume was influenced more by the amount of gluten proteins than by the total amount of protein. In our investigation WW had strong positive correlation of LV with PC ( $r=0.74^{***}$ ) and WGC ( $r=0.72^{***}$ ). Surprisingly there was no correlation between the LV and protein and gluten characteristics of SW.

Four years the average DST of SW was higher than that of WW (SW: 8.8 min, WW: 4.7 min). The variation of this trait was high (CV 63-88%). The influence of the year was greater than the influence of type for DST ( $R_E^2 = 46.0^{***}$ ;  $R_T^2 = 15.5^{***}$ ), but also the residual part was quite high. For WW and SW the effect of the genotype wasn't significant, the effect of the year was  $R_E^2 = 53.8^{***}$  and  $R_E^2 = 54.1^{***}$  respectively. There were positive relationships between DST- PC and DST-WGC for both types of wheat (SW:  $r=0.75^{***}$ ,  $r=0.66^{***}$  respectively; WW  $r=0.90^{***}$ ,  $r=0.82^{***}$  respectively). A strong positive correlation between LV and DST was found only for WW ( $r=0.69^{***}$ ).

## Conclusion

The results, based on the data of the 15 WW and 14 SW varieties during 2004-2007 indicated, that WW had higher yield potential and bigger kernels under Estonian conditions. Quality data were better for SW: higher volume weight, protein and gluten content. But there were not found significant differences between the gluten index and the falling number between the two wheat types. Although the bred loaf volume was bigger for SW, the difference wasn't significant. Dough stability was better for SW.

The value of the yield and 1000 kernel weight were determined by the wheat type but other characteristics were more affected by the environment (year). If the two wheat types are compared separately, yield and kernel size were determined by the environment for both types of wheat. Volume weight was influenced by the genotype for WW and by the year for SW. The influence of the year was greater for the bread loaf volume and dough stability of WW. The most important measurement of bread quality may be considered to be the final loaf volume. It was predictable better by the protein and gluten content and gluten index of WW there wasn't a correlation of SW

between these traits. But there were positive and strong correlations between dough stability and protein and gluten content for both types of wheat.

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## ZIEMAS UN VASARAS KVIEŠU RAŽAS UN KVALITĀTES PAZĪMJU SALĪDZINĀJUMS

**Koppel, R., Ingver, A.**

Ziemas kvieši tradicionāli ir pazīstami ar augstāku ražas potenciālu un vasaras kvieši – ar labākām maizes cepamīpašībām. Šajā pētījumā tika pārbaudīts, kā atšķirās ziemas un vasaras kviešu ražas



un kvalitātes pazīmes Jogevas Augu Selekcijas institūta izmēģinājumos laikā no 2004. līdz 2007. gadam. Ziemas kviešu raža un 1000 graudu masa pārsniedza vasaras kviešu rādītājus katru gadu. Vasaras kviešiem bija augstāks proteīna saturs un tilpummasa. Graudu raža un 1000 graudu masa bija atkarīgas no kviešu sezonālā tipa, bet citas pazīmes vairāk ietekmēja attiecīgo gadu meteoroloģiskie apstākļi. Gada ietekme abiem kviešu tiptiem bija lielāka uz ražu, 1000 graudu masu, proteīna un lipekļa saturu, maizes kukuļa apjomu un mīklas stabilitāti. Gada ietekme uz krišanas skaitli vasaras kviešiem bija lielāka nekā šķirnes ietekme, bet ziemas kviešiem – otrādi. Tilpummasa vasaras kviešiem bija vairāk atkarīga no gada, bet ziemas kviešiem - no šķirnes.

## SELECTION CRITERIA IN TRITICALE BREEDING FOR ORGANIC FARMING

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### Abstract

For creating varieties suitable for organic farming a special breeding programme has been started in Priekuli Plant Breeding Institute. The evaluation of triticale genotypes in organic farming was done in Priekuli during 2005 - 2007. The aims of research are:

Estimating possibility for selecting genotypes desirable for organic farming in conventional fields.  
To find desired traits for the organic triticale varieties breeding programme.

There were included 25 different winter triticale (*xTriticosecale Wittm.*) breeding lines in our trials, selected from the conventional breeding programme. The different traits were tested for each genotype. The influence of different traits on yield and grain quality was analyzed. Every year the best 25 different triticale breeding lines from the organic and conventional growing conditions were compared.

The results showed that different breeding lines reacted differently to growing conditions. It is possible to select genotypes suitable for organic conditions in the conventional field. To select genotypes with better stability of the traits (especially in the years with unfavorable weather conditions) and suitability for organic farming, selected breeding lines must be tested in organic growing conditions.

For organic farming only genotypes with good winterhardiness and resistance to snow mould should be selected.

Triticale genotypes with different plant height, growth habit, leaf size would be suitable for organic growing conditions.

**Key words:** triticale, organic breeding, trait

### Introduction

For the further development of organic agriculture, more attention is being focused on the creation of better adapted varieties. As organic conditions are less controllable and more variable, breeding should be aimed on improved yield stability and product quality by being adapted to organic soil fertility, sustainable weed, pest and disease management (Lammerts van Bueren., 2002; Lammerts van Bueren *et al.*, 2007). Therefore the traits required for the varieties in organic and conventional farming differ. Some breeding programmes were started in the last years with aim to evaluate genotypes adaptation to organic agriculture for characteristic traits required in organic farming systems and to elaborate the selection criteria that facilitate the breeding of proper varieties for organic agriculture (Schneider *et al.*, 2007; Legzdina and Skrabule, 2005).

The main objectives in the breeding programmes for small grains cultivars for organic farming are: to improve the nutrient efficiency, weed suppression ability (new ideotype of plant), as well as the resistance to leaf, spike and soil born diseases, the efficient use of manure, reducing risk of diseases (long stem, ear high above flag leaf, ear not too compact, last leaves green for the longest time possible), reducing risks at harvest, higher stress tolerance to abiotic causes (Ittu *et al.*, 2007, Legzdina and Skrabule, 2005; Lammerts van Bueren, 2002; Kopke, 2005; Goyer *et al.*, 2005).

Long years work experience with the newest small grains species - triticale (*xTriticosecale Wittm.*) shows that this crop is very suitable for growing in Latvian agroclimatic conditions. Such traits as high yield potential, good nutrient efficiency, resistance to diseases are advantages for growing triticale in Latvian organic and conventional fields. Triticale breeding for organic farming was started in Latvia at the same time as in some other countries – for example, Romania, Germany (Skrabule, 2005; Ittu *et al.*, 2007).

The winter triticale breeding programme for organic farming was initiated in the State Priekuli Plant Breeding Institute in 2004. The limited area allowed for organic agriculture as well as limited financing will be the bottleneck for the establishment of specific breeding programmes for organic farming systems (Lammerts van Bueren *et al.*, 2002). The financing of a special triticale breeding programme for organic farming is problematical in Latvia too. Due to this problem the first tasks of the breeding programme in Priekuli are: 1. Estimating possibility for selecting genotypes desirable for organic farming in conventional fields; 2. Determination of the most important traits for the selection criteria in plant breeding for organic farming.

### Materials and Methods

The investigation was conducted at the State Priekuli Plant Breeding Institute experimental fields. The breeding material was evaluated over three years (2004-2005, 2005-2006 and 2006-2007) in a randomized block design with 4 replicates, plot size 12.6 m<sup>2</sup>. The field was certified as being organic from 2003. Soil properties: sod-podzolic soil, soil type –sandy loam, organic matter content 19- g kg<sup>-1</sup>, pH<sub>KCl</sub> – 5.9- 6.0, P - 69-111 g kg<sup>-1</sup>, K - 114-165 g kg<sup>-1</sup>, preecrop– peas for green manure. No agrochemicals and fertilizers were used. Trial varieties were sown on 26 September 2005, 14 September 2006 and 16 September 2007. The seeding rate was 450 germinating seeds per m<sup>2</sup>.

The following field trials were performed:

1. The breeding lines according traits assessment which are significant for organic farming (stable yield, disease resistance a.o.) were selected in the conventional field and tested in the organic field, with the aim to establish the efficiency of the organic breeding if the first steps are done in conventional field. Every year the best 25 different triticale breeding lines from the organic and conventional growing conditions were compared. Soil properties in conventional field: sod podzolic soil, soil type –sandy loam, organic matter content 19-23 g kg<sup>-1</sup>, pH<sub>KCl</sub> – 5.9- 6.0, P - 79-84 g kg<sup>-1</sup>, K - 110-160 g kg<sup>-1</sup>, preecrop – white clover for seed. Basic fertilizer (N:P:K 6:26:30) 300 kg ha<sup>-1</sup>, additional fertilizing (N 60+30) as well as herbicide, was applied in the conventional field in every ear of the investigation. The trial varieties in the conventional field were sown on 24 September 2005, 14 September 2006 and 15 September 2007. The seeding rate was 400 germinating seeds per m<sup>2</sup>.

2. With the aim to find the most desirable traits for the triticale organic crop ideotype for Latvian growing conditions 25 different winter triticale breeding lines in the organic field were tested and about 30 different traits were evaluated: yield, heading, maturity, winter hardiness, lodging, growth habit, infection with leaf diseases (*Septoria* spp., *Puccinia* spp., *Rhynchosporium* spp. a.s.o), infection with snow mould (*Microdochium nivale*), weed amount in the plot, grain quality parameters (1000 kernel weight, protein, starch content, volume weight, falling number). Five plants from every replication were planted and such morphological traits as plant height, ear length, spike density were measured. The importance of the influence of different traits on yield and quality traits (protein content) was tested. The correlation analysis was used to find out the most acceptable traits for organic crop ideotype of triticale. Descriptive statistics and correlation analysis were used for analyzing the obtained results.

During the study years, the meteorological conditions were significantly different. Due to a cold and dry autumn in 2004 the tillering of triticale was delayed. The spring of 2005 was cold and the vegetation renewed itself comparatively late and the infection of plants with snow mould was very strong. Triticale flowering in 2005 was very late – from 20 of June (about 10 days later than usually). July was warm and dry, but the beginning of August was very rainy – as a result of these conditions triticale grains sprouted in the ears.

The weather conditions in autumn and winter months of 2005/2006 were favorable for triticale tillering and for over wintering. However April was very cold and the triticale plants began to

wither away. Snow mould was spread widely. July was dry and warm. Such weather conditions significantly influenced yield – yield level was lower than in another years.

Some days of January 2007 had very low air temperature (-22.5°C), without a mantle of snow on the soil. These conditions negatively influenced triticale overwintering. Vegetation renewed comparatively early in spring 2007. Triticale flowered earlier than in other years. The weather conditions in June, July, August was typical for the Latvian climate and the triticale yield level was high. The weather conditions in 2007 were very favorable for leaf disease development - septoria (*Septoria tritici*), leaf rust (*Puccinia recondita f.sp. tritici*), powder mildew (*Erysiphe graminis*).

## Results and Discussion

The obtained results show very similar triticale genotypes traits average yield value and their variance in organic and conventional fields. Triticale adapted oneself good to conventional and to organic conditions (Table 1). The mean and maximum yield of triticale genotypes was slightly higher in the organic field in the two years of the investigation (2005 and 2007). Only in the year with unfavorable weather conditions (cold winter and dry and hot summer) the triticale yield in organic field was lower.

Table 1. Characteristics of triticale genotypes yield in organic and conventional fields

Year	Growing conditions	Mean, t ha <sup>-1</sup>	Standart deviation	Coefficient of variation, %	Minimum, t ha <sup>-1</sup>	Maximum, t ha <sup>-1</sup>
2005	Org. field	4.6	0.8	17.0	3.1	6.4
	Conv.field	4.3	0.7	16.3	2.5	5.7
2006	Org. field	2.9	0.6	20.6	1.5	3.8
	Conv.field	4.7	0.7	14.8	3.5	5.8
2007	Org. field	5.6	0.8	14.2	4.1	7.6
	Conv.field	5.7	0.7	12.2	5.0	7.5

Although many investigations point out that triticale samples are characterized by a high amount of crude protein in grains, this was not proved in Latvia agroclimatic conditions (Kronberga, 2001), therefore it is necessary by breeding to increase protein content in triticale grains, especially in the organic field where the mean protein content was lower than in the conventional field (Table 2). Mean protein level was higher in the organic field only in 2005, nonetheless in all three years in the organic field maximum protein level in grains was similar to them on conventional field.

Table 2. Characteristics of protein content (in %) in grains in organic and conventional fields

Year	Growing conditions	Mean, % in grains	Standart deviation	Coefficient of variation, %	Minimum, % in grains	Maximum, % in grains
2005	Org. field	11.3	0.8	7.1	9.9	12.9
	Conv.field	10.1	0.7	6.9	8.5	11.5
2006	Org. field	13.2	0.7	5.3	12.1	15.3
	Conv.field	14.0	0.7	5.0	12.7	15.1
2007	Org. field	10.4	0.8	7.6	9.1	11.7
	Conv.field	10.9	0.6	5.5	9.8	11.9

Each year of the testing the genotypes with a high starch or protein content in the grains, good winterhardiness, resistance to snow mould, 1000 kernel weight and other tested traits were in the organic field. This verifies that it is possible to select genotypes in conventional field, which would have good testing results in the organic field.

Triticale traits were influenced by weather conditions also. So the triticale in the organic field overwintered better than in the conventional field however the yield level in organic field was lower in 2006. Very hot and dry weather conditions during ripening may disturbed effective utilizing of nutrients in the organic field. This shows the mean and maximum value of 1000 kernel weight was lower in organic field (Table 3).

The mean protein content in grains in 2006 was higher than in the other years of investigation in both fields due too warm and dry summer, however, trait variance in the organic and the conventional field was very similar. Therefore it is necessary especially evaluate genotype stability

in the organic field in the years with unfavorable weather conditions with aim the to find genotypes which have stable trait values in these conditions.

Table 3. Characteristics of triticale genotypes 1000 kernel weight (g) in organic and conventional fields

Trait	Growing conditions	Mean, g	Standart deviation	Coefficient of variation, %	Minimum, g	Maximum, g
2005	Org. field	46.1	3.4	7.3	40.0	52.9
	Conv.field	43.0	3.5	8.1	36.8	49.1
2006	Org. field	38.1	2.7	7.1	33.2	43.7
	Conv.field	40.8	4.4	10.8	35.6	51.4
2007	Org. field	44.2	3.1	7.0	39.6	51.0
	Conv.field	41.6	4.4	10.6	33.1	51.2

Different triticale genotypes varied differently according to their response to organic and conventional growing conditions. The correlation between yield of the same genotypes in the organic and conventional field was positive and significant only in 2007. This trial year had suitable weather conditions for winter cereal development ( $r_{2005}=0.0217 < r_{0.05}=0.396$ ;  $r_{2006}=-0.077 < r_{0.05}=0.396$ ,  $r_{2007}=0.675 < r_{0.05}=0.396$ ). It was possible to select lines with a higher yield and grain quality in organic growing conditions. For example, line 9402-32 had lower yield, protein content in grains, 1000 kernel weight in the organic field in the all years of investigation (Table 4). But line 9540-1 was better in organic conditions. However in 2006, when weather conditions were unfavorable for triticale growing, the yield of line 9540-1 in the organic field decreased significantly. There were some cultivars that had high yield performances in both types of testing conditions (for example, 9405-23).

Table 4. Results of some triticale lines testing in organic and conventional growing conditions

Genotype	Year	Yield, t ha <sup>-1</sup>		Protein, %		1000 kernel weight, g	
		org.cond.	conv. cond.	org.cond.	conv. cond.	org.cond.	conv. cond.
9402-32	2005	3.11	4.89	10.8	11.2	49.7	52.3
	2006	3.22	4.56	12.8	14.6	43.7	48.0
	2007	5.89	6.11	10.8	11.4	51.0	49.3
9540-1	2005	5.49	4.74	11.1	9.7	42.1	39.3
	2006	2.73	5.48	13.1	13.7	34.4	35.6
	2007	5.62	5.44	10.4	10.4	42.7	37.1

To find the most desirable traits for organic farming, the correlation between all the tested traits and the yield as well as quality traits (protein) was calculated. Winterhardiness is one of most important trait for organic varieties. There was a positive and significant correlation between yield and winterhardiness in all years of the investigation (Table 5).

Table 5. Correlation between traits in organic field

Traits	Year		
	2005	2006	2007
Yield-winterhardiness	0.504*	0.605**	0.529**
Yield –resistance to snow mould	-0.300	-0.655**	-0.646**
Winterhardiness –resistance to snow mould	0.634**	0.604**	0.467*
Yield-plant height	0.041	0.063	0.153
Yield-weed amount in the plot	-0.639**	-0.849**	-0.563**
Yield-protein content in grains	-0.153	-0.086	-0.458*
Leaf area -1000 kernel weight	0.674**	0.104	0.218
Growth habit-weed amount in the plot	0.354	-	-0.244

\*, \*\* - significant at the 0.05 and 0.01 probability level respectively

A significant correlation between winterhardiness and resistance to snow mould in all the years of the investigations prove, that good resistance to snow mould is one of most important components of good overwintering of triticale in Latvia. Genotypes with better winterhardiness have a larger amount of ears on the 1 m<sup>2</sup> and as result – better weed competitiveness and a higher yield. This shows that for organic farming only genotypes with good winterhardiness and resistance to snow mould should be selected.

The obtained results do not prove the necessity to select for organic farming triticale genotypes with long stems. There was not found a significant correlation between yield and plant height ( $r_{2005}=0.041 < r_{0.05}=0.396$ ;  $r_{2006}=0.063 < r_{0.05}=0.396$ ,  $r_{2007}=0.153 < r_{0.05}=0.396$ ,  $n=25$ ). It was observed, that genotypes with longer stems were less infected with leaf diseases, however in our trials there was a significant negative correlation between infection with leaf diseases and plant height only in the year 2005 ( $r_{2005}=-0.409 > r_{0.05}=0.396$ ;  $r_{2006}=-0.363 < r_{0.05}=0.396$ ,  $r_{2007}=0.271 < r_{0.05}=0.396$ ,  $n=25$ ). The obtained results were similar with triticale testing results in Rumania, with the conclusion, that both tall and short straw genotypes are between genotypes suitable for organic farming (Ittu *et al.*, 2007).

According to the obtained results, the yield was not significantly influenced by leaf size or growth habits. Triticale resistance to diseases decreased in the last years (Arseniuk *et al.*, 2006) but the influence of diseases on the yield was not found. There was not a significant correlation between yield and infection with leaf diseases (*Septoria* spp., *Puccinia* spp., *Rhynchosporium* spp), powder mildew (*Erysiphe graminis*). Good resistance to these diseases make triticale very suitable as an organic field crop for the present, and by selecting genotypes it should not be lost.

There were negative correlation between the yield and protein content in the grains; however only in 2006 this correlation was significant. It indicates possibility to find genotypes with high yield and protein content in grains.

No stable and significant influence of the tested traits was found on the protein content in the grains. There was not proved influence of growth habit on the weed competitiveness.

The obtained results indicated that it is possible to find triticale genotypes suitable for organic farming with different traits as plant height, growth habit, maturity time, leaf size. However these genotypes have to be with good winterhardiness and resistance to snow mould, as well as resistance to other diseases.

## Conclusions

It is possible to select triticale genotypes suitable for organic conditions in the conventional field. However the testing of selected breeding lines have to be done in organic growing conditions. Genotypes with better stability traits must be selected (especially in the years with unfavorable weather conditions).

For organic farming only genotypes with good winterhardiness and resistance to snow mould should be selected.

Triticale genotypes with different plant heights, growth habits, leaf size would be suitable for organic growing conditions.

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## IZLASES KRITĒRIJI TRITIKĀLES ŠĶIRŅU SELEKCIJĀ BIOĻĢISKAJAI LAUKSAIMNIECĪBAI

### Kronberga A.

Priekuļu laukaugu selekcijas institūtā uzsākta bioloģiskajai lauksaimniecībai piemērotu šķirņu selekcija. Tās sākuma etapā tika veikta dažādu tritikāles genotipu izvērtēšana bioloģiskajā laukā ar mērķiem:

Novērtēt, vai iespējama bioloģiskajai lauksaimniecībai piemērotu genotipu izlase konvencionālās selekcijas laukā;

Atrast pazīmes, pēc kurām jāvērtē tritikāles genotipi, lai nodrošinātu to labu piemērotību bioloģiskajai lauksaimniecībai.

Izmēģinājums veikts trīs gadus (no 2005. līdz 2007.gadam). 25 dažādas tritikāles selekcijas līnijas atlasītas konvencionālās selekcijas laukā un izvērtētas bioloģiskajā laukā. Katram genotipam novērtētas 30 dažādas pazīmes, tai skaitā arī pazīmes, kas tiek uzskatītas kā nozīmīgas bioloģiskajai lauksaimniecībai (auga garums, cera forma u.c.). Novērtēta šo pazīmju korelācija ar ražu un kopproteīna saturu graudos. Katru gadu salīdzināti 25 genotipi bioloģiskajā un konvencionālajā laukā un novērtēta dažādu pazīmju variācija atkarībā no audzēšanas veida.

Iegūtie rezultāti liecina, ka dažādu genotipu reakcija uz bioloģiskajiem un konvencionālajiem audzēšanas apstākļiem atšķiras. Bioloģiskajai lauksaimniecībai piemērotu līniju atlasīti iespējams veikt konvencionālās selekcijas laukā. Tomēr šīs līnijas pēc tam nepieciešams pārbaudīt bioloģiskajā laukā, lai novērtētu pazīmju stabilitāti un atlasītu bioloģiskajai lauksaimniecībai vispiemērotākās.

Pēc izmēģinājuma rezultātiem konstatēts, ka svarīgākie izlases kritēriji, veidojot bioloģiskajai lauksaimniecībai piemērotas ziemas tritikāles šķirnes ir to laba ziemciētība un izturība pret sniega pelējumu. Pētījumā netika pierādīts, ka bioloģiskajiem laukiem ir piemēroti genotipi tikai ar garu stiebru un klājenisku cera formu.

## TESTING RESULTS OF THE SPRING BARLEY VARIETY ‘RUBIOLA’

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### Abstract

One of problems for barley (*Hordeum vulgare* L.) in organic farming is its infection with seed born diseases, particularly with loose smut (*Ustilago nuda*). Currently in the Latvian Plant Variety

Catalogue there are no varieties completely resistant to this disease. Our aim was to offer a resistant variety acceptable for growing in organic conditions. The first step in breeding for organic farming was the evaluation of breeding lines from conventional breeding program under organic growing conditions. The breeding line PR-2797 ('Rubiola') was derived from a cross with a loose smut resistant parent. 'Rubiola' was tested under conventional growing conditions during 5 seasons in 1 site and under organic conditions during 3 seasons in 2 sites. The Latvian barley varieties 'Abava', 'Idumeja', 'Ansis' and 'Ruja' were used for comparison. The yield of 'Rubiola' under conventional growing conditions was superior to extensive type varieties, but it could not surpass the intensive check 'Ansis'. The mean yield of 'Rubiola' under organic conditions did not significantly differ from the check varieties. The results indicate that a significant yield gain for 'Rubiola' under organic growing conditions could be achieved by increasing the seed rate. Resistance to the loose smut of 'Rubiola' was improved by artificial inoculation and by PCR-based markers. The most important advantage of 'Rubiola' for organic farming was its resistance to loose smut; notable was also its relatively low infection with leaf diseases, high spike productivity and grain volume weight. 'Rubiola' was entered for VCU (Value for Cultivation and Use) and DUS (Distinctness, Uniformity and Stability) testing starting from 2007.

**Key words:** barley breeding, organic farming, agronomic traits, loose smut resistance, seed rate

### Introduction

Two types of breeding programs dealing with organic farming exist: conventional breeding for organic agriculture with the testing of advanced lines in organic conditions in the later stages and organic breeding programs where all steps are performed under organic conditions using breeding techniques in conformity with organic principles (Lammerts van Bueren *et al.*, 2007). For example in Austria the breeding of winter wheat for organic agriculture is carried out combining to conventional (until F<sub>5</sub> generation) and organic growing conditions (Loshenberger, 2007). Barley (*Hordeum vulgare* L.) breeding for organic farming is a developing direction in the Priekuli Plant Breeding Institute. The first steps were the evaluation of the registered varieties (Legzdina *et al.*, 2005) and breeding lines from conventional breeding programs under organic growing conditions and the investigation of the most efficient selection criteria and methods differing from conventional breeding.

The main requirements for cereal varieties adapted to organic farming are related to improved nutrient uptake and use efficiency due to limited nutrient availability in soil, competitive ability with weeds and resistance to diseases (Lammerts van Bueren, 2002). One of problems for barley in organic farming is infection with seed born diseases, particularly loose smut (*Ustilago nuda* (Jens.) Rostr.). The regulations for seed production in organic farming are the same as in conventional farming, but chemical seed treatment is not allowed. According to the research results of acceptable seed treatment methods in organic farming, only treatment with hot water can reduce infection with loose smut to the acceptable level, but it is not economic and it is difficult for farmers to organize such treatment. The choice of resistant varieties is an important component of preventative strategy. Loose smut of barley can be mainly controlled by choosing resistant varieties. (Borgen, 2004) Currently there are no varieties included in the Latvian Plant Variety Catalogue that are resistant to loose smut; only the variety 'Rasa' is recommended for organic farming (Latvian Catalogue of Plant Varieties, 2007). The aim of this study is to summarize the testing results of the new barley variety 'Rubiola'.

### Materials and Methods

The spring barley breeding line PR-2797 ('Rubiola') was derived from the cross combination of Ruja/Run 458. 'Ruja' is a medium late maturing Latvian variety and Run 458 is a Canadian line with the loose smut resistance gene Un8. At first the line was tested in regular breeding nurseries under conventional conditions (until 2003) and discarded. Due to resistance to loose smut the line was selected for testing under organic conditions (2004-2006). Additional testing under conventional conditions was performed during the preparation of the line for registration (2006-2007).

Trial 1 summarizes the testing results of the breeding line PR-2797 under conventional growing conditions in breeding nurseries in Priekuli during 2001-2003 and 2006-2007 in 6 replications and 23.1 m<sup>2</sup> plots. The variety 'Abava' was used as a check during 2001-2003, but 'Ansis' and 'Idumeja' were checks during 2006-2007. Soil characteristics: sod-podzolic (2001-2003, 2006) and sod-podzolic gley (2007) soil, loamy sand and sandy loam, organic matter 11-27 g kg<sup>-1</sup>, pH<sub>KCl</sub> 5.2-5.5 (2003, 2007) and 5.8-6.1 (2001, 2002, 2006), P 58-119 mg kg<sup>-1</sup>, K 105-163 mg kg<sup>-1</sup>. Fertilizer N 81-86, P 40.5-43, K 43-67.5 kg ha<sup>-1</sup> and herbicides Granstar and Primus and the insecticide Fastac were applied. The seed rate was 400 seeds able to germinate per m<sup>2</sup>; the seed was not treated.

Trial 2 was arranged in Priekuli and Vecauce in fields certified for organic farming in 4 replications during 2004-2006. Plot size in Priekuli was 12.3 m<sup>2</sup> and in Vecauce 25 m<sup>2</sup>. The varieties 'Abava', 'Idumeja' and 'Ruja' (Latvia) were used for comparison. Soil characteristics in Priekuli: sod-podzolic soil, loamy sand and sandy loam, organic matter 21-23 g kg<sup>-1</sup>, pH<sub>KCl</sub> 5.4-6.3, P 69-124 mg kg<sup>-1</sup>, K 100-191 mg kg<sup>-1</sup>. Soil characteristics in Vecauce: sod-gleysolic soil, sandy loam, organic matter 19-32 g kg<sup>-1</sup>, pH<sub>KCl</sub> 6.8-7.5, P 21-40 mg kg<sup>-1</sup>, K 50-71 mg kg<sup>-1</sup>. The seed rate 400 seeds able to germinate per m<sup>2</sup> was applied. Harrowing was used in the tillering stage. Protein and starch were determined by a Infratec grain analyzer (Foss) and expressed in % in dry matter. Sprouting was evaluated in Vecauce in 2005 by calculating % of the sprouted kernels in 75 spikes from two replications. Traits related to weed suppression ability were determined in Priekuli during 2006-2007. Emergence was calculated in % after counting emerged plants in 2 x 0.1 m<sup>2</sup> per plot. Soil shading was estimated visually in % at the end of the tillering stage. Plant height in the shooting stage was measured once per plot. The length and width of flag leaf was measured for 10 plants per plot after flowering.

Trial 3 was performed under organic growing conditions in Priekuli during 2005-2006 (growing conditions as in trial 1) in 4 replications with plot size 23.1 m<sup>2</sup>. Three seed rates (250, 400 and 550 seeds able to germinate per m<sup>2</sup>) were compared for the varieties 'Rubiola', 'Ruja' and 'Idumeja'.

Infection with powdery mildew (*Blumeria graminis* f.sp. *hordei*) and netblotch (*Drechslera teres* (Sacc.) Shoem) was scored in Priekuli according to a 0-4 scale (0-no infection, 4-strong infection) and in Vecauce disease severity in % was assessed. Lodging was scored according to a 1-9 scale (1-completely lodged, 9-no lodging).

The particularities of the meteorological conditions: in 2001 higher air temperature and an amount of rainfall larger than the long-term average was observed in July when severe lodging was caused by heavy thunderstorms 2002 was overall warm and dry (air temperature higher by 2.6 °C and the amount of rainfall 36% lower than the long-term data). The beginning of the growing season in 2003 was wet, very dry conditions were observed in the beginning of June, middle of July and the beginning of August. A high amount of precipitation in August (270% of the long-term average) caused lodging and sprouting. In 2004 dry conditions in April and May hindered plant emergence, a high amount of rainfall was registered at the end of June (245 and 232% of the long-term data in Priekuli and Vecauce) and in the middle of August and the mean air temperature in May, June and July was below the long-term data. In 2005 enduring and heavy rainfalls were registered in the middle of May and the extremely high amount of rainfall was recorded in the beginning of August (398% of the long term data in Priekuli and 142% in Vecauce) caused sprouting. 2006 was very dry (47% rainfall of the long term data in the growing season) and the mean temperature in the growing season was 2 °C above the long term average.

'Rubiola' was tested by artificial inoculation with loose smut during 2004-2006; its parent variety 'Ruja' which is susceptible to loose smut was used for comparison. Each flower in 3 spikes per genotype was infected during anthesis with a syringe containing a local loose smut population spore suspension of 1 g l<sup>-1</sup>. The infected seeds were harvested and sown next season; the presence of plants with loose smut symptoms was stated.

DNA was extracted from 1 week old seedlings of the varieties 'Rubiola', 'Ruja' and 'Run 458' using the Genomic DNA Purification Kit (Fermentas). PCR (polymerase chain reaction) amplification using SCAR (sequence characterized amplified region) primers for detection of resistance and susceptibility to loose smut was carried out according to the protocol of Eckstein *et al.* (2002). The forward primer T800F4 was used for both resistant and susceptible allele. The



primer T800R was used to prime the resistant allele and to amplify a single 716 bp band. The primer H700R was used to prime the susceptible allele and to amplify a single 714 bp band. Two and three way ANOVA was applied for statistical data analysis.

## Results and Discussion

The testing results of 'Rubiola' under conventional growing conditions (trial 1) are summarized in Table 1. The yield of the line was significantly higher than that of the check variety 'Abava' in 2001 and 2003; it did not significantly differ from the checks 'Ansis' and 'Idumeja' in 2006, but the differences were significant in 2007 ( $p=0.05$ , Figure 1).

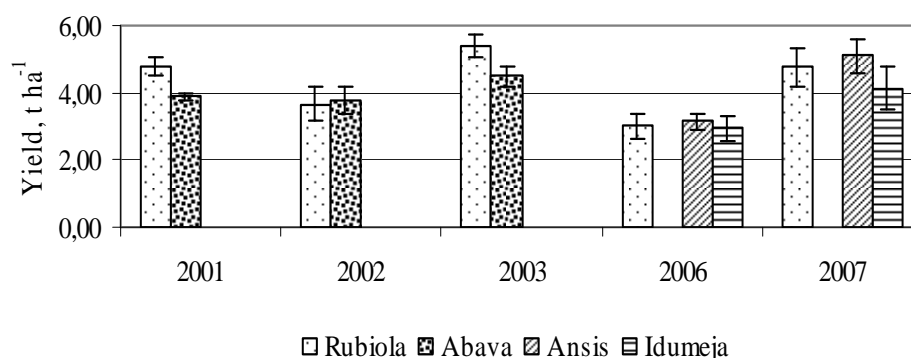


Figure 1. Yield of 'Rubiola' and check varieties under conventional growing conditions.

The length of vegetation of 'Rubiola' was similar to the medium-late varieties 'Abava' and 'Ansis'. Slightly better resistance to lodging was observed for 'Rubiola' if compared to 'Abava' in the years when lodging problems accrued. 'Rubiola' provided the highest volume weight of all the compared varieties.

Table 1. Testing results under conventional growing conditions (Priekuli)

Variety	Grain yield, t ha <sup>-1</sup>	Vegetation, days	Lodging, 1-9 <sup>1</sup>	TKW, g	Volume weight, g l <sup>-1</sup>	Powdery mildew, 0-4 <sup>2</sup>	Net-blotch, 0-4 <sup>2</sup>	Loose smut, spikes per m <sup>2</sup>	Number of kernels per spike	Kernel weight per spike, g
2001-2003										
Rubiola	4.62	96	7.7	45.2	684	1.3	0.9	0.1	22.7	1.07
Abava	4.05	96	6.5	44.1	659	2.6	2.2	0.9	19.9	1.03
LSD <sub>0.05</sub>	0.23									
2006-2007										
Rubiola	3.90	99	9	45.8	706	0.8	0.8	0	21.4	1.00
Ansis	4.13	100	9	44.6	701	0.3	0.5	0.8	19.0	0.90
Idumeja	3.55	93	9	48.2	660	1.7	2.2	2.5	17.8	0.85
LSD <sub>0.05</sub>	0.40									

<sup>1</sup> 9-no lodging; 1-completely lodged; <sup>2</sup> 0-no infection, 4-very high infection

The infection level of 'Rubiola' with powdery mildew and netblotch was lower than 'Abava' and 'Idumeja'. Only 'Ansis' had less infection. Practically no loose smut infection was found in 'Rubiola'; only a few infected plants were found more likely to admixtures. Superior spike productivity was ascertained for 'Rubiola' in comparison to all other checks.

The mean grain yield of 'Rubiola' during its three years under organic growing conditions in the two locations (trial 2, Table 2) did not significantly differ from the check varieties. The yield of 'Rubiola' surpassed significantly that of 'Abava' (Vecauce, 2004;  $p=0.05$ , Figure 2), 'Ruja' (Priekuli, 2005) and 'Idumeja' (Priekuli and Vecauce 2005), but it was significantly lower than the

yield of 'Abava' and 'Idumeja' in Vecauce in 2006. Extremely dry conditions in 2006 had a particularly negative influence on the yield of 'Rubiola' and its parent variety 'Ruja'.

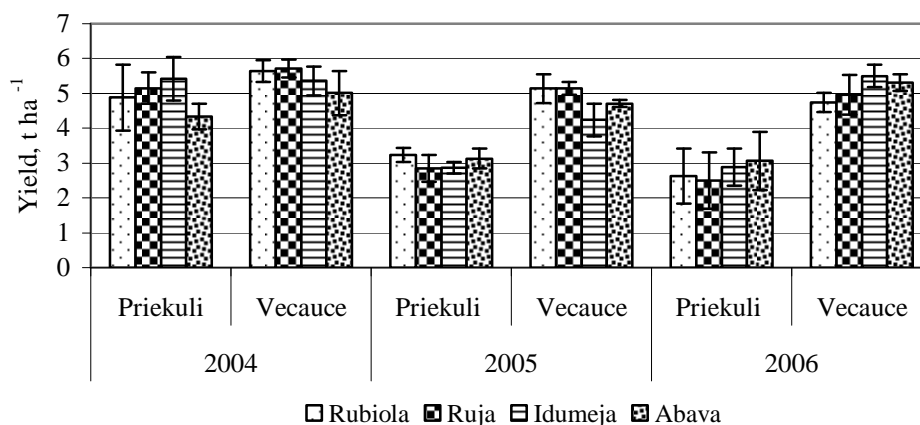


Figure 2. Grain yield under organic growing conditions

The vegetation period of 'Rubiola' was one day shorter than that of the parent variety 'Ruja', equal to 'Abava' and 8 days longer than for the early maturing variety 'Idumeja'. The medium-tall plants of 'Rubiola' can be considered as an advantage for organic growing conditions because of their weed suppressing ability. Superior volume weight was stated for 'Rubiola' similarly to conventional growing conditions (significant advantage over 'Idumeja' and 'Ruja'). No significant differences in grain chemical composition between the varieties was found.

Table 2. Yield, length of vegetation, plant height and grain quality under organic growing conditions (average from Priekuli and Vecauce, 2004-2006)

Variety	Grain yield, t ha <sup>-1</sup>	Vegetation, days	Plant height, cm	TGM, g	Volume weight, g l <sup>-1</sup>	Protein, g kg <sup>-1</sup>	Starch, g kg <sup>-1</sup>
Rubiola	4.38	101	82	46.3	688	126	602
Ruja	4.39	102	78	48.2	668	123	604
Abava	4.26	101	85	45.7	685	126	602
Idumeja	4.38	93	72	47.1	646	126	605
LSD <sub>0.05</sub>	0.28	1.6	4.3	2.4	19.4	n.s.	n.s.
p genotype	0.4	<0.001	<0.001	0.2	<0.001	0.3	0.7
p environment	<0.001	<0.001	<0.001	<0.001	0.3	<0.001	<0.001

Although the differences were not significant, infection with powdery mildew was lowest for 'Rubiola' if compared to the check varieties (Table 3). The infection level with netblotch was the lowest for 'Rubiola' also; showing significant superiority over 'Abava' and 'Idumeja' in the Priekuli tests. Significantly lower in infection with loose smut was 'Rubiola' (few infected plants are more likely due to admixtures) in comparison to 'Abava' and its parent variety 'Ruja' as stated in Priekuli. In Vecauce the infection level with loose smut was very low and no infected plants were found for 'Rubiola'. The lowest sprouting rate among the tested varieties was found for 'Rubiola'; more than half of the kernels were sprouted for the early maturing variety 'Idumeja'.

Table 3. Infection with diseases and sprouting in organic growing conditions (average from Priekuli and Vecauce, 2004-2006)

Variety	Powdery mildew, Priekuli, 0-4	Powdery mildew, Vecauce, %	Netblotch, Priekuli, 0-4	Netblotch, Vecauce, %	Loose smut <sup>1</sup> , spikes per m <sup>2</sup>	Sprouting <sup>2</sup> , %
Rubiola	1.25	6.04	1.42	0.92	0.08	5.5
Ruja	2.50	9.75	1.92	3.11	4.62	5.9
Abava	2.75	11.92	2.75	6.37	5.75	9.7
Idumeja	1.75	10.97	2.67	1.46	0.11	54.3

LSD <sub>0.05</sub>	n.s.	n.s.	1.21	n.s.	4.5	7.1
p genotype	0.2	0.4	0.1	0.1	0.04	<0.001
p year	0.01	0.007	0.3	0.05	0.2	-

<sup>1</sup> in Priekuli; <sup>2</sup> 2005 in Vecauce

The expression of traits related to the weed suppression ability of 'Rubiola' are close to the average values of all tested varieties and breeding lines in most of the cases (Table 4). This can be explained by the fact that 'Rubiola' was not selected under organic growing conditions and no special attention was paid to such traits. The soil shading of 'Rubiola' was only a little better than the average; plant height in shooting was equal to the average value or a little higher. Genotypes with significantly longer and wider flag leaves than 'Rubiola' were found among the tested breeding lines.

Table 4. Weed suppression ability determinative traits (Priekuli, 2006-2007)

Genotypes	Soil shading in tillering, %		Plant height in shooting, cm		Length of flag leaf, cm		Width of flag leaf, mm	
	2006	2007	2006	2007	2006	2007	2006	2007
Rubiola	65	73	36	33	11.7	11.1	8.6	8.1
Varieties and average	61	70	36	30	12.3	11.4	8.5	8.0
breeding lines <sup>1</sup> min-max	45-71	55-79	30-47	25-38	9.3-14.5	8.6-13.7	6.5-10.2	5.4-10.5
LSD <sub>0.05</sub>	n.s.	9.3	4.4	4.3	1.75	1.20	1.20	0.71

<sup>1</sup> n=21 in 2006 and n=18 in 2007

The results of Trial 3 indicate that a significant yield gain can be achieved for 'Rubiola' by increasing the seed rate from 250 to 400 and from 400 to 550 seeds able to germinate per m<sup>2</sup> (Table 5). Similar results were obtained for 'Ruja', but for 'Idumeja' a seed rate of 400 provided the highest yield and the further increase of seed rate was not effective. The reason for this is not clearly recognizable yet.

Table 5. Effect of seed rate increase under organic growing conditions (Priekuli, 2005-2006)

Variety	Yield, t ha <sup>-1</sup>			TGW, g			Volume weight, g l <sup>-1</sup>			Infection with netblotch, 0-4 <sup>2</sup>		
	250 <sup>1</sup>	400 <sup>1</sup>	550 <sup>1</sup>	250	400	550	250	400	550	250	400	550
Rubiola	2.29	2.92a	3.36ab	51.1	49.0a	48.0a	682.8	683.1	690.0a	0.81	0.95	1.13
Ruja	1.93	2.33a	2.83ab	52.6	49.9a	49.7a	661.4	662.6	668.6a	1.35	1.75	2.01a
Idumeja	2.37	3.17a	3.21a	50.6	49.4	48.8a	650.4	651.6	655.5	2.19	2.50	2.75a
□year	17.5			11.5			12.7			32.7		
□genotype	12.3			9.5			60.2			37.7		
□seed rate	29.6			39.4			2.7			4.3		

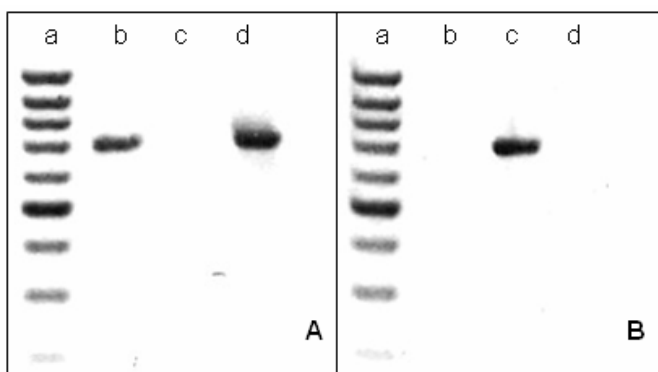
<sup>1</sup> seed rate; a- significant difference from seed rate 250, b-significant difference from seed rate 400 (p=0.05)

<sup>2</sup> 0-no infection, 4-very high infection

Gruber *et al.* (2003) reported that higher seed rate (200-450 seeds able to germinate per m<sup>2</sup> tested for spring barley) resulted in higher yield for cereals, but it is not always economical. The possible explanation could be the not very high productive tillering coefficient of 'Rubiola' (during 2006-2007 under conventional conditions it was 2.7 and 3.5 for 'Rubiola' and 'Idumeja' respectively). On the other hand, the number of productive tillers per m<sup>2</sup> for 'Rubiola' was higher than that of 'Idumeja' under organic growing conditions (418 and 366, respectively, average from 2006-2007). The difference between early maturing 'Idumeja' and medium-late maturing 'Ruja' and 'Rubiola' can be possible explained by the longer lasting influence of the higher weed biomass in the case of lower seed rates for late maturing varieties. The decrease of TGW and the increase of volume weight was observed along with the increase of seed rate for all varieties. A slight increase of infection with netblotch was found with increased seed rate (the difference is not significant for 'Rubiola'). The results show that the increase of seed rate had a more positive influence on yield

gain than the yield reduction caused by netblotch infection. The influence of seed rate on TGW and grain yield was high, but it was less on volume weight and infection with net blotch.

The results of artificial inoculation improve the resistance of 'Rubiola' against loose smut; 0% infected plants were found during 3 cycles of testing and we presume that the line has the *Un8* resistance gene. For the susceptible parent variety 'Ruja' most of the infected plants (80-90%) had loose smut symptoms. Primers T800F4 and T800R amplified a single band in varieties the 'Rubiola' and 'Run 458' (Fig. 3A). Primers T800F4 and H700R amplified a single band in the variety 'Ruja' (Fig. 3B). These results indicate that the variety 'Rubiola' carries the resistance gene *Un8* which is obtained from the parent 'Run 458'.



(a) 100 bp GeneRuler™ DNA ladder (Fermentas); (b) 'Rubiola'; (c) 'Ruja'; (d) 'Run 458'.

Figure 3. Amplification of the genomic DNA of the barley varieties 'Rubiola', 'Ruja' 'Run 458' using primers specific for (A) the resistant allele of (B) the susceptible allele.

### Conclusions

The grain yield of 'Rubiola' in conventional growing conditions was able to surpass the extensive type varieties ('Abava' and 'Idumeja'), but it could not surpass the intensive type variety ('Ansis'). The most important advantage of 'Rubiola' for organic farming is its resistance to loose smut; notable are also its relatively low infection with leaf diseases, optimal plant height, spike productivity and grain volume weight. 'Rubiola' was not the most optimal genotype regarding a number of traits desirable for organic growing conditions like the length of vegetation and traits determining weed suppression ability.

The increasing of seed rate might be beneficial for obtaining a higher yield for the variety 'Rubiola' under organic growing conditions.

'Rubiola' was entered for VCU (including organic testing) and DUS testing from 2007. If the testing results will be successful, the variety will be recommended for growing in organic farms.

### Acknowledgement

We are thankful for professional assessment of infection with leaf diseases in location Vecauce to assoc. prof. Dr. Biruta Bankina.

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## VASARAS MIEZU SKIRNES ‘RUBIOLA’ PARBAUDES REZULTATI

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Viena no miežu (*Hordeum vulgare* L.) audzēšanas problēmām bioloģiskajā lauksaimniecībā ir inficēšanās ar slimībām, kuru ierosinātāji saglabājas sēklas materiālā, īpaši ar putošo melnplauku (*Ustilago nuda*). Pašlaik neviena no Latvijas Augu šķirņu katalogā iekļautajām šķirnēm nav izturīga pret šo slimību. Mūsu mērķis bija piedāvāt audzētājiem izturīgu šķirni, kas būtu piemērota bioloģiskajiem audzēšanas apstākļiem. Pirmais solis, uzsākot selekciju bioloģiskās lauksaimniecības vajadzībām, bija konvencionālajā selekcijas programmā izveidotu selekcijas līniju pārbaude bioloģiskos apstākļos. Selekcijas līnija PR-2797 (‘Rubiola’) izveidota no krustojuma kombinācijas, kurā viens no vecākaugiem ir ar izturības gēnu Un8 pret putošo melnplauku. Rakstā apkopoti rezultāti par ‘Rubiolas’ pārbaudes rezultātiem konvencionālos apstākļos (5 gadi) un bioloģiskos apstākļos (3 gadi). Salīdzināšanai izmantotas Latvijā selekcionētas miežu šķirnes ‘Abava’, ‘Idumeja’, ‘Ansis’ un ‘Rūja’. Konvencionālos apstākļos ‘Rubiolas’ raža pārspēja ekstensīva tipa šķirnes, taču tā atpalika no intensīvā tipa šķirnes ‘Ansis’. ‘Rubiolas’ vidējā raža bioloģiskos apstākļos būtiski neatšķīrās no standartšķirņu ražas. Rezultāti rāda, ka būtisks ražas pieaugums šķirnei ‘Rubiola’ bioloģiskos audzēšanas apstākļos var tikt sasniegts, palielinot izejas normu. Šķirnes izturība pret putošo melnplauku pierādīta, veicot mākslīgo inficēšanu un izmantojot molekulāros marķierus. Nozīmīgākā šķirnes ‘Rubiola’ priekšrocība bioloģiskajā lauksaimniecībā ir tās izturība pret putošo melnplauku; vērā ņemama ir arī relatīvi nelielā inficēšanās ar lapu slimībām, vārpu produktivitāte un augstā graudu tilpummasa. Šķirnei ‘Rubiola’ SĪN (saimniecisko īpašību noteikšanas) un AVS (atšķirīguma, viendabīguma un stabilitātes) pārbaudes uzsāktas no 2007.gada.

## EVALUATION OF SPRING BARLEY MALTING VARIETIES FOR BREEDING PROGRAMMES

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### Abstract

During the period 2005-2007 grain yield stability and the malt quality characteristics of malting spring barley varieties were investigated at the Lithuanian Institute of Agriculture. The growing conditions in 2005 and 2006 were dry and in 2007 were rather wet compared with the long-term mean. The high temperatures and drought in June of 2006 impacted low grain >2.5 mm yield and high protein content. The relationships between the lengths of spring barley growth periods and quality traits were evaluated. Using the software STABLE the stability of malting barley grain, coarse grain and extract yields as affected by the weather conditions during the crop year, genotype properties for varieties, as well as the interactions of variety and weather conditions were estimated. The selection of varieties promising in terms of grain yield, >2.5 mm grain yield and extract yield, was based on their ability to realize their genetic potential in various meteorological

conditions. Consequently, the highest sum of integral assessment of grain yield and grain >2.5 mm yield was identified for the varieties 'Tocada', 'Justina', 'Cruiser', 'Scarlett' and 'Annabell'. The varieties which exhibited high grain yield stability and were distinguished by high grain quality, disease resistance and other agronomic traits were involved in further breeding programmes.

**Key words:** *Hordeum vulgare*, L., disease resistance, yield stability, malting quality traits

## Introduction

The agromorphological and grain quality traits of barley are commonly influenced by environmental factors and growing conditions and therefore are of limited use for assessing the levels of variability. The genotypic peculiarities of a variety and the climatic conditions over a growing period are the key factors influencing grain yield and quality (Tamm, 2003; Paynter and Young, 2004; Lazauskas *et al.*, 2005). The evaluation of the genetic diversity of initial material and the matching of individuals with suitable traits for the malting barley breeding programmes are of primary importance (Bail and Meynard, 2003). The coarse grain output (grain on 2.5 x 20 mm sieve) is a specific requirement for malting barley. According to the present brewing requirements it must be not less than 90.0 %. The weather humidity and temperature over the grain filling period affect it (Paynter and Young, 2004; Passarella *et al.*, 2005). Low grain protein content positively correlates with good malt quality (Eagles *et al.*, 1995; Molina-Cano *et al.*, 1997; Leach *et al.*, 2002; Zhang *et al.*, 2006). A strong correlation exists between grain protein content and extract content. Spring barley varieties differ among themselves according to their reaction to the growing conditions. Therefore the variation of yield and grain quality traits is wide in range (Mašauskienė *et al.*, 2001; Costa and Bollero, 2001). In Lithuania, temperatures noticeably vary during the summer months and every second or third summer is much colder or much hotter than the long-term mean (Galvonaitė *et al.*, 2007). Therefore the weather conditions during the growing period are among the factors of primary importance which impact malting barley yield and especially grain and malt quality.

Even though the yield components of cereals are partly determined in the vegetative stage, the actual process of grain formation and filling takes place between the heading and physiological maturity stages. Distinctly shorter grain filling periods of barley were observed under drought stress (Long *et al.*, 1998). Low yields of barley have often been observed when grain filling was shortened as a consequence of high temperatures and/or insufficient water supply (Savin *et al.*, 1997a). Besides yield reductions, significant negative effects on malting quality have been observed as a consequence of stress situations during grain filling. Increased protein concentrations in the grain have been found in association with elevated temperatures and/or drought stress (Savin and Nicolas, 1996). A smaller average grain size has been related to stress factors.

Grain yields over a wide range were relatively independent from the total precipitation in the time span from January 1 to yellow ripeness. High temperatures also tended to reduce grading percentages and to a smaller extent increase protein concentrations. Precipitation amounts within certain periods are not as decisive as the occurrence of drought stress (Schelling *et al.*, 2003).

However, moist weather also favours rapid development of fungal pathogens. Net blotch infection suppresses plant height and reduces 1000-grain weight (Robinson, 2000). Khan (1987) established a statistically significant relationship between yield loss and net blotch infection under Australian conditions using values for mean foliar damage on the top three leaves.

In Lithuania the sufficiently humid (normal monthly amount of precipitation in June and July is 69-79 mm) climate and warm summers enable barley to perform well. Therefore the investigation of varieties and their suitability for malting barley breeding programmes is of great relevance. The main objectives of this study is to evaluate phenological traits as well as grain, coarse grain and the extract yield stability of spring barley genetic resources and to select the most suitable varieties for malting barley breeding.

## Materials and Methods

During the period 2005-2007, malting spring barley varieties were studied for yield, disease resistance and malt quality characteristics at the Lithuanian Institute of Agriculture. The genotypes tested were Lithuania-registered varieties, varieties from genetic resources collections, and new

varieties for initial breeding. The experimental plot for each cultivar was 20 m<sup>2</sup>. The plots were arranged in a random design with four replications. The soil of the experimental site was sod gleyic (*Endocalcari-Endohypogleyic Cambisol* (CMg-n-w-can) light loam, with a clay content of 240-270 g kg<sup>-1</sup>, pH<sub>KCl</sub> 6.0-7.0. The organic matter by Tyurin in the plough layer was 25-27 g kg<sup>-1</sup>; content of available P – 83-109 and K – 166-216 mg kg<sup>-1</sup> by Egner-Riehm-Domingo. Fertilizers at a rate of N<sub>90</sub>P<sub>60</sub> K<sub>60</sub> were applied annually before seeding.

The period 2005-2007 was rather favourable for spring barley versatile evaluation because of the variable weather conditions. The conditions for seed germination and initial growth were favourable, but the growth period from booting till maturity in 2005 was warm and dry, in 2006 it was hot and dry and in 2007 - warm and wetter than the long-term mean.

In the trials we evaluated grain yield (t ha<sup>-1</sup>), 1000 kernel weight (TKW) (g), hectolitre weight (HLW) (g l<sup>-1</sup>) and grain grading >2.5 mm (%). Malt extract content (%), protein content (g kg<sup>-1</sup>) and starch content (g kg<sup>-1</sup>) were calculated in dry matter. Protein content was measured by the Kjeldahl method, starch content by hydrochloric acid dissolution. Malt extract content was determined on the basis of EBC (Analytica-EBC, 1987). Coarse (>2.5 mm) grain and malt extract yields per hectare were calculated. During the growth period earliness was defined as the number of days from germination (BBCH 10) to heading (BBCH 58) (pre-anthesis period) and the post-anthesis period was marked as the number of days from heading to maturity (BBCH 89).

Resistance to diseases was estimated. Powdery mildew (*Blumeria graminis* DC) and net blotch (*Pyrenophora teres*) were assessed once at the plant early-medium milk development stage (BBCH 73-75). The severity of the diseases were measured in scores, using the scale 1-9, where 1 meant full resistance, and 9 - complete susceptibility.

The level of statistical significance of data was calculated by the analysis of variance using the software package STATISTICS. The coefficients of linear correlation, trait mean  $\bar{x}$ ,  $\left[ \frac{\sigma_y}{\sigma_x} \right]$  and the least significant difference LSD<sub>0.05</sub> was introduced. The stability of traits was evaluated by the mathematical model STABLE (Kang, Magari, 1995) adapted at LIA by P. Tarakanovas (2004) and used for the analysis of varietal grain yield stability.

## Results and Discussion

Grain yield, 1000 kernel weight, coarse grain, extract and the starch percentage of malting barley varieties were significantly and negatively impacted by the length of the growing period from germination to heading (Table 1).

Table 1. Phenotypic correlation coefficients between the length of growth stages, resistance to diseases and spring barley grain yield and quality, Dotnuva, 2005-2007.

Trait	Grain yield, t ha <sup>-1</sup>	Grain grading, % (>2.5 mm)	Extract content, %	1000 kernel weight, g	Hectoliter weight, g l <sup>-1</sup>	Protein content, g kg <sup>-1</sup>	Starch content, g kg <sup>-1</sup>
Germination – heading, days	-0.267*	-0.493**	-0.254*	-0.392**	-0.194	0.186	-0.254*
Heading – maturity, days	-0.438**	0.565**	0.638**	0.345**	0.156	-0.847**	0.472**
Resistance to powdery mildew, scores	-0.121	0.311*	0.086	0.271*	0.227	-0.196	0.086
Resistance to net blotch, scores	-0.431**	0.034	0.485**	-0.159	0.076	-0.649**	0.485**

Significant at \*P<0.05; \*\*P<0.01

However, the influence of the post-anthesis period was much more substantial on these indices than that of pre-anthesis period. The resistance to powdery mildew impacted grain grading and 1000 kernel weight. The higher grain yield matured and a higher protein content was accumulated for resistant to net blotch varieties, but the extract percentage in that case was lower. It led to the

explanation that the number of grain per spike for resistant varieties might have been too high and therefore the smaller-sized grain matured compared with the varieties affected by the net blotch. The correlation between the length of the growing periods and resistance to disease was insignificant.

Varieties of Lithuania origin 'Alsa', 'Aura DS', 'Aidas' had the highest protein content and the varieties 'Ūla', 'Luokė' were characterized by lower protein content (Table 2).

Table 2. The characteristics of grain quality and disease resistance of spring barley varieties, Dotnuva, 2005-2007.

Variety	Country of origin*	Duration in days		Resistance to diseases in scores**		Grain quality traits		
		BBCH 10 – 58	BBCH 10 – 89	Powdery mildew	Net blotch	1000-kernel weight, g	Protein, g kg <sup>-1</sup>	Starch g kg <sup>-1</sup>
Auksiniai 3	LT	41.7	80.7	4.7	2.7	44.7	129	608
Aidas	LT	43.7	83.0	4.3	2.0	47.1	135	585
Luokė	LT	40.7	80.3	5.3	2.3	49.7	127	564
Ūla	LT	40.7	80.3	5.3	2.7	51.2	131	587
Aura DS	LT	43.3	81.0	5.0	2.3	48.6	134	575
Alsa	LT	43.7	82.3	4.0	2.7	46.4	136	590
Henni	DE	42.3	80.7	4.3	2.7	44.8	127	600
Annabell	DE	42.7	79.3	3.7	2.3	43.4	132	605
Scarlett	DE	43.3	79.3	2.3	5.3	41.9	124	586
Pongo	SE	42.7	80.7	1.3	3.7	46.9	124	585
Jersey	NL	43.0	82.0	4.0	2.3	47.3	133	577
Tolar	NL	41.0	80.3	1.3	4.0	48.5	128	588
Prestige	FR	41.0	78.7	1.3	3.0	45.8	127	596
Breamer	UK	41.7	79.7	1.3	4.0	48.0	131	607
Barke	DE	41.7	79.7	1.7	3.3	46.4	124	587
Justina	DE	42.0	79.3	4.0	2.7	44.2	123	618
Sebastian	DK	42.7	81.3	3.0	3.7	47.3	128	593
Tocada	DE	43.7	81.7	2.7	3.3	45.8	127	598
Class	FR	42.3	80.0	1.0	6.7	46.5	126	591
Cruiser	DE	42.7	80.0	1.0	3.7	44.7	128	598
Power	UK	41.0	79.0	2.3	3.3	44.1	125	601
$\bar{x}$		42.25	80.44	3.05	3.27	46.35	128.4	592.3
LSD <sub>0.05</sub>		1.492	1.726	1.875	1.326	3.071	11.82	22.04

\* DE – Germany, DK – Denmark, FR – France, LT – Lithuania, NL – the Netherlands, SE – Sweden, UK – United Kingdom.

\*\* 1 - full resistance, 9 - complete susceptibility.

The low protein content could be an inherent property of both these varieties. However, their resistance to powdery mildew was low. According to the protein percentage almost every registered foreign malting variety could be used as a donor for the development of the malting barley lines. However, their resistance to net blotch should be taken into account because of their negative relationship with grain yield. The varieties 'Annabell' and 'Tocada' demonstrated satisfactory resistance to both diseases. Net blotch infection was most severe in 2007. Therefore, although the weather conditions were conducive to grain formation, all the varieties investigated formed better grain >2.5 mm yield in 2005, except for 'Annabell' and 'Tolar'. They yielded better in 2007. Disease infection in 2006 (powdery mildew, net blotch and leaf spots) were low because of the high temperatures and drought. But this did not have any impact on yield as lack of



precipitation was the main limiting factor. The resistance to net blotch positively correlated with the extract and starch content and negatively with the protein content.

Table 3. Mean-square of the analysis of variance of spring barley grain, grain >2.5 mm and extract yields, Dotnuva 2005-2007.

Source of variability	DF	Mean square of the yield (MS)		
		grain t ha <sup>-1</sup>	grain >2.5 mm t ha <sup>-1</sup>	extract t ha <sup>-1</sup>
Varieties (V)	21	2.501**	1.498	2.465**
Year (Y)	3	3,237.368**	1,812.767**	485.574**
Interaction (VxY)	62	63.849**	23.908**	12.9005**
Heterogeneity	20	0.211	0.789	0.186
Standard error	180	0.007	0.026	0.019

Significant at \*P<0.05; \*\*P<0.01

According to grain yield an especially high assessment was given for the 'Tocada', 'Justina', 'Cruiser', 'Annabel', 'Power', 'Tolar' and 'Henni' (Table 4). These varieties combined high yield with a low variance of stability. They were developed in countries that have warm and wet weather conditions. Therefore there is some uncertainty about their properties in relation with climate warming.

Table 4. Assessment of spring barley varieties according to yield, grain >2.5 mm and extract yield and stability, Dotnuva, 2005-2007.

Variety	Grain yield, t ha <sup>-1</sup>		Grain>2.5 mm yield, t ha <sup>-1</sup>		Extract yield, t ha <sup>-1</sup>	
	Means	YS(i)*	Means	YS(i)*	Means	YS(i)*
Auksiniai 3	3.644	-8	3.071	-4	2.869	-6
Aidas	3.324	-10	2.448	-2	2.514	-6
Luokė	3.904	1	3.229	7+	2.954	4
Ūla	3.671	-7	3.167	4	2.762	-9
Aura DS	3.898	0	3.163	3	2.999	1
Alsa	3.763	-4	2.988	-6	2.878	-5
Henni	4.092	10+	3.177	5	3.188	8+
Annabell	4.306	13+	3.308	14+	3.364	18+
Scarlett	3.921	2	2.578	-9	3.148	7+
Pongo	3.584	-9	3.389	11+	2.830	-7
Jersey	3.944	4+	3.416	12+	3.110	4
Tolar	4.254	11+	3.130	5	3.358	11+
Prestige	3.695	-6	3.147	8+	2.906	-4
Breamer	3.742	-5	3.281	8+	2.932	5
Barke	3.773	5+	3.443	14+	2.980	8+
Justina	4.429	15+	3.142	-1	3.397	15+
Sebastian	3.924	3	3.581	24+	3.112	5
Tocada	4.525	16+	3.214	6+	3.553	24+
Class	3.986	7+	3.136	-2	3.122	10+
Cruiser	4.311	14+	3.507	23+	3.382	17+
Power	4.256	12+	2.834	-8	3.387	14+
LSD <sub>0,05</sub>	0.068		0.130		0.129	

\*- The varieties that surpassed the average integral evaluation YS(i) of the trial are indicated by (+)

The lowest assessment for grain yield stability was obtained for the Lithuanian varieties. However over the experimental period the grain yield and yield stability for the modern varieties 'Luokė' and 'Aura DS' was higher than that for 'Pongo', 'Prestige' and 'Breamer'.

Grain grading is an important parameter both for food and malt barley. A high integral assessment for this parameter was given to the varieties 'Sebastian', 'Cruiser', 'Barke', 'Annabell', 'Jersey',

'Pongo'. The integral assessment of course grain output per hectare for 'Luokė' was the highest among Lithuania-bread varieties. For the extract yield varieties 'Tocada', 'Annabell', 'Cruiser', 'Justina', 'Power' and 'Tolar' did well.

Over the experimental period the crop-year weather conditions varied considerably. Despite the fact that according to the averaged three years data the growth period of spring barley varieties varied between 79-83 days, the length of that period among varieties in some years was greater.

As a result, the impact of length of specific growth periods on yield and grain qualities was noted. The influence of the post-anthesis period was much more substantial on these indices than that of the pre-anthesis. In Lithuania's weather conditions the long post-anthesis period is frequently related to wet and cloudy weather which is favourable for starch and unfavourable for protein synthesis, therefore the long post-anthesis period positively affected the traits which are important for spring barley malting qualities. In several studies low barley grain yield has been related to the short duration of the heading-maturity period (Savin *et al.*, 1997a; Savin *et al.*, 1997b, Schelling *et al.*, 2003). According to the climate classification Lithuania belongs to the southwestern sub area of the Atlantic wood continental area. Only the Lithuanian coast of the Baltic Sea from the point of climate is similar to Western Europe and can be linked to the separate South Baltic sub area (Galvonaite *et al.*, 2007). However, the climate is influenced by the global warming phenomena. Our study showed that the influence of the weather conditions on spring barley yield and malting qualities is much more considerable than that of genotype properties. As a result, the genotypes with shorter pre- and post-anthesis and simultaneously low protein content like 'Scarlett', 'Cruiser' or 'Justina' will have priority. The Lithuanian varieties were more often characterized by longer growth and post-anthesis periods than the other investigated ones, but the grain yield and malting qualities were lower. The varieties 'Annabell' and 'Tocada' demonstrated satisfactory resistance to powdery mildew and net blotch. The selection of varieties promising for malting barley breeding programmes in terms of grain yield, >2.5 mm grain yield and extract yield stability was based on their ability to realize the genetic potential in various growing conditions. Consequently, the highest sum of integral assessment of grain yield and grain >2.5 mm yield was identified for the varieties 'Tocada', 'Justina', 'Cruiser', 'Scarlett' and 'Annabell' and therefore these genotypes are acceptable for the breeding of varieties intended for Lithuanian weather conditions.

## Conclusions

The genotypes with shorter pre- and post-anthesis and simultaneously low protein content will have priority in breeding programmes for growing in the south-west sub area of the Atlantic continental forest area. The influence of the length of the post-anthesis period on grain yield and malting qualities was more pronounced than that of the pre-anthesis.

The impact of varietal resistance to net blotch on grain yield, protein, starch, extract content was greater than that of their resistance to powdery mildew.

According to their ability to realize their genetic potential in various weather conditions in terms of grain yield, >2.5 mm grain yield and extract yield stability the varieties 'Tocada', 'Justina', 'Cruiser', 'Scarlett' and 'Annabell' are best for breeding programmes in Lithuanian meteorological conditions.

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## VASARAS MIEŽU ALUS ŠĶIRŅU IZVĒRTĒJUMS SELEKCIJAS PROGRAMMU VAJADŽĪBĀM

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Laika periodā no 2005. līdz 2007. gadam Lietuvas Zemkopības institūtā tika pētīta vasaras alus miežu šķirņu graudu ražas stabilitāte un iesala kvalitātes pazīmes. Augšanas apstākļi 2005. un 2006. gadā bija sausi, bet 2007. gadā tie bija samērā lietaini, salīdzinot ar ilglaicīgi novērotajiem vidējiem rādītājiem. Augstās temperatūras un sausuma ietekmē 2006. gadā veidojās zema graudu frakcijas >2.5 mm raža un augsts proteīna saturs. Tika novērtētas sakarības starp vasaras miežu veģetācijas perioda garumu un kvalitātes pazīmēm. Izmantojot datorprogrammu STABLE, tika noteikta alus miežu graudu, rupjās graudu frakcijas un ekstraktvielu ražas stabilitāte audzēšanas gadu meteoroloģisko apstākļu, šķirņu genotipisko īpašību, kā arī šķirņu un meteoroloģisko apstākļu mijiedarbības ietekmē. Tika veikta daudzsološāko šķirņu atlase pēc graudu, graudu frakcijas >2.5 mm un ekstraktvielu ražas, pamatojoties uz šķirņu spēju realizēt ģenētisko potenciālu dažādos meteoroloģiskos apstākļos. Rezultātā augstākā integrālā novērtējuma summa graudu ražai un graudu frakcijas >2.5 mm ražai tika konstatēta šķirnēm ‘Tocada’, ‘Justina’, ‘Cruiser’, ‘Scarlett’ un ‘Annabell’. Šķirnes ar augstu graudu ražas stabilitāti, izturību pret slimībām un citām agronomiskām īpašībām tika tālāk izmantotas selekcijas programmās.

## RENEWAL AND THE MOLECULAR CHARACTERISATION OF THE LATVIAN MELON (*Cucumis melo* L.) GENETIC RESOURCES

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### Abstract

Latvia is at the northern boundary of melon (*Cucumis melo* L.) growing in open-field conditions. The first local varieties were bred at the beginning of the 20th century using Russian and Western European varieties. These varieties were well adapted to Latvian climatic conditions, with tolerance to low temperature and resistance to different pathogens. In the following years seed production was of insufficient quality and therefore genotypes were destroyed because of the cross-pollination of different varieties. The homogenisation and dividing of the local melon population into distinctive lines was started in 2003 at the Pūre Horticultural Research Station with the aim to derive homogeneous lines that had excellent taste and rich aroma and characteristics. That included early ripening and high resistance to pathogens.

In 2006 and 2007, five of the most promising inbred lines and sibilines were evaluated according to phenology, morphological and organoleptical features. Molecular markers (SSRs) were used to fingerprint these melon lines acquired from the local population and Europe. Successive generations of inbred lines were fingerprinted to determine the success of homogenization. Using SSR fingerprinting, the genetic diversity and relationships within the Latvian melon germplasm could be determined.

**Key words:** morphological markers, molecular markers, ssr, inbreeding, sibling, fingerprinting

### Introduction

At the beginning of the last century the melon growing boundary rapidly spread to the northern regions of Europe (Sukatnieks, 1954). Latvia is at the northern boundary of melon (*Cucumis melo* L.) growing in open-field conditions. At the beginning of the 20th century the first local varieties in Latvia were bred utilising both Russian and Western European genetic material. These varieties were well adapted to Latvian climatic conditions combining earliness, high tolerance to low temperature, sharp fluctuations of temperature and late spring frosts, as well as resistance to different pathogens. In addition, the taste of these varieties was characterised as good. Latvian melons were maintained by enthusiasts in home gardens, without ensuring proper seed production. Due to the outcrossing nature of melons (McCreigh, 2000), these locally adapted varieties were lost. During the 1990s the enthusiast Ē. Piļka and the breeders U. Dēķens and I. Drudze started to grow the local melon material retained by the gardening enthusiast P. Sukatnieks. At the beginning the seed material gave very uneven plants: from very early and tasty to late and totally inedible. In 2003 the material was sown in the Pūre Horticultural Research Station with the aim of clarifying the homogeneity of the material and to evaluate the morphological features of the genotypes. According to the results obtained, it was determined that homogenisation was necessary. So in 2003 inbreeding and sibling crossing was started with the aim of obtaining homogeneous melon lines. Melon lines acquired from the local population and also European and USA varieties were analysed with simple sequence repeat (SSR) markers to distinguish their genetic relationships, and to assess the success of homogenisation.

### Material and Methods

The samples were collected from the growers in the Latgale region and during 2003 - 2007, in the Pūre Horticultural Research Station. Close related breeding (inbreeding and sibling) was done for the homogenisation of the material. The seeds of the local melon accessions were sown in plastic

pots in peat substrate in mid April, and transplanted into a plastic tunnel mid-May. Plants were grown in peat substrate and spaced 1 x 0.5 m. Ten plants from each line or variety were planted. Plants were tied up on the support system, pruned so that two fruit yielding side branches were left. Agro-ecological conditions were ensured according to the melon requirements. Every season the phenological and morphological features of the plants were recorded and evaluated: germination speed, days from sowing to flowering and first harvesting. According to the UPOV descriptors colour immature and mature fruit shape was determined, grooving, the colour of the grooves, weight, cork netting and organoleptic evaluation was carried out for the taste and aroma of the mature fruit, the content of soluble solids was measured according to the Brix scale and flesh softness measured by a penetrometer. Five fruits per plant were evaluated. The plants with the highest quality fruits were selected for further homogenisation by inbreeding and sibling crossing. Closely related pollination was continued for four years.

DNA was extracted by using the "Fermentas" DNA purification kit. SSR primer sequences were obtained from Danin-Poleg *et al* (2001) and Katzir *et al* (1996) (Table 1). The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a fluorescent sequencer. The PCR reactions contained: 20 - 50 ng DNA, 10 x Taq buffer with KCl - MgCl<sub>2</sub>, 2.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP Mix, 0.5 u Taq DNA Polymerase (recombinant), 0.2µM each primer (forward and reverse) in a final volume of 25 µl. PCR was conducted in the following conditions: denaturation at 94°C for 2 min, 35 cycles of denaturation at 94 °C for 45 sec, annealing at 51 °C, 45 sec, elongation at 72 °C for 45 sec, final elongation at 72 °C for 5 min. PCR products were analysed using an ABI 3100 capillary DNA sequencer and genotyped using GeneMapper software. Genetic analyses were conducted using GenAlEx (Peakall and Smouse, 2006), and NTSYSpc 2.11.

Table 1. SSR primers utilised in this study

Primer	Sequence	Label	Repeat	Reference
CMCTT144F	CAAAAGGTTTCGATTGGTGGG	6-FAM	(CTT) <sub>10</sub> CTAC(CTT) <sub>4</sub>	Danin-Poleg et al 2001
CMCTT144R	AAATGGTGGGGTTGAATAGG			
CMACC146F	CAACCACCGACTACTAAGTC	HEX	(ACC) <sub>9</sub>	Danin-Poleg et al 2001
CMACC146R	CGACCAAACCCATCCGATAA			
CMGA104F	TTACTGGGTTTTGCCGATTT	NED	(GA) <sub>14</sub> AA(GA) <sub>3</sub>	Danin-Poleg et al 2001
CMGA104R	AATTCCGTATTCAACTCTCC			
CMAT141F	AAGCACACCACCACCCGTAA	6-FAM	(AT) <sub>7</sub> (GT) <sub>6</sub>	Danin-Poleg et al 2001
CMAT141R	GTGAATGGTATGTTATCCTTG			
CMCCA145F	GAGGGAAGGCAGAAACCAAAG	HEX	(CCA) <sub>5</sub>	Danin-Poleg et al 2001
CMCCA145R	GCTACTTTTGTGGTGGTGG			
CMTC13F	TGGATGGATAAGGTGGTAAG	NED	(TC) <sub>12</sub> (CG) <sub>5</sub> (AG) <sub>3</sub>	Katzir et al 1996
CMTC13R	TTCCCCTAGTCGCTCTCT			
CMGT108F	CTCCTTCAAACATTGTGTGTG	6-FAM	(GT) <sub>9</sub> N <sub>65</sub> (CT) <sub>7</sub>	Danin-Poleg et al 2001
CMGT108R	GAGATAGGTATAGTATAGGGG			
CMCT134bF	GCTCCTCCTTAACTCTATAC	HEX	(TA) <sub>2</sub> (CT) <sub>8</sub> (AT) <sub>7</sub>	Danin-Poleg et al 2001
CMCT134bR	GCATTATTACCCATGTACGAG			
CMCT44F	TCAACTGTCCATTTCTCGCTG	NED	(CT) <sub>10</sub> TGTT(CT) <sub>3</sub>	Danin-Poleg et al 2001
CMCT44R	CCGTAAAGACGAAAACCC TTC			
CMTC168F	ATCATTGGATGTGGGATTCTC	6-FAM	(TC) <sub>14</sub>	Danin-Poleg et al 2001
CMTC168R	ACAGATGGATGAAACCTTAGG			
CMCT170bF	ATTGCCCAACTAACTAAACC	HEX	(CT) <sub>8</sub>	Danin-Poleg et al 2001
CMCT170bR	CACAACAATATCATCCTTG			
CMAG59F	TTGGGTGGCAATGAGGAA	NED	(GA) <sub>2</sub> A(AG) <sub>8</sub>	Katzir et al 1996
CMAG59R	ATATGATCTTCCATTTCCA			
CMTC47F	GCATAAAAGAATTTGCAGAC	6-FAM	(TC) <sub>9</sub> (CT) <sub>6</sub>	Danin-Poleg et al 2001
CMTC47R	AGAATTGAGAAGAGATAGAG			
CMGA15F	CGGCAAGACGATTGGCAGC	HEX	(GA) <sub>7</sub>	Danin-Poleg et al 2001
CMGA15R	ATCACCGTAGCGAAGCACC			
CMTA170aF	TTAAATCCCAAAGACATGGCG	NED	(TA) <sub>9</sub> T(TA) <sub>3</sub>	Danin-Poleg et al 2001
CMTA170aR	AGACGAAGGACGGTTAGCTTT			

## Results and Discussion

At the beginning of the phenotypic investigations in 2003, very high heterogeneity was determined in local melon lines. In 2004, the lines of the 1<sup>st</sup> inbred generations had less homogeneity, but it was still notable. Distances and relationships between the plants of the investigated lines according to 16 phenotypic traits are shown in Figure 1. In 2005 lines were less heterogeneous according to the phenotypic traits (data not shown). In 2006 and 2007, the phenotypic variation within the lines was eradicated – all the lines consisted of morphologically homogeneous plants.

The phenotypic analysis of the inbred Latvian melon lines revealed differences between lines 4(3), 5(2), 8 and 14. Lines 4(3) and 5(2) are morphologically the most similar – fruits with yellow skin, oval shape and medium cork netting. The flesh is orange and floury with average taste. These two lines differ according to earliness – line 4(3) is earlier than 5(2), but nevertheless both these lines are the earliest of all melon lines. Line 8 is characterized by a small, round, grey-green fruit with darker grey grooves. The flesh is orange, juicy, aromatic and sweet. Line 14 is characterized by small (up to 14cm diameter), round fruit, with yellow-green skin and green coloured grooving. The flesh is green, soft, sweet and juicy. Earliness of lines 8 and 14 is evaluated as mid-early.

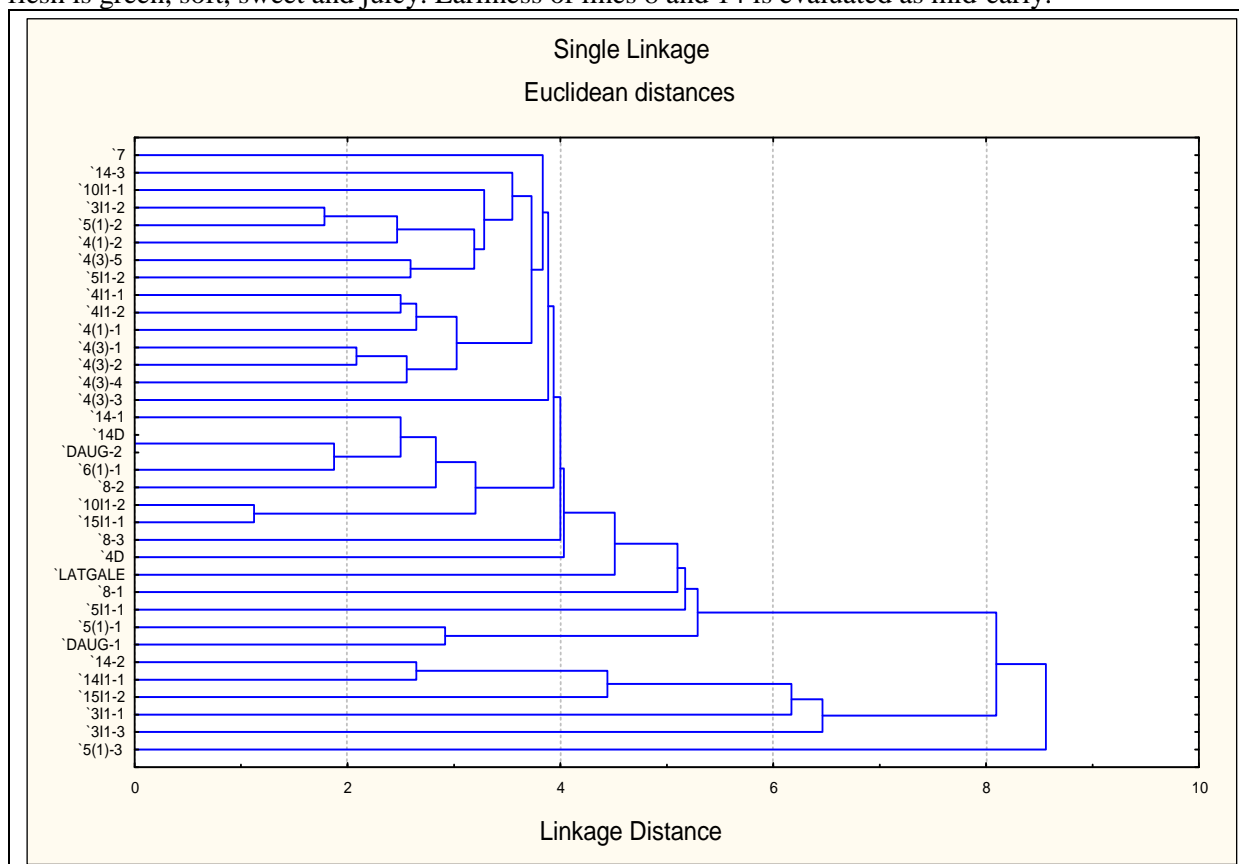


Figure 1. Relationships between lines in 2004 according to morphological traits

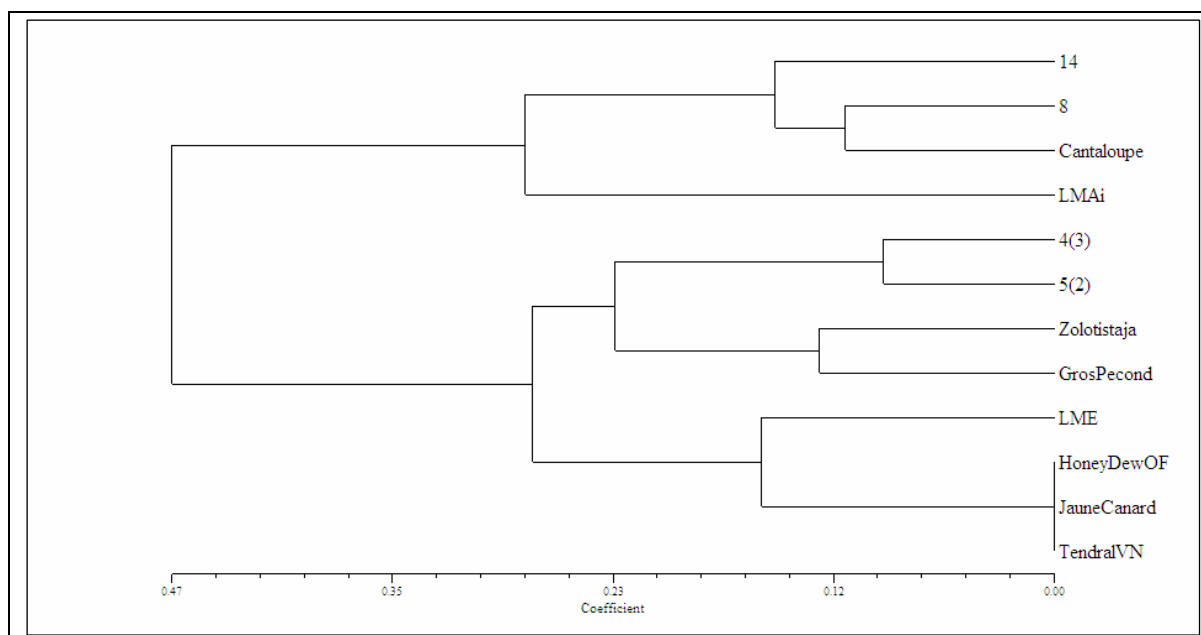
In 2006, a total of 15 SSR primer pairs were used to analyse bulked DNA from 8 melon lines and varieties. These included the local lines 4(3), 5(2) and 8, as well as the southern European and American varieties ‘Cantaloupe De Bellagarde’, ‘Gros Pecond’, ‘Honey Dew Orange Flesh’, ‘Jaune Canaris’ and ‘Tendral Verde Negro’. Of the 15 loci, only 10 were polymorphic in the lines tested. The number of alleles detected by the polymorphic loci ranged from 2-7 (mean 2.67) and the polymorphism information content (PIC) values ranged from 0.13-0.83, (mean 0.59) (Table 2). Table 2. Allele number, polymorphic information content (PIC) and observed heterozygosity for the 15 SSR loci surveyed

Locus	Allele #	PIC	Obs het
CMCTT144	4	0.72	0.00
CMACC146	1	-	-
CMGA104	3	0.65	0.00
CMAT141	1	-	-

CMCCA145	1	-	-
CMTC13	3	0.59	0.00
CMGT108	2	0.13	0.14
CMCT134b	5	0.78	0.00
CMCT44	1	-	-
CMTC168	2	0.43	0.13
CMCT170b	4	0.72	0.00
CMAG59	3	0.61	0.00
CMTC47	2	0.47	0.00
CMGA15	1	-	-
CMTA170a	7	0.83	0.71
Mean	2.67	0.59	0.10

Of the 10 polymorphic loci, heterozygous lines were detected by only 3 loci (CMGT108, CMTC168 and CMTA170a). As bulked DNA was used from the lines, at this point it is not possible to determine if these represent heterozygous loci within the individuals, or loci segregating within the lines.

In 2007, SSR analysis was undertaken using DNA extracted from individual plants rather than bulked samples. The lines 14i3, D14i3, 4(3)Ai4, 4(3)i2, 4(3)i3, 5(2)i2, 5(2)i3, 8i2, 8i2herm, 8i3, LMAi, LME were tested and a Russian cultivar 'Zolotistaja' was also analysed. 11 SSR markers were tested (CMACC146, CMGA104, CMAT141, CMCCA145, CMGT108, CMCT44, CMTC168, CMCT170b, CMAG59, CMGA15). Of these, six were polymorphic on the tested material (CMGA104, CMGT108, CMCTT144, CMTC168, CMCT170b and CMAG59). The heterozygosity was very low, with only four heterozygous loci detected (8i2/CMGT108; LME and 'Zolotistaja'/CMCTT144; LMAi/CMAG59). Only one of these lines was in the process of being homogenised (8i2), and in the next inbred generation (8i3), this locus was homozygous. The genetic relationships of these Latvian line and the other cultivars analysed is shown in Figure 2.



**Figure 2. UPGMA dendrogram of genetic distances between Latvian melon lines and other varieties. Genetic distances were calculated using the proportion of shared alleles.**

At the beginning of the evaluation of the Latvian melon genetic resources collection each line was split into two or three phenotypes according to their morphological traits, such as fruit size, shape, colouring, groove, cork netting, flesh colour and thickness, skin thickness, taste and aroma. Inbreeding and sibling crossing increased the homogeneity of the samples with every generation. The lines with the best horticultural traits were 8 and 14, and as they also had tasty fruits, they were determined as the most promising for further breeding.

Genetic diversity among melon cultivars has been reported as being high, especially when detected by SSR markers (Danin-Poleg *et al*, 2001; López-Sesé *et al*, 2002; Nakata *et al*, 2005). Overall, we detected similar numbers of alleles per SSR marker, and the PIC values and heterozygosity levels were similar to those reported. Other markers types such as RAPDs and RFLPs were found to detect low levels of polymorphism within cultivated melons (Katzir *et al*, 1996). However, we found low heterozygosity in the material tested, even when we were using bulked material from the early stages of line homogenisation. The SSR markers utilised were sufficient to distinguish all the lines and cultivars tested, therefore, for our purposes of genetic fingerprinting and assessment of line homogenisation, the markers tested were sufficiently polymorphic.

LMAi and LME are samples collected from a garden in Latgale, and are probably descended from the original Latvian-bred cultivars. However, they have not been propagated via controlled crosses, and therefore cannot be considered as pure lines or cultivars. This is reflected in the fact that two of the total four heterozygous SSR markers were found in these lines. The genetic relationships of the Latvian lines corresponds to their phenotypic characteristics, with lines 4(3) and 5(2) grouping together, while the lines 8 and 14 also were found in the same cluster. These two groups were found on separate clusters on the dendrogram. The line LMAi and the variety 'Canteloupe de Bellagarde' clustered with lines 8 and 14, while the other tested varieties and the line LME clustered with the lines 4(3) and 5(2). This dissimilarity of the genetic relationships with the region of origin could be indicative of the diverse origins of Latvian melon genetic resources, in which European and Russian germplasm was combined. The clustering of genotypes in relation to the melon morphological types is very weak.

The Russian cultivar 'Zolotistaja', while not known to be directly utilised in the development of the original Latvian melon cultivars, was included in this study in order to investigate the genetic relationship of this cultivar with the Latvian germplasm and other varieties.

The amount of intra-varietal polymorphism was not directly assessed at this stage of the project, however, the low level of heterozygosity, as well as the lack of phenotypic variation within the lines, indicates that these lines are approaching homogeneity. Further work will extend these fingerprinting analyses to further Latvian melon lines, as well as investigate intra-varietal polymorphism. Due to the low number of alleles and low heterozygosity detected by these SSR markers, it may be necessary to increase the number of SSR loci surveyed in order to distinguish closely between the related melon lines. These SSR markers have been used to fingerprint the Latvian melon germplasm, and to assess the success of melon line homogenisation. In the future, these markers can also be used in the Latvian breeding program to assess levels of genetic diversity, and to allow the targeted introgression of genetic material in order to broaden the genetic base of Latvian melon germplasm.

#### Acknowledgements:

This project was funded by the Latvian Ministry of Agriculture.

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## LATVIJAS MELOŅU (*Cucumis melo* L.) ĢENĒTISKO RESURSU ATJAUNOŠANA UN MOLEKULĀRAIS RAKSTUROJUMS

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Latvija ir meloņu (*Cucumis melo* L.) izplatības areāla ziemeļu robeža Eiropā, kur iespējams melones audzēt lauka apstākļos. 20.gs sākumā, izmantojot Rietumeiropas un Krievijas materiālu, tika izveidotas pirmās vietējās šķirnes. Izveidotās šķirnes bija labi piemērotas Latvijas apstākļiem – salcietīgas un izturīgas pret slimībām. Turpmākos gados netika veikta kvalitatīva sēklu ieguve un šķirnes pamazām izvirta nekontrolētas savstarpējās apputeksnēšanās rezultātā.

2003.g. Pūres Dārzkopības izmēģinājumu stacijā tika uzsākta meloņu populāciju sadalīšana līnijās un to homogenizēšana ar mērķi iegūt agras, viendabīgas un izturīgas pret slimībām meloņu līnijas, kuru augļi izceltos ar labām garšas īpašībām un aromātu.

2006.g. un 2007.g. piecas perspektīvākās meloņu līnijas tika izvērtētas pēc fenoloģiskajām, morfoloģiskām īpašībām un to augļu kvalitātes. Šīs līnijas tika pasportizētas ar molekulāriem marķieriem, tām izpētīta ģenētiskā daudzveidība un savstarpējā saistība. Lai noskaidrotu homogenizācijas gaitu un pakāpi, ar molekulārajiem marķieriem tika pētītas dažādas līniju paaudzes.

## THE MAIN TRAITS OF WINTER WHEAT FOR BREEDING FOR ORGANIC FARMING IN LITHUANIA

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### Abstract

The investigations were done at the Lithuanian Institute of Agriculture in the field certified for organic agriculture during 2006-2007. Winter wheat genotypes were tested in 2006 and 2007. Overwintering in most cases strongly correlated ( $r=0.71-0.81$ ) with spring re-growth, and later soil covering intensity. Yield medium correlated ( $r=0.60$ ) with overwintering, and strongly correlated with spring re-growth ( $r=0.79$ ) in 2006 when winter frosts damaged the wheat. Analysis of data of 2007 revealed lower correlations among traits. Intensive autumn growth weakly correlated ( $r=-0.48 - 0.45$ ) with the soil covering during spring and summer time. Good overwintering positively influenced ( $r=0.10 - 0.62$ ) the later development of wheat. The highest impact on yield was determined by the autumn growth type ( $r=-0.67$ ) and soil covering at the flowering stage ( $r=0.54$ ). Resistance to powdery mildew was satisfactorily for many genotypes as the area under the disease progress curve (AUDPC) did not exceeded 100. Resistance to leaf spot diseases (tan spot and Septoria leaf blotch) was lower. Some genotypes had this value up to 500 and higher. The most problematic disease for winter wheat was common bunt. The most infected cultivar in the ecological field was 'Seda' – 70%, in 2006. The next year trials were sown with clean from common bunt seeds and this disease did not cause damage. Investigations in the common bunt nursery during 2006-2007 showed that less than 1% of cultivars and lines were infected lower than 5%. The other genotypes were less resistant. The most resistant lines in their pedigree were the cultivars 'Bill', 'Lut.9329', 'Lut.9392' 'Strumok', 'Dream'. Among the investigated cultivars the most resistant were 'Z-296', 'Sana', 'Boval', 'Stava', 'Penta', 'Quebon', 'Tommi'.

**Key words:** vegetative growth, soil covering, resistance, fungal diseases, common bunt

### Introduction

The competitive ability of cereals against weeds depends on specific traits. For winter cereals, the first trait is lush autumn growing. On the other hand, overgrowing until winter time is undesirable due to the possible weakening of plants. However, selection for this trait in breeding nurseries with conventional growing systems should be done considering that plants will be weaker in the organic

system. Therefore, it is desirable to select the thriest genotypes. A limiting factor for successful over-wintering is resistance to cold. In the case of poor over-wintering, even the genotypes with the best competitive traits will not be superior. Another trait is fast growing after resumption of vegetative growth. It should be part of the complex traits depressing weeds during spring and summer. Other mandatory traits are good tillering ability, strong stems, at least of medium height, non-erect and broad leaves. The desirable height of a cereal cultivar for every location and field will depend on the specific population of weed species. The growing season should be short or medium short. This trait would give more time for mechanical weed control after the yield harvest and prevent yield losses due to drought. (Walker *et al.*, 1988; Seavers and Wring, 1999; Feledyn-Szewczyk and Huer, 2006; Lemerle *et al.*, 2006; Olsen *et al.*, 2006).

The other complex of mandatory traits is resistance to fungal diseases. The resistance of many genotypes of winter cereals to powdery mildew is satisfactory at the present moment and the selection of cultivars for this trait is not complicated. In case of leaf spot disease, especially tan spot, resistance is lower (Anonymous, 2006), although, organic growing technology is more favourable for plants. Leaf spot diseases develop at lower rates than in the conventional growing system and resistance of some cultivars are satisfactory. The most dangerous disease is common bunt of wheat. When effective synthetic seed treatment materials were created and introduced for practical exploitation the breeding for the development of common bunt resistant cultivars become very scarce. At the present moment many wheat cultivars are very susceptible to this disease. Effective wheat growing under organic conditions can be realised if resistant cultivars are grown, or effective organic seed treatments are used. However neither the first nor the second systems work at present moment. The most realistic is a combined technique which involves the growing of cultivars with adequate traits for organic growing and possess some resistance to common bunt and the treatment of seeds with the most effective organic means (Bonman *et al.*, 2006; Dumalasova and Bartoš, 2006; Waldow and Jahn, 2007).

The present study was designed to evaluate the genotypes of winter wheat in terms of their main traits for organic growing.

## Materials and Methods

The study was conducted at the Lithuanian Institute of Agriculture during 2006-2007. Ten wheat (*Triticum L.*) varieties were tested in 2006 and 16 wheat varieties and breeding lines were tested in 2007. The soil of the experimental site is *Endocalcari-Epihypogleyic Cambisol* (CMg-p-w-can) (55°24'N, 23°50'E), with a pH<sub>KCl</sub> 7.0, amount of organic matter 21 g kg<sup>-1</sup>, available P 65-80 mg kg<sup>-1</sup> and K 83-125 mg kg<sup>-1</sup>. The experimental varieties were grown in three replications and with a plot size of 5.0 x 1.5 m<sup>2</sup>. The crop was sown on a well-prepared seedbed with Hege 80 at a rate of 5 million seed ha<sup>-1</sup> in the middle of September. Seeds used for sowing in 2005 were from the yield of 2005 of organically grown cereals. The next years seeds were from conventionally grown breeding nurseries. The cereals were preceded by a black fallow. The field was certified for organic agriculture. No agrochemicals and fertilizers were used. The soil was checked for the amount of mineral nitrogen present in spring before the resumption of vegetation. The low amount of available mineral nitrogen (sum of nitrate and ammonium nitrogen) (20-30 kg ha<sup>-1</sup>) was determined. Nitrate nitrogen was determined by the ionometric method and ammonia nitrogen was determined by the spectrophotometric method.

Overwintering was evaluated using the scale: 1- all plants alive; 9- died more than 80%.

The ability of cultivars to shadow soil was visually assessed using the following scales:

*Scale for autumn and spring growing:* 1 – soil covered up to 20%, very slow growth rate; 5 – up to 100%, very fast growth rate. Genotypes were evaluated in autumn at the end of intensive vegetation; at spring when intensive growth started (BBCH29-30).

*Scale for evaluation of later development:* 1 - soil covered up to 20%, plants are very thin; 9 – up to 100%, plants are very lush; genotypes were evaluated at stem elongation and flowering stages (BBCH 32-34; 41-42; 60-65).

The leaf diseases were assessed from stem elongation (BBCH 30-31) to late milk development (BBCH 77). Resistance to diseases was measured in scores, using 1-9 scale. Score – 1 no visible symptoms of diseases, 2 – 0.1%, 3 – 1%, 4 – 5%, 6 – 20%, 7 – 40%, 8 – 60%, 9 – ≥80%. The highest disease severity was used as disease index (DI). Wheat was infected by powdery mildew

(*Blumeria graminis* f. sp. *tritici* Em. Marchal.), tan spot (*Pyrenophora tritici-repentis* (Died.) Drechsler) and Septoria leaf blotch (*Septoria tritici* Rob. ex Desm).

The area under the disease progress curve (AUDPC) was calculated as the total area under the graph of disease severity against time, from the first scoring to the last.

$$AUDPC = \sum_{i=1}^{n-1} [(t_{i+1} - t_i) (y_i + y_{i+1})/2]$$

Where “t” is time in days of each reading, “y” is the percentage of affected foliage at each reading and “n” is the number of readings (Campbell and Madden, 1990).

Wheat resistance to common bunt (*Tilletia tritici* (Bjerk.) was investigated in a common bunt nursery with artificial infection. Inoculation was carried out by shaking seeds with teliospores (5 g spores/1000 g seed) in a flask for 5 min. In October, when the soil temperature had dropped below 7°C, the inoculated seeds were sown 15 g per genotype per a 3- m- long row at a depth of 10 cm in four replications arranged in different parts of the field. The common bunt incidence was measured at the medium milk development stage (BBCH 75) as the number of infected ears from the total ears harvested. The following scale was used to estimate varietal resistance: infected ears 0.0 = very resistant, 0.1-5.0 = resistant, 5.1-10.0 = moderately resistant, 10.1-30.0 = moderately susceptible, 30.1-50.0 = susceptible, 50.1-100.0 = very susceptible (Szunics, 1990).

Correlation analysis was applied to calculate relations between different traits at the probability levels 0.05 and 0.01.

### Results and Discussion

Good overwinter survival strongly influenced many traits. A strong correlation (r=0.71-0.81\*\*) was between over-wintering and spring re-growth, soil covering at later development stages, the height of plants and heading time in 2005/2006 (Table 1).

Table 1. Correlation between overwintering, soil covering traits and the plant growth peculiarities of winter wheat, 2005/2006

Traits	2	3	4	5	6	7	8	9
Overwintering	0.80**	0.81**	0.53*	0.80**	0.70**	0.71**	-0.71**	-0.50*
Re-growth, BBCH29-30	xxx	0.66**	0.42*	0.82**	0.50**	0.66**	-0.80**	-0.63*
Soil covering, BBCH32-34	xxx	Xxx	0.46*	0.80**	0.81**	0.76*	-0.57*	-0.52*
Soil covering, BBCH41-42	xxx	xxx	xxx	0.66**	0.52*	0.55*	-0.58*	-0.62**
Soil covering, BBCH60-65	xxx	xxx	xxx	xxx	0.83*	0.87**	-0.74**	-0.68*
Productive tillers	xxx	xxx	xxx	xxx	xxx	0.74*	-0.50**	-0.36*
Plant height	xxx	xxx	xxx	xxx	xxx	Xxx	-0.36*	0.38*
Heading	xxx	xxx	xxx	xxx	xxx	Xxx	xxx	0.85**
Maturity	xxx	xxx	xxx	xxx	xxx	Xxx	xxx	xxx

\*- probability level 0.05, \*\* - probability level 0,01

Analysis of the correlation between investigated traits showed that autumn growth type in 2007 had no influence under organic conditions on overwintering capability (r=-0.24). Autumn growth weakly correlated with spring re-growth and soil covering at stem elongation, r=0.45\* (Table 2).

Table 2. Correlation between overwintering, of soil covering traits and the plant growth peculiarities of winter wheat, 2006/2007

Traits	2	3	4	5	6	7	8	9
Autumn growth	-0.24	0.45*	0.45*	-0.48*	0.03	-0.11	0.22	-0.42**
Overwintering	xxx	0.51**	0.10	0.32*	0.62**	0.28	-0.21	-0.56*
Spring re-growth, BBCH29-30	xxx	xxx	0.82**	0.17	0.32*	0.53**	-0.31*	-0.60**
Soil covering, BBCH41-42	Xxx	xxx	xxx	0.16	0.01	0.68*	-0.42*	-0.33*
Soil covering, BBCH60-65	Xxx	xxx	xxx	Xxx	0.17	0.39*	-0.30*	0.03
Productive tillers	Xxx	xxx	xxx	Xxx	xxx	-0.19	-0.13	-0.43*
Plant height	Xxx	xxx	xxx	xxx	xxx	Xxx	-0.20	-0.27
Heading	Xxx	xxx	xxx	xxx	xxx	Xxx	xxx	0.33*
Maturity	Xxx	xxx	xxx	xxx	xxx	Xxx	xxx	xxx

\* - probability level 0.05, \*\* - probability level 0.01

The results of the over-wintering of 2006/2007 showed a medium correlation ( $r=0.51^{**}$ ) between spring re-growth and the number of productive tillers ( $r=0.62^{**}$ ).

Spring re-growth showed the strong correlations between soil covering at plant flowering and heading time in 2006,  $r=0.82^{**}$  and  $-0.80^{**}$ , respectively. Correlations with other traits were weaker ( $r=-0.63^*$  -  $0.42^*$ ). Spring re-growth strongly correlated ( $r=0.82^{**}$ ) with the soil covering at the booting stage, and the medium correlated ( $r=0.53^{**}$ ) with plant height and negatively with maturity time ( $r=-0.60^{**}$ ).

Correlation analysis of vegetation peculiarities and resistance to leaf diseases with yield showed that the strong positive influence ( $r=0.79^{**}$ ) on yield in 2006 was exerted by plant spring re-growth (Table 3). However, strong correlations were calculated only in 2006. A medium positive influence on yield expressed overwintering ( $r=0.60^{**}$ ) in 2006.

Table 3. The correlation of winter wheat yield with the main agronomic traits

Traits	Yield		Traits	Yield	
	2006	2007		2006	2007
Autumn growth type	-	-0.67**	Plant height	0.27	-0.22
Overwintering	0.60**	0.25	Heading	-0.55**	-0.34*
Spring re-growth, BBCH29-30	0.79**	-0.26	Maturity	-0.27	0.31*
Soil covering, BBCH32-34	0.29	-0.38*	Powdery mildew. scores	-0.47**	-0.25
Soil covering, BBCH41-42	0.20	0.45*	Powdery mildew. AUDPC	-0.43**	-0.04
Soil covering, BBCH60-65	0.37*	0.54**	Leaf spots. Scores	-0.26	0.06
Productive tillers	0.05	0.47*	Leaf spots. AUDPC	-0.13	-0.06

\*- probability level 0.05, \*\* - probability level 0.01

Among negative traits the highest effect was calculated for heading data ( $r=-0.55^{**}$ ) and leaf diseases ( $-0.13$  -  $-0.47^{**}$ ) in 2006. This mainly depended on plant photosynthetic activity which in turn affected the amount of assimilates in the yield. Autumn growth in 2007 showed a medium negative ( $r=-0.67^{**}$ ) influence on yield. Soil covering at the flowering stage influenced the yield medium ( $r=0.54^{**}$ ). The yield in 2007 correlated with other traits weakly or did not correlate. Highly ranging correlations were mainly influenced by different weather condition during the investigation period. In 2006 wheat was severely damaged by cold and summer drought. Whereas, wheat overwintered well in 2007 and precipitation during the summer was satisfactory for wheat development. Susceptible to powdery mildew winter wheat genotypes can be characterized by AUDPC values exceeding 1000 (Ruzgas and Liatukas, 2006). Therefore, resistance to powdery

mildew for many cultivars was sufficient in 2006, when 10 cultivars of 14 were evaluated by AUDPC value up to 100 (Table 4.). The four cultivars were evaluated by AUDPC value up to 500. The year 2007 was less favourable for powdery mildew and all genotypes were evaluated by AUDPC values only up to 10. The leaf spot diseases were not problematic for the majority of cultivars in 2006, when 12 cultivars of 14 were evaluated by AUDPC values only up to 100. The resistance of 2 cultivars was evaluated by values from 100 to 500. The actual resistance to leaf spot diseases was revealed in 2007, when all cultivars were evaluated by AUDPC values more than 100. The four cultivars were medium resistant and had values up to 200.

Table 4. Resistance to fungal diseases of winter wheat genotypes tested for organic growing

AUDPC value	Number of genotypes				CB level	Number of genotypes		
	PM		LS			2006*	2006**	2007**
	2006	2007	2006	2007				
0-10.0	0	16	0	0	0	0	0	
10.1-50.0	3	0	3	0	0.1-5.0	1	0	1
50.1-100.0	3	0	1	0	5.1-10.0	2	0	1
100.1-200.0	2	0	2	2	10.1-30.0	4	1	0
200.0-500.0	2	0	4	10	30.1-50.0	1	0	1
≥500.1	0	0	0	4	≥50.1	2	9	13
Total No.	10	16	10	16	Total No.	10	10	16

PM – powdery mildew, LS – leaf spot diseases, CB – common bunt

\*-organic field, \*\*- common bunt nursery

The main problem concerning fungal diseases resistance was susceptibility to common bunt. There were no highly resistant cultivars among the winter wheat genotypes tested under organic conditions. Winter wheat genotypes were sown in the organic field with naturally infected seeds. Under the conditions of the organic field 3 cultivars were infected up to 10% in 2006. Although testing of these genotypes in common bunt nursery with artificial infection revealed that only 1 genotype of 10 possessed some resistance. The results of 2007 were similar, 2 genotypes of 16 were infected less than 10%. The overall infection mean in the common bunt nursery was 81% and 64% in 2006 and 2007, respectively. Therefore, cultivars infested less than 10% possessed some resistance level.

Data from common bunt nursery presented in Table 5 shows the most resistant genotypes among all those tested. Among the tested genotypes in 2006 only cv. 'Quebon' was resistant.

Table 5. The most common bunt resistant genotypes of winter wheat in 2006-2007, common bunt nursery

Genotypes in 2006					
	Avg.*	SD**		Avg.*	SD**
Quebon	5.0	2.6	Watson	18.0	10.3
Tommi	15.0	9.6	Dream / Lut.9329	18.5	19.0
Bill	15.5	10.2	Dream / Lut.9329	28.8	22.4
Bill/Aspirant	17.2	15.5	Flair / Lut.9329	29.5	20.5
Genotypes in 2007					
Bill / Aspirant	1.9	1.7	Globus	5.5	2.9
Strumok / Lut.9392	2.0	0.8	Tarso / Bussard	5.5	1.3
Z-296	2.8	1.7	Bill / Dream	6.0	2.9
Sana	3.0	1.6	Strumok / Lut.9313	6.3	1.7
Boval	3.3	1.3	Sj 05-15	7.5	1.3
Sj 020196-050	3.5	1.3	Dream / Lut.9329	7.8	3.2
Stava	3.6	0.9	Penta	8.5	1.3
Strumok / Lut. 9392	4.5	1.9	Dream / Bill	8.5	3.1

\*Avg. - average, \*\*SD – standart deviation

The least damaged seven were evaluated as medium susceptible and evaluated as being 15.0 to 29.5% of infected ears. The genotypes tested in 2007 were more resistant. The eight genotypes were evaluated as resistant; the other 9 were medium resistant.

The distribution of winter wheat genotypes by resistance to common bunt revealed that only minority of germplasm can be used for resistance breeding. Only 1 cultivar of 175 was resistant in 2006. Among the tested genotypes in 2007 not one was highly resistant. Eight genotypes of 474 were resistant and 10 were medium resistant. Among the tested genotypes in both years the very susceptible germplasm dominated: 92% in 2006 and 74.6% in 2007 (Table 6).

Table 6. Distribution of winter wheat genotypes by common bunt resistance reaction in 2006-2007, common bunt nursery

Year	Resistance groups												Total No.
	0		0.1-5.0		5.1-10.0		10.1-30.0		30.1-50.0		50.1-100.0		
	No.*	%**	No.	%	No.	%	No.	%	No.	%	No.	%	
2006	0	0.0	1	0.6	0	0.0	7	4.0	6	3.4	161	92.0	175
2007	0	0.0	8	1.7	10	2.1	48	10.1	55	11.6	353	74.6	474

\*- number of genotypes per group, \*\* - percent of genotypes per group

The first trait in autumn which can be assessed is the autumn growth rate of cereals. This trait is the capability of plants to compete with weeds from the initial growth stages. This trait did not correlate with overwintering in cereals grown under organic conditions in 2007. Under conventional growing conditions too lush growth is not desirable because it negative influences overwintering. Autumn growth type is strongly correlated with spring re-growth and soil covering at the stem elongation stage. This trait medium correlated with plant height. Considering the correlations obtained the autumn growth rate can be one of traits allowing for the selection of genotypes with higher competition abilities in regard to weeds. This trait is relatively stable under different environments as well as its plant height. Therefore genotypes can be selected at early generation stages if the environment is suitable enough for longer development in autumn.

Overwintering is one of essential traits for winter cereals under the organic growing system. Due to a low amount of available nitrogen the development of plants in spring is slower. Therefore, weakened plants hardly can compete with weeds. Such crop stands later will be depressed by weeds. Correlation analysis showed that this trait strongly affected spring re-growth intensity and later soil covering.

Autumn growth and overwintering can be considered as the two main traits which affect crop stand development. Autumn growth type defines further plant growth more or less and successful overwintering warrant adequate development of crop stand and its soil covering capability. Plant height expressed strong correlations in many cases. However, high correlation with autumn growth type showed that this trait is closely related and is found even in early plant growing stages. The main reason is that plant height reducing genes more or less affect all the vegetative parts of the plant. At the present moment many cultivars of winter wheat are too short for growing under organic conditions. Moreover, these cultivars have erect leaves, which poorly shadow the soil surface.

Our results revealed that leaf diseases were not problematic for many of the tested genotypes during the investigation period. The majority genotypes of wheat in 2006 in the case of powdery mildew had a AUDPC value up to 100, whereas the most susceptible wheat genotypes in breeding nurseries (data not shown) had a AUDPC up to 1800. The present cultivars of wheat possess satisfactory resistance to powdery mildew (Anonymous, 2006). The research of resistance types to powdery mildew showed that partial resistance dominates. This resistance type is durable. Therefore, determined resistance level will be determined mostly by environment influences (Miedaner and Flath, 2007).

Dry weather conditions in 2006 negatively influenced the development of leaf spot disease. The next wet year revealed that only two genotypes of 16 were medium resistant. Leaf spot dominated among other diseases. It developed to a considerable level only at the end of vegetation, therefore did not influence the yield negatively. At the present moment breeding for resistance of wheat to leaf spot diseases are progressive. The best results are achieved in the case of Septoria leaf blotch,

some the newest cultivars do not have a considerably higher yield without fungicides when infection pressure is not very high (Anonymous, 2006). Therefore, perspectives for the use of resistant cultivars are optimistic.

The main problem was the common bunt of winter wheat. Examining miniscule parts of resistant genotypes of winter wheat under organic conditions is very complicated. An epidemic of common bunt did not occur in organic farms up to now only due to the reason that certified seed production under organic conditions consist only of small part of the productive of all organic farms. This situation is similar in all Baltic states as well as Europe. As the organic farms buy conventional certified seed they break the infection chain which could create epidemics. This scheme could be suitable to stop an epidemic if such occurs. The recent investigations showed that at the present moment the most realistic is the situation when seeds of cultivars possessing some resistance are treated with treatments allowed for organic farming. Moreover, seed material must be investigated from the point of view of pathogens and appropriate means should be selected individually for every seed lot (Waldow and Jahn, 2007).

So complicated cultivation of winter wheat can be avoided if the considers the quality of grain of cultivars grown under organic farming. In many cases grain quality is too low for bread making. Considering complication with common bunt and low grain quality instead of wheat triticale or rye could be chosen. In this case, cultivars of winter triticale and rye should be evaluated for soil covering traits as described above. Moreover, resistance to ergot must be checked as some cultivars of triticale and the majority of rye are susceptible.

## Conclusions

Autumn growth and over-wintering can be considered as the two main traits which affect later crop stand development. Autumn growth type are strongly correlated with spring re-growth and soil covering at the stem elongation stage. Considering the correlation obtained autumn growth type can be one of traits allowing for the selection of genotypes with higher resistance to weeds. Overwintering was one of essential traits for winter cereals under the organic growing system. Correlation analysis showed that this trait strongly affected spring re-growth intensity and later soil covering. The main problem concerning the resistance of wheat to fungal diseases was susceptibility to common bunt. There were no highly resistant cultivars among winter wheat genotypes tested under organic conditions.

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### ZIEMAS KVIEŠU NOZĪMĪGĀKĀS PAZĪMES SELEKCIJAI BIOĻĢISKAJAI LAUKSAIMNIECĪBAI LIETUVĀ

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Pētījumi tika veikti Lietuvas Zemkopības institūtā bioloģiskajai lauksaimniecībai sertificētos laukos laika periodā no 2006. līdz 2007. gadam. Ziemcietība lielākajā daļā gadījumu cieši korelēja ( $r=0.71-0.81$ ) ar ataugšanas intensitāti pavasarī un augsnes noseģšanas intensitāti vēlākās attīstības stadijās. 2006. gadā, kad tika novēroti ziemas sala bojājumi, raža vidēji korelēja ar ziemcietību ( $r=0.60$ ) un cieši ( $r=0.79$ ) – ar ataugšanas intensitāti pavasarī. Datu analīze 2007. gadā parādīja vājākas korelācijas starp pazīmēm. Augšanas intensitāte rudenī korelēja ar augsnes noseģšanu pavasarī un vasarā ( $r=-0.48 - 0.45$ ). Laba ziemcietība pozitīvi ietekmēja vēlāko kviešu attīstību ( $r=0.10 - 0.62$ ). Lielākā ietekme uz ražu tika konstatēta augšanas tipam rudenī ( $r=-0.67$ ) un augsnes noseģšanai ziedēšanas fāzē ( $r=0.54$ ).

Izturība pret miltrasu bija apmierinoša vairumam genotipu, jo laukums zem slimības progresa līknes (AUDPC) nepārsniedza 100. Izturība pret lapu plankumainībām (kviešu lapu dzeltenplankumainību un septoriozi) bija mazāka. Dažiem genotipiem AUDPC vērtība bija 500 un lielāka. Visproblemātiskākā ziemas kviešu slimība bija cietā melnplauka. Visvairāk inficētākā šķirne bioloģiskajā laukā bija „Seda” – 70% 2006. gadā. Nākamā gada izmēģinājumos tika izmantots no cietās melnplaukas tīrs sēklas materiāls un slimības bojājumi netika novēroti. Pētījumi atsevišķi iekārtotā cietās melnplaukas audzētavā 2006.-2007.g. parādīja, ka mazāk nekā 1% no šķirnēm un līnijām inficēšanās ar šo slimību bija mazāka par 5%. Citi genotipi bija mazāk izturīgi. Izturīgākajām līnijām viens no vecākaugu starpā bija genotipi ‘Bill’, ‘Lut.9329’, ‘Lut.9392’ ‘Strumok’ un ‘Dream’. Visizturīgākās šķirņu starpā bija ‘Z-296’, ‘Sana’, ‘Boval’, ‘Stava’, ‘Penta’, ‘Quebon’ un ‘Tommi’.

### BREEDING ASPECTS OF NARROW – LEAVED LUPINE (*Lupinus angustifolius* L.) GROWING FOR GREEN MANURE

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#### Abstract

The individual selection method has been employed in narrow-leaved lupine breeding at the Lithuanian Institute of Agriculture's Vokė Branch since 1993, when a collection from the N. Vavilov All-Russian Institute of Plant Industry was obtained. During the period 1995 – 2005, using the individual selection method two narrow – leaved lupines varieties VB Derliai and VB Ugniai intended for green manure and two narrow – leaved forage varieties VB Vilniai and VB Antaniai were bred. The varieties VB Derliai, VB Ugniai and VB Vilniai during the 2006-2007 years were included in the EU common catalogue of plant varieties. Also during the period 1995 – 2005 not only the afore-mentioned varieties were developed, but also five lines of narrow-leaved lupine for green manure that passed all the stages of the breeding process and were assessed according to international standards. All of the five breeding lines are characterised by a high resistance to fungal diseases, a fast growth rate at all growth stages, high seed yield (2.1-2.9 t ha<sup>-1</sup>) and a short vegetative growth period (82-98 days). They are a valuable material from the genetic, breeding and agronomic point of view. They will be used in further lupine breeding programs, and the most valuable lines will be transferred to official testing centres.

**Key words:** narrow-leaved lupine for green manure, individual selection, variety, resistance to fungal diseases.



## Introduction

The breeding of lupine in Lithuania was started at the Dotnuva plant breeding station in 1934 (Lazauskas *et al.*, 1992). In 1947, lupine breeding was transferred from Dotnuva to the Karoliniškės station near Vilnius, and after the reorganisation of research institutions, lupine breeding was transferred from the Karoliniškės station to the Vokė Branch in 1957 where continuous breeding with – yellow lupine (*Lupinus luteus* L.) has been carried out until now. The only narrow-leaved lupine variety Snaigiai for green manure (breeder A. Subačius) was bred in 1962 using the intervarietal crossing method. The greater interest was given to the narrow - leaved lupine in 1993 when a collection was obtained from the N. Vavilov All-Russian Institute of Plant Industry. Comprehensive research into this species of lupine, conducted during the period 1993 – 1997 was of special importance since a high resistance to the new fungal disease of lupine anthracnose (*Colletotrichum gloeosporoides* (Penz.) Penz & Sass.) was identified in this species (Kurlovič *et al.*, 2002, Maknickienė *et al.*, 2001). Lupine anthracnose affects all lupine species without exception, and yellow lupine is especially heavily damaged by the disease (Ivaniuk *et al.*, 2001, Evsikov *et al.*, 1999). Currently, there are no yellow lupine varieties notable for a high resistance to anthracnose. Therefore yellow forage lupine can only be replaced by a narrow-leaved forage lupine and lupine for green manure, which are characterised by exceptional characteristics such as fast growth rate at all developed stages, a short growing season and high yield.

During the period 1995 – 2005, using the individual selection method four narrow-leaved lupine varieties were developed: two narrow-leaved lupine varieties for green manure VB Derliai (breeder Z.Maknickienė) and VB Ugniai (breeders Z.Maknickienė, A.Ražukas), and two narrow-leaved forage lupine varieties VB Vilniai (breeders Z. Maknickienė, J. Lazauskas, A. Subačius, A.Ražukas), and VB Antaniai (breeder Z.Maknickienė). During the period 1995 – 2005 not only the afore-mentioned varieties were developed, but also numerous valuable breeding lines that were assessed according to international standards. Using the individual selection method, five narrow-leaved lupine breeding lines for green manure have been developed so far. They are a valuable material from the genetic, breeding, and agronomic point of view and will be used in subsequent breeding work. The most valuable lines will be transferred to the state variety testing.

## Materials and Methods

Lupine breeding crops are grown at the LIA Vokė Branch in a six-course crop rotation, with spring cereals as a preceding crop. The soil – Haplic Luvisols (LVh), moderately acid, low in humus (2.0-2.1), nitrogen 0.096-0.117 %, phosphorus 113.2-147.3 mg kg<sup>-1</sup>, potassium 126.4-153.3 mg kg<sup>-1</sup> soil. A conventional soil preparation technology was employed: deep autumn ploughing, two soil cultivations in spring. The herbicide gezagard (2–2.5 kg ha<sup>-1</sup>) was applied for weed control. The fungicide kemikar-T (2 l/t seed) was used for seed treatment.

The chief breeding methods employed were individual selection and intervarietal hybridization. The lupine variety Snaigiai was used as a control. Lupine breeding is performed according to the approved methodology, following this scheme: 1) collection nursery, 2) hybrid nursery, 3) breeding nursery, 4) control nursery, 5) initial variety trials, 6) competitive variety trials. In the collection, hybrid and breeding nurseries – in the plots 0,8 – 2,4 m<sup>2</sup>. In the control nursery plots of 9,6 m<sup>2</sup>. Initial and competitive variety trials involve 3 – 4 replications, the size of a record plots is 15,0 – 20,0 m<sup>2</sup> respectively. During the vegetative growth period various assessment methods (IBPGRI *et al.*, 1981, CЭB *et al.*, 1985) are applied in all stages of breeding work for the identification of resistance to fungal diseases at three plant growth stages: seedling, bud formation – flowering and shiny pods. 1 to 9 point scale was used: a 1 – very low resistance, diseased plants over 50 %, 3 – low resistance, diseased plants 26-50 %, 5 – moderate resistance, diseased plants 11-25 %, 7 – high resistance, diseased plants 2.5-10 %, 9 – very high resistance, diseased plants less than 2.5 %. With this end in view, at complete emergence plants were counted in A and C replications, at seedling, bud formation – flowering, and shiny pods stages anthracnose-affected plants were counted and removed from the plot. At the complete maturity stage healthy plants were counted and their productivity was estimated. The percent of fungal-disease affected plants was identified according to the formula:

$P = (n / N) \cdot 100$ , where n – number of affected plants, N – number of assessed plants.

Morphological, genetic and agronomically important biochemical characteristics were estimated. Biochemical composition content was estimated in the periods schining legumes. Alkaloid content was determined by the gravimetric method LST 1560, total nitrogen content (N) was determined by the Kjeldahl method (LST 1523), P – by wet combustion, colorimetric method using *Technikon* instrument, K and Ca – by flame photometry. All analyses were done at the Center Of Agrochemical studies of the Lithuanian Institute of Agriculture. The results thus obtained were statistically analyzed using ANOVA software (Tarakanovas *et al.*, Raudonius *et al.* 2003).

At the end of the breeding process the best selected variety is transferred to the state variety testing centre for final evaluation.

## Results and Discussion

Development of breeding lines. The success of the breeding work depends on the abundance and value of the initial material.

The variety VB Derliai was produced from the variety Deter-3. The variety Deter-3 is of a determinant type, very early, low-yielding, with a low alkaloid content and a low resistance to fungal diseases. The variety VB Derliai significantly differed in morphological and biochemical properties from the parental variety. This variety is characterised by monopodic branching, high seed and green material yield, high content of alkaloids and high resistance to fungal diseases.

The variety VB Ugniai was produced from the variety DM-15. The selected genotypes differed in blossom and seed colour. The blossom colour of the collection accession DM-15 is light blue and that of the seeds sandy. The variety VB Ugniai 1679 is characterised by a dark blue blossoms and the dark sandy colour of the seeds and by a high resistance to anthracnose.

The breeding line 1669 was selected from the variety Serebrianskij. Three genotypes were selected according to phenotypical traits. The blossoms of the parental variety had a light pink colour, the seeds were sandy coloured. The selected genotypes were distinguished by white blossoms and the white colour of the seeds. Both the parental variety and the line 1669 are characterised by high yield and resistance to fungal diseases.

The breeding line 1682 was selected from the variety F-2, which is characterised by light blue blossoms and light brown seeds. According to the phenotype the selected genotypes differed in the colour of blossoms (dark blue) and in seed colour (dark brown with white dots). Individual genotypes were distinguished by a high resistance to fungal diseases and a short growing season.

The breeding line 1685 was selected from the variety AT- 4. The selected genotype is characterised by sympodic branching and long and narrow pods. A significant difference is noted in the small seeds. 1000 seed weight is 101g, and that of the variety AT- 4 139.4 g.

The breeding line 1688 was selected from the collection accession 3210. The collection number 3210 is characterised by light blue blossoms and light brown seeds. The breeding line 1688 was distinguished by dark blue blossoms and bright brown seeds. A significant advantage of the breeding number is its high resistance to pod bursting.

The breeding line 1709 was selected from the variety Velikan – 4. One very productive (56 pods, 219 seeds), early, fusarium and anthracnose-resistant plant was selected from the collection accessions.

*Green material yield.* Narrow-leaved lupine needs a fairly large amount of moisture for growth and development. During the growing season lupine requires a high amount of moisture from sowing to complete sprouting and from flowering to the whitening of the pods, the optimal amount of precipitation being 250 mm . In the high precipitation of 2003 the highest green material yield was obtained. The yield of the narrow-leaved lupine varieties VB Derliai, VB Ugniai, and their breeding lines ranged between 65.6-69.7, and that of the standard 71.6  $\text{tha}^{-1}$  (Table 1). The breeding line N1685 (69.7  $\text{tha}^{-1}$ ) lagged behind the standard only inappreciably. The averaged experimental data from 2003 – 2005 suggest that the varieties VB Derliai, VB Ugniai, and their breeding lines lagged behind the standard in terms of green material yield by 2.4 – 6.0  $\text{tha}^{-1}$ . Our experimental findings show that the reduction in green material yield depends on lupine genetic characteristics and hydrothermal factors. Lupine plant height determines the yield of green material. The height of all the tested breeding lines and the varieties VB Derliai, VB Ugniai ranged between 57.0- 66.1 and that of the standard was 93.0-95.0 cm. When there is an excess of precipitation the height of the

lupine variety Snaigiai can exceed 100 cm. Due to these genetically inherent characteristics the green material yield of the variety is very high.

Ploughing in the green material of lupine is recommended when the plants have reached their highest productivity. Nitrogen accumulation in the vegetative mass and roots becomes more intensive from the beginning of budding to the shiny pod stage. The variety Snaigiai and the tested breeding lines reach the shiny pod stage at different times. The breeding lines and the varieties VB Derliai, VB Ugniai reach this stage depending on hydrometeorological conditions during the second ten-day period of July and the first ten-day period of August. It is a perfect green manure fallow for winter cereals, while the standard variety reaches this stage 2–3 weeks later.

The efficacy of lupine as green manure depends not only on the yield of the incorporated green material but also on its chemical composition. Our experimental evidence indicates that the content of total nitrogen in narrow-leaved lupine at the shiny pod stage is higher (3.2-3.7 %) than that of yellow lupine (2.3-2.7 %). According to chemical composition indicators the tested breeding lines and the varieties VB Derliai and VB Ugniai are similar to the standard.

Table 1. Green material yield of narrow-leaved lupine with green manure, (Trakų Vokė, 2003-2005)

Variety, breeding line	2003	2004	2005	Mean, t ha <sup>-1</sup>	Diference from the standard,
	green material, t ha <sup>-1</sup>	green material, t ha <sup>-1</sup>	green material, t ha <sup>-1</sup>		
Snaigiai	71.6	65.0	57.0	64.5	0.0
VB Derliai	68.2	60.0	52.0	60.1	-4.4
VB Ugniai	65.6	61.2	51.8	59.5	-5.0
1669	67.6	63.0	54.7	61.8	-2.7
1685	69.7	62.6	54.0	62.1	-2.4
1688	67.6	58.0	50.0	58.5	-6.0
1682	66.0	58.8	55.1	60.0	-4.5
1709	66.3	59.2	56.2	60.6	-3.9
LSD <sub>05</sub>	1.3	3.73	3.08	1.56	

Seed yield. Currently, when the occurrence of lupine anthracnose is high in Lithuania the seed yield is one of the chief criteria for the assessment of breeding material. The world's lupine gene fund does not comprise any varieties that are 100 % resistant to fungal or viral lupine diseases, and it is possible that the varieties characterised by partial resistance do not lose this trait longer, and in the years of the weak epiphytoty of the disease such varieties either do not catch the disease at all or are only slightly affected. Depending on the growth stage at which the disease appears, lupine anthracnose can destroy all of the seed yield. The later the plants are affected, the higher the chances to secure at least a small seed yield. The weather conditions during the experimental years were diverse therefore the spread of fungal diseases of lupine also differed.

Table 2. Anthracnose-affected narrow-leaved lupine with green manure, % (Trakų Vokė, 2003-2005)

Variety, breeding line	% of affected plant		
	2003	2004	2005
Snaigiai	9.6	100	31.0
VB Derliai	1.0	2.1	1.1
VB Ugniai	1.1	2.0	1.0
1669	0.9	2.0	0.8
1685	1.2	2.2	1.8
1688	1.0	2.4	1.7
1682	0.8	2.2	1.4
1709	1.2	2.4	1.0

In 2003 narrow-leaved lupine was least affected by anthracnose. A dry and cold June suppressed the spread of the pathogen. In the narrow-leaved lupine the first sporadic anthracnose lesions were recorded at the beginning of the third ten-day period of July when the breeding lines were at the wax maturity stage and the standard was at the end of the milk maturity stage. The breeding lines

matured on August 7-12, the standard – on August 19. At the end of maturity the anthracnose-affected plants in the breeding lines amounted to 0.8-1.2 %, in the standard 9.6 %. In 2003 the highest yield (1.1 t ha<sup>-1</sup>) of the standard was obtained. The yield of the breeding lines (2.1-2.7 t ha<sup>-1</sup>) exceeded the standard by 191-245 %.

The highest severity of anthracnose was recorded in 2004. The weather conditions in July were conducive to the occurrence of anthracnose. The weather was rainy with frequent downpours. Anthracnose occurred in the lupine stands at the beginning of the third ten-day period of July. When the anthracnose began to spread all breeding lines had reached the stage of wax maturity, therefore this fungal disease did not have any marked effect on seed yield (affected plants made up 2.0-2.4 %) (Table 2), seed yield (2.2-3.1 t ha<sup>-1</sup>) (Table 3). The variety VB Derliai and N1685 were distinguished by a high seed yield (2.8-3.1 t ha<sup>-1</sup>). In the control treatment anthracnose occurred at the lupine milk maturity stage. At the wax maturity stage all the plants in the control treatment were affected by anthracnose and the seed yield amounted to 0.3 t ha<sup>-1</sup>. The tested breeding lines matured at the end of the third ten-day period of July, the standard matured on August 22.

Table 3. Seed yield of narrow-leaved lupine with green manure, (Trakū Vokē, 2003 – 2005)

Variety, breeding line	2003	2004	2005	Mean, t ha <sup>-1</sup>	Diference from the standard, (+-) t ha <sup>-1</sup>
	seed yield, t ha <sup>-1</sup>	seed yield, t ha <sup>-1</sup>	seed yield, t ha <sup>-1</sup>		
Snaigiai	1.1	0.3	0.8	0.73	0.0
VB Derliai	2.6	3.1	2.6	2.8	+2.07
VB Ugniai	2.4	2.2	2.2	2.3	+1.57
1669	2.3	2.9	2.5	2.6	+1.87
1685	2.4	2.8	2.5	2.6	+1.87
1688	2.5	2.6	2.3	2.5	+1.77
1682	2.7	2.5	2.7	2.6	+1.87
1709	2.1	2.3	2.1	2.2	+1.47
LSD <sub>05</sub>	0.14	0.21	0.22	0.11	

In the year 2005 the first symptoms of anthracnose were recorded at the end of the third ten-day period of June. The breeding lines reached wax maturity at the beginning of the second ten-day period of July. At the end of maturity anthracnose-affected plants in the breeding lines amounted to 0.8-1.8 % - of the standard 31 %. The breeding lines matured on July 26 - on August 8. The standard matured on August 29. In terms of seed yield, the breeding lines and the variety Derliai exceeded the standard variety (0.8 t ha<sup>-1</sup>) by 262-337 %. N1682 was found to be the most productive 2.7 t ha<sup>-1</sup>.

Average data from 2003-2005 suggest that all the tested breeding lines exhibited a rapid growth rate during all development stages, which resulted a high resistance to anthracnose and a high seed yield 2.2-2.6 t ha<sup>-1</sup> (of the standard 0.73 t ha<sup>-1</sup>). Due to its short growing season, (82-98 days) this lupine species can be successfully grown all over the country without any additional methods of cultivation.

## Conclusions

1. Since the resumption of narrow-leaved lupine breeding at the LIA's Voke Branch, five breeding lines and the varieties VB Derliai and VB Ugniai were developed using the individual selection method during the period of 1995-2005.

2. All of the narrow-leaved lupine breeding lines are characterised by a rapid growth rate during all development stages, high anthracnose resistance (affected plants 0.8 – 2.4 %, of the standard 9.6 – 100 %), high seed yield 2.1 - 2.9 t ha<sup>-1</sup>, (of the standard 0.73 t ha<sup>-1</sup>), short growing season 82 - 98 days, (of the standard 100 - 114 days).

3. The varieties VB Derliai and VB Ugniai 2006-2007 years have been included in the EU common catalogue of plant varieties.

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## ŠAURLAPU LUPĪNAS SELEKCIJAS PERSPEKTĪVAS (*Lupinus angustifolius* L.) ZAĻMASAS AUDZĒŠANAI

**Maknickienė Z.**

Šaurlapu lupīnu selekcijā Lietuvas Zemkopības institūta Vokes nodaļā tiek izmatota individuālā selekcijas metode kopš 1993, kad tika iegūta kolekcija no N. Vavilova Viskrievijas Augu Rūpniecības institūta. Laika periodā no 1995. – 2005. tika selekcionētas divas šaurlapu lupīnu šķirnes, kas paredzētas zaļmasas ražošanai - VB Derliai un VB Ugniai, kā arī divas lopbarības šķirnes - VB Vilniai un VB Antaniai. Šķirnes VB Derliai, VB Ugniai un VB Vilniai iekļautas ES Augu Šķirņu katalogā 2006.-2007. gadā. Laika posmā 1995. – 2005. ir izveidotas ne tikai iepriekšminētās šķirnes, bet arī piecas šaurlapu lupīnu līnijas izgājušas visas selekcijas procesa pakāpes un tika izvērtētas saskaņā ar starptautiskiem standartiem. Visām piecām selekcijas līnijām konstatēta augsta izturība pret sēņu slimībām, ātrs augšanas temps visās augšanas stadijās, augsta sēkļu raža (2.1–2.9 t ha<sup>-1</sup>), īss veģetatīvās augšanas periods (82-98 dienas). Tās ir vērtīgs materiāls no ģenētikas, selekcijas un agronomijas viedokļa. Līnijas var tikt izmantotas tālākā lupīnu selekcijas programmā bet vērtīgākās līnijas tiks nodotas oficiālām pārbaudes iestādēm.

## THE EVALUATION OF BUCKWHEAT HYBRIDS FOR THE INHERITANCE OF VALUABLE AGRONOMIC FEATURES

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### Abstract

Buckwheat (*Fagopyrum esculentum* Moench.) breeding work has been done at the Voke Branch of the Lithuanian Institute of Agriculture during 2004 – 2007. The general idea of buckwheat breeding was to create high yielding, big size grains and short stem buckwheat cultivars. Buckwheat varieties are bred using hybridization, massive and individual selection methods. The selected best buckwheat genotypes were assessed evaluating grain yield and size, plant stability and the ability to lie flat and other valuable biometric features.

The main aim of this research work was to explore buckwheat hybrid seedling phenotype inheritance. The general idea of buckwheat breeding was to create high yielding, big size grains and short stem buckwheat cultivars. Trials were performed with 144 hybrid numbers. 40.0 – 47.6% the selected hybrids had big sized grain and short stem inheritance. Buckwheat inheritance consistent patterns have a great influence in the breeding work for big size and short stem buckwheat cultivars.

**Key words:** buckwheat breeding, buckwheat features inheritance, new buckwheat hybrids

## Introduction

Buckwheat is one of the main cereal plants grown on the light soils in Lithuania. Traditionally they are used in the food industry as well as in pharmacy and the chemical industry (Пироговская, 2000). The land area for buckwheat covers over 21000 ha in Lithuania (Batulevičiūtė, 2006). The economical importance of buckwheat increases every year as more and more attention is given to human health problems. Due to this buckwheat scientific research work and the breeding of new buckwheat varieties has started to increase on the world wide level (Campbell, 2003).

Improvement programs for buckwheat varieties have existed now for over a hundred years. But yield and other quality feature changes were small in comparison with other crops. Buckwheat is a cross pollinated plant, but also self pollination is possible. But self pollinated grain is inefficient and plant production from such seeds is low (Савицкий, 1970).

The main aim of the research work at the Voke Branch of the Lithuanian Institute of Agriculture was to explore buckwheat hybrid phenotypes and their valuable farming features such as grain size and plant height inheritance in the breeding process for high yielding, big size and resistant to low flat varieties during the 2001-2004 research years.

## Materials and Methods

The experimental plots were established on sandy loam on carbonaceous fluvial-glacial gravel eluviated soil (IDp), according to FAO-UNESCO classification *Haplic Luvisols (LVh)*. Soil agrochemical characteristics: pH<sub>KCl</sub> 5.2 – 6.2, humus – 2.11 – 2.18%, mobile P<sub>2</sub>O<sub>5</sub> 108 – 152 mg kg<sup>-1</sup>, mobile K<sub>2</sub>O 150 – 165 mg kg<sup>-1</sup>. Soil for buckwheat trials was ploughed in the autumn, cultivated twice and harrowed in the spring. Fertilization – N<sub>60</sub>P<sub>50</sub>K<sub>40</sub>. Herbicide – agroxone 750, 1,6 l ha<sup>-1</sup> was applied after buckwheat sowing. Test field area for collection trials – 2 m<sup>2</sup>, for competitive varieties trials – 12 m<sup>2</sup> in 4 replications. Planting rate – 3 mln ha<sup>-1</sup> of fertile seeds. Buckwheat is a short vegetation period plant. The growing period is June, July and August. So summer meteorology conditions have the most important influence on buckwheat growth and development. During the research June meteorology conditions were unfavourable for buckwheat plant growth till the bud stage and flowering start. Because of high rainfall and low air temperature 2004 was a very bad year for buckwheat and also because of drought in 2006. Meteorology conditions data are presented in 1 Table.

The buckwheat flowering period and grain formation physiologically proceed at the same time – from the end of June till mid September, but the most intensive period is July. Buckwheat ripens about one month from the beginning of the flowering period, so for yield formation July and August are very important months. During the research (2004 and 2005) the July months were dry (hydrothermic coefficient HTK 0.87 and 0.70), but 2007 had higher rainfall (HTK 4.22). Meteorology conditions during August of 2004-2006 were unfavourable for buckwheat growth because of high rainfall and in 2007 due to drought.

Buckwheat varieties are bred using hybridization, massive and individual selection methods. Selected buckwheat hybrid numbers 1000 grain mass and plant height was compared with parent plants in accordance with the coefficient of phenotype domination *hp*:  $hp < -1.0$  – negative hyper domination,  $-1.0 < hp < -0.5$  – partial domination of lower index,  $-0.5 < hp < 0.5$  – no or low domination,  $hp > 1.0$  – full heterosis (Волотович, 2006). The phenotype domination coefficient *hp* was calculated in accordance with the formula:

$$hp = \frac{(F_1 - MP)}{(P_{max} - MP)}$$

$F_1$  – hybrid features value

$MP$  – parent's features mean value

$P_{max}$  – parent parent's with the higher characters display feature value

According to the phenotype domination coefficient four hybrids groups were evaluated:

- I. 20 hybrids, bred in 2001.
- II. 20 hybrids, bred in 2002.
- III. 53 hybrids, bred in 2003.
- IV. 51 hybrids, bred in 2004.

## Results and Discussion

The buckwheat breeding program was created with varieties and hybrids consisting of the valuable agronomic features. 4 buckwheat varieties were used for the crosses: Volma, Zaleika, Zniajarka, VB Vokiai with the hybrids, selected during the research work. During the research work 144 buckwheat hybrids were selected and tested. The buckwheat selected according to the phenotype domination coefficient, and valuable farming features from parent plants inherited hybrids, are explored in the second and third groups. Results show that 50% of the hybrids, selected in 2002, inherited big size grain and 60% – short stem. Data results are presented at Fig. 1 and Fig. 2. In the third group 60.4% of the hybrids inherited big size grain features. In the first group, the buckwheat hybrids selected in 2001 had weaker valuable agronomic features: domination was found only in 10% of the plants, so there was no heterosis effect. Most buckwheat hybrids in the fourth group had an intermediate size and short stem inheritance.

Buckwheat plants have one of the longest flowering periods and produce a high number of flowers. But compared with other cereals buckwheat yield is usually lower. So the most important way to increase buckwheat yield is to select productive varieties.

Buckwheat breeding data results show that 2001 hybrids did not inherit large seeds. About 80% of the tested hybrids had small grain and there was no heterosis effect (Fig. 1). It was determined that buckwheat selection for large grain size is one of the main measures in new variety breeding, because of polymorphism domination inheritance (Алексеева, Паушева, 1988; Анохина, 1990). Grain size which has 1000 grain weight is one of the most important buckwheat variety features. But it can vary depending on growing conditions. For example, the trial data of 2000-2001 gave 1000 grain mass – 32.2 g to the variety VB Vokiai (Almantas, 2002). But during the 2001-2004 period the conditions for plant growing were extreme, so 1000 grain weight was lower – 31.0 g (Romanovskaja, Ražukas, 2006). Buckwheat breeding trials performed at the Voke branch of Lithuanian Institute of Agriculture data show that 1000 grain mass variation was very low. The varieties variation coefficient was 0.5 – 5.7%, hybrids 1.0 – 6.5% (Romanovskaja, Ražukas, 2006). Buckwheat hybrids plant height inheritance evaluation data of the first group show that 70% of the tested hybrids had heterosis (Fig. 2). Selected hybrids did not inherit short stem height. Multiple populations were produced during the first two years of the breeding work, in 1999 and 2000. The mother plants were selected as hybrid numbers and the foreign varieties as father plants. First year hybrids were used as the primary material for the new cultivar breeding. The buckwheat hybrids of 2002 – 2004, the parental crossings were eliminated and the higher hybrid numbers inherited big size grain and low plant height up to 60% (Fig. 1 and Fig. 2). In the fourth group the hybrids received after crossings with the Lithuanian variety VB Vokiai and the best hybrids of 1999 – 2001 were tested. Evaluating buckwheat hybrids valuable farming features and evaluating the phenotype domination coefficient it was determined that the hybrids which had intermediate big size grain inheritance dominated. An 39.2% of the hybrids had such an inheritance type (Fig. 1). Low height parental plants were chosen for the crossing combinations, which have genetic features for earliness and resistance against the plants lying flat. For example, the Lithuanian variety VB Vokiai is of low height, the average stem height is 87 cm and even in the wet seasons plants are not high (Almantas, 2002; Romanovskaja, Ražukas, 2006). The greatest number of 2004 buckwheat hybrids were taller than the parental plants. 39.2% of the tested hybrids had the heterosis effect (Fig.2). Low plant height was inherited by more than one third of the selected hybrids – 37.3%.

Research results show that choosing low height varieties and hybrids for the new low height varieties the planned results can not always be obtained because of the heterosis effect. But most of all the new bred varieties had lower than medium height, less than one meter.

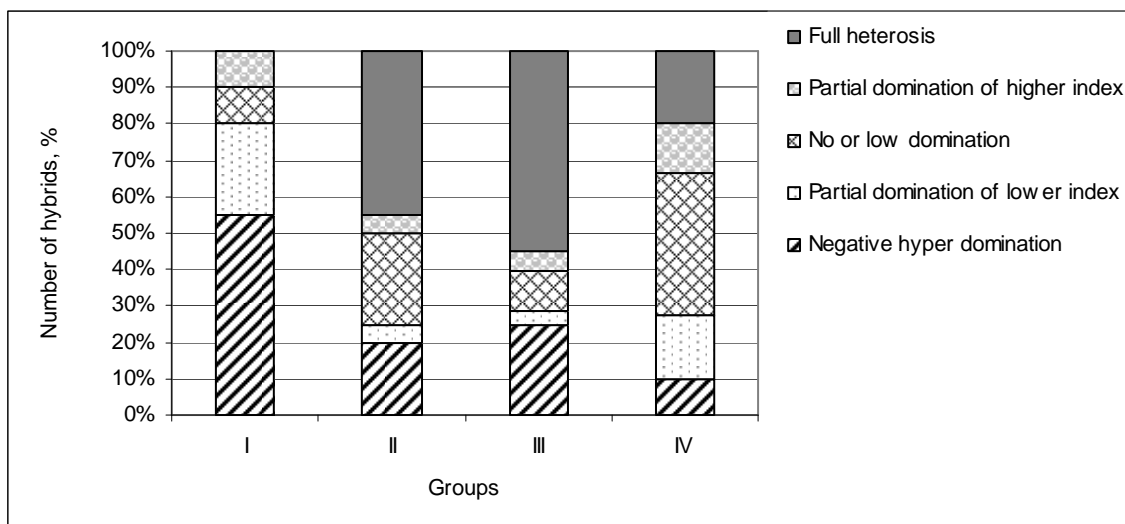


Figure 1. Hybrids inheritance estimation of 1000 grain weight (Trakų Vokē, 2004 – 2006)

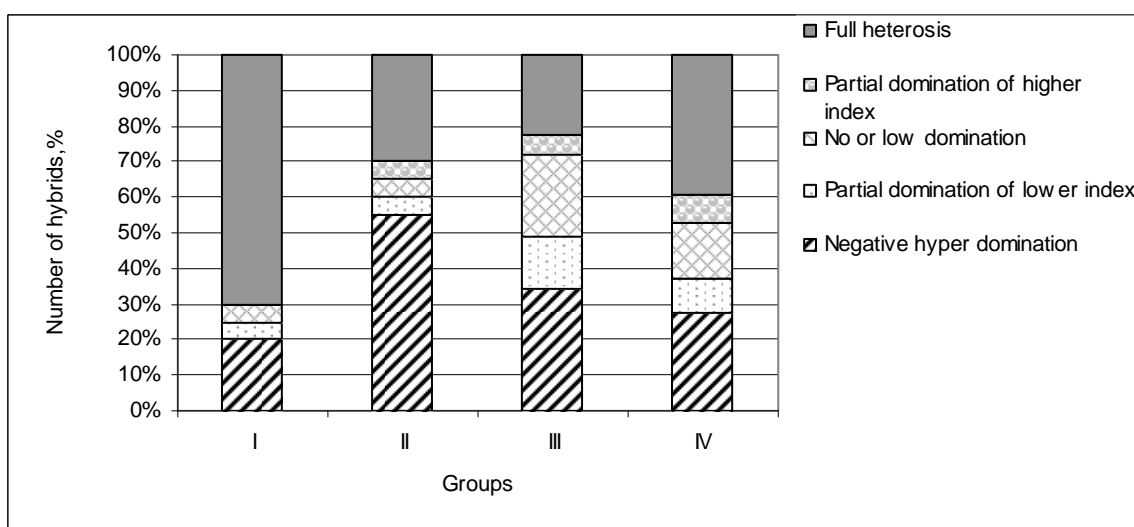


Figure 2. Hybrids inheritance estimation of plant height (Trakų Vokē, 2004 – 2006)

Estimating the last three years research results, when parental crosses were eliminated in the buckwheat breeding work, the data show that 47.6% of the inherited big grain size hybrids and low stem height – 46% of the hybrids. Research of the buckwheat hybrids quantitative features evaluation show that by choosing the right parent plants good results can be achieved in big size grain productive buckwheat varieties breeding.

### Conclusions

The buckwheat breeding program performance for the selection of big size grain and low height plant varieties demonstrated that 46.0 – 47.6% of the selected hybrids inherited the most valuable inheritable features. Using the parent crossing breeding method, only 10% of the buckwheat hybrids inherited big size grain feature and 25% – low plant height. The inheritance of the most valuable agronomic features was very low.

144 buckwheat hybrids were tested during the six research years. Very important genetic material was collected for further buckwheat breeding and research work. The buckwheat hybrids quantitative features consistent pattern will be of great significance for the breeding of new big size grain and low plants height buckwheat varieties.



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## NOZĪMĪGU AGRONOMISKU PAZĪMJU IEDZIMŠANAS IZVĒRTĒJUMS GRIĶU HIBRĪDIEM

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Laika posmā no 2004 līdz 2007.gadam Lietuvas Zemkopības institūta Voke filiālē veikta griķu (*Fagopyrum esculentum* Moench.) selekcija. Galvenās griķu selekcijas metodes ir: iekšsugu hibridizācija, masu un individuālā izlase. Labākajiem atlasītajiem genotipiem tiek vērtēta graudu raža, augu garums, sēklu lielums un citas agronomiski nozīmīgas pazīmes.

Galvenais pētījuma mērķis bija novērtēt, kā griķu hibrīdiem iedzimst dažādas agronomiski svarīgas pazīmes. Galvenais selekcijas mērķis ir izveidot augstražīgas, īsstiebrainas griķu šķirnes ar rupjām sēklām. Izmēģinājumā vērtētas 144 hibrīdās kombinācijas. Rupji graudi un īss stiebrs iedzima 46.0 - 47.6% hibrīdu. Saistītai rupju sēklu un īsa stiebra iedzimšanai hibrīdos ir nozīme griķu selekcijā, jo iespējams iegūt šķirnes ar iepriekšminētajām pazīmēm.

## VARIATION OF POTATO MERISTEM CLONES - NEW FACTS FOR SCIENCE AND PRACTICE

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### Abstract

*In vitro* regenerated plantlets obtained through the virus-eradication procedure were preserved as meristem clones *in vitro*. The progeny of each meristem was the basis for the meristem clone. The agronomic traits of meristem clones were evaluated in field trials. The late blight resistance of meristem clones was determined on the *in vitro* level, where the plants were inoculated with a pure culture of *Phytophthora infestans* and as well as in the field. More than 600 meristem clones of 40 varieties were studied over a long period of time. The results showed that meristem clones differed in their yield, starch content and disease resistance. For example, the yield of 16 meristem clones of the variety Agrie Dzeltenie varied from 32.4 to 51.4 t ha<sup>-1</sup> as an average during the three trial years. The one meristem clone of the variety Bintje was superior to the others for a higher resistance to late blight, for yield and for starch content. For the variety Ants there was no clear correlation between susceptibility to late blight and yield. The most resistant meristem clone produced a yield of 38,1 t ha<sup>-1</sup> and the moderately susceptible clone – 51.8 t ha<sup>-1</sup>. We can conclude

that it is possible to improve the agronomic traits of potato varieties by selecting the meristem clones and this phenomenon can be used in seed production and in plant breeding.

**Key words:** potato meristem, meristem clones, yield, late blight resistance

## Introduction

The viruses of potato are still a great problem despite the fact that the diagnosis of viruses has been improved and rapid methods of eradication and multiplication of plants have been developed. The meristem method has been used as a tool for the eradication of potato initial seed material from virus diseases for about 40 years. The method is principally the following: a slice of meristematic tissue is cut from the bud of a plant and cultivated in a culture medium in conditions that are favourable for the regeneration of the whole new plant. The exact method varies according to the aim of cultivation process. Several treatments for plants or sprouts are used prior the cultivation of meristem, like thermotherapy, chemotherapy, x-rays etc. The meristematic tissue can be excised from the sprout or shoot; it can be lateral or apical.

In EVIKA a lot of factors that affect virus eradication and the regeneration of plants are studied. As a result research of a long duvalim the technology for virus eradication was created according to which the meristem is excised from the lateral and apical buds only from shoots of green plants that have passed through the thermotherapy treatment. The optimal size of the meristem explant that guarantees the highest regeneration percentage and the highest number of disease-free plants is 0.2–0.3 mm. The selection for the best plants starts already on the level of first regenerants. The offspring of all meristem explants is preserved and propagated separately, this way the meristem clones are formed. Before the selection for seed production is made, the meristem clones are evaluated in the fields. Simultaneously one part of each meristem clone offspring is preserved *in vitro* (Kotkas, Rosenberg, 1999).

The results of the preliminary trials where the seed potato was propagated by the conventional clone method demonstrated that some meristem clones differed from others by their yield and level of infection with late blight. Similar information was obtained from the districts of Moscow and Tomsk, where the seed potato propagated in EVIKA was compared to the potato eradicated in another laboratory. These results inspired us to study the characteristics of meristem clones as closely as possible. The goal was to find out if there was any correlation between the characteristics of the meristem explant and the yield and other qualities of the meristem clone. We also wanted to know if the difference between meristem clones was a constant phenomenon or a random.

In the current study we present the comparative data of the agronomical traits of potato meristem clones. The number of publications of possible similar trials is very limited. Our further aim is to do the genetic analysis of potato meristem clones. Up to now the factors increasing the variability of meristem clones and possibilities to create clones with positive variations have been unclear. Therefore the importance of thermotherapy, growth hormones and the genotype in variation development will be studied.

Despite the large number of potato varieties available and the fast breeding of new varieties, some old varieties are still important and are the carriers for valuable genetic information (e.g. Bintje, King Edward, Russet Burbank etc). Meristem clones with improved traits would be helpful for all potato growers – disease resistant meristem clones with uniform tuber shape and size and with high yield capacity would enable one to reduce the utilization of chemicals and other costly inputs. Consequently production would become more favourable ecologically and economically.

The variability of potato genotypes was placed under detailed observation from the disease resistance point of view. The genetic resistance to bacterial and fungal disease enables one to produce potatoes using environmentally friendly methods. Therefore the genotypic reaction of breeding material and old varieties to diseases was studied (Pasco *et al.*, 2006; Lebecka *et al.*, 2006).

Our current knowledge says that the selection of meristem clones with favourable characteristics enables us to improve old as well as new potato varieties. Having a close cooperation with local potato breeders, the promising new selections are brought to our lab for examination, meristem culture establishment and for the selection of meristem clones with the best characteristics. Only

then the breeding procedures with the improved material continue. Such cooperation makes our research directly applicable in practice.

### Materials and Methods

The cultivation of meristem clones and the preparation of test material. Meristem tissue of 0.2–0.3 mm in size was cut from apical or lateral buds of green plants that had passed through 6–8 weeks of thermotherapy treatment 37–39°C 16h and 33–35°C 8h. Usually 20–30 explants were cut per variety. The explants were cultivated on Murashige-Skoog medium modified by EVIKA. The plants regenerated within 4–12 weeks. Fast developing plants with stem-length 8–12 cm and with a developed root primordial were selected for further studies. Regenerants with chlorotic and abnormal leaf or stem shape that remained short and did not develop any roots were eliminated from the tests. The first selection was made at the stage of first meristem plants. The selected meristem plants were propagated *in vitro* by means of microcuttings. The progeny of each meristem plant was the basis for the meristem clone. One part of the meristem clone plants remained *in vitro*, the others, usually 3 of each clone, were used for virus diagnostics. The ELISA-test was used for PVX; PVS, PVM, PVA, PVS and PLRV identification. The virus-free plants were selected for further trials. The plants of meristem clones were propagated *in vitro* and in plastic rolls and were used for the cultivation of the first generation tubers in the field. Simultaneously one part of each meristem clone plant was preserved *in vitro*

The varieties. The following potato varieties were used in the tests: Agrie Dzeltenie- from Latvia, released in 1962, medium early; Juku -Estonia, medium late; Piret - Estonia 1998, medium late; Eba, Holland, medium late; Ants, Estonia 1980, medium late; Bintje - Holland, 1910, medium late; Vigri -Estonia 1980, medium late.

Field trials. The field trials were conducted in the test field of EVIKA in 1994–2003 in North-Estonia. First generation tubers were used for first year trials and every following year the second, third or fourth generation tubers were used respectively. The trials were set in 4 replicas, 40 tubers per plot. The random block- placement in 4 repetitions was used.

The same agrotechnical procedures as in production fields were used in the trials. The potatoes were fertilized with the complex fertilizer Cropcare 10-10-20, 50 kg N, 50 kg P and 100 kg K ha<sup>-1</sup>. The soil type was different every year. The planting time depended on weather and varied from May 8<sup>th</sup> to 25<sup>th</sup> as well as the harvesting time – from September 2<sup>nd</sup> to 20<sup>th</sup>. Chemical weed control was used when needed and the potatoes were hilled up 2–3 times during the vegetation period. There was no chemical disease or pest control made in the test fields.

The potato plants were monitored during the whole growing period. The speed and quality of emergence, the intensity of flowering and infection with late blight was evaluated. The number of tubers per plant was counted and the weight of the tubers was measured at harvesting. The results were analyzed statistically using dispersion analysis.

The tests of the meristem clones for resistance to late blight. The infection rate of *in vitro* plants with pure cultures of various stems of *P. infestans* was tested in the lab. Pure cultures were cultivated on rye agar and the layer of fungus was washed with sterile water to make the suspension necessary for inoculation. The liquid was then filtrated through 2 layers of cheesecloth. The concentration of conidia was determined under the microscope at 120-fold magnification. Plants developed *in vitro* from microcuttings were infected with a suspension of conidia with a concentration of 25–50 conidia at the field of view of microscope. After inoculation the plants were maintained *in vitro* at a temperature of 20–21°C and photoperiod 18 hours. The resistance to *P. infestans* was evaluated visually and expressed as the percentage of late blight injured leaf area, starting on the 3<sup>rd</sup> day after the inoculation until the death of the plants. In the field the infection rate of potato late blight and its development was evaluated visually. The percentages of injured leaf area before potato harvest and infected tubers are given.

## Results and Discussion

The aim of our research was to study the agronomic traits of potato meristem clones like yield capacity, the starch content of tubers, resistance to potato late blight as well as the morphologic characteristics of plants. We studied many varieties of different origin and age, new as well as old varieties. Special consideration was bestowed upon the old and widely grown variety in Europe – Bintje. The yield capacity of meristem clones was studied. Special attention was given to the tolerance of late blight since the variety is known to be especially susceptible to *P. infestans* infection. The data in figure 1 show that the yield as well as the number of tubers of all 7 varieties varied. The large variations occurred in the case of old as younger varieties. In figure 1 among the presented varieties, the oldest are Agrie Dzeltenie (from 1962) and Bintje (1910).

In the same figure the varieties Juku, Piret and Ants are the younger varieties, all less than 20 years. During field monitoring it was observed that the plants of the meristem clones with a higher yield capacity had more shoots, uniformed bush and more intensive flowering than other clones.

Several studies with other varieties have shown that meristem clones may differ in their starch content in tubers but also in their disease resistance (Rosenberg *et al.*, 2007<sup>-1</sup>, 2007<sup>-2</sup>, 2004). Interesting results were obtained with the varieties Bintje and Ants (Tables 1, 2).

Four meristem clones of the variety Bintje were studied thoroughly and one was found to be more resistant to late blight than the others. The higher resistance was detected on *in vitro* plantlets and in the field, where less infection was observed on the veins, leaves and on the tubers. In the same plots the infection started later. The same meristem clone was superior to the others also for yield, the starch content of tubers and for the uniformity of the plant canopy.

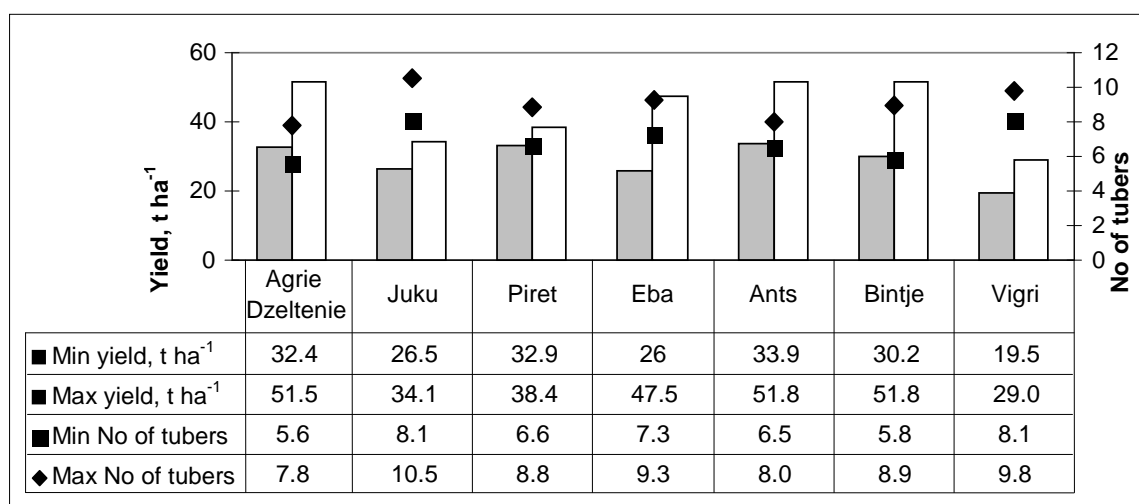


Figure 1. Variation of yield and number of tuber per plant on meristem clones\* of different potato varieties

\*Agrie Dzeltenie-16 clones, PD 95 % = 4.0 t ha<sup>-1</sup>; PD 95 % = 0.7 tubers plant<sup>-1</sup>; Juku-10 clones, PD 95 % = 3.2; PD 95 % = 0.4 tubers plant<sup>-1</sup>; Piret- 10 clones, PD 95 % = 2.6; 0.8 tubers plant<sup>-1</sup>; Eba- 6 clones, PD 95 % = 4.2 t ha<sup>-1</sup>; PD 95 % = 0.4 tubers plant<sup>-1</sup>; Ants-5 clones, PD 95 % = 4.3 t ha<sup>-1</sup>; PD 95 % = 0.7 tubers plant<sup>-1</sup>; Bintje-4 clones PD 95 % = 3.6 t ha<sup>-1</sup>; PD 95 % = 0.8 tubers plant<sup>-1</sup>; Vigri-11 clones, PD 95 % = 2.7 t ha<sup>-1</sup>; PD 95 % = 0.3 tubers plant<sup>-1</sup>.

In the variety Ants there was no clear correlation between resistance to late blight and yield. For instance, the most resistant meristem clone gave the yield of 38.1 t ha<sup>-1</sup> as an average and the moderately susceptible clone – 51.8 t ha<sup>-1</sup>. Variety Ants is relatively resistant to late blight itself and therefore the infection by *P. infestans* did not affect the yield remarkably.

Table 1. The resistance of the meristem clones of the variety Bintje to *P. infestans in vitro* and in field

Clone No	<i>P. infestans</i> injured leaf area on <i>in vitro</i> plants, %					Late blight in the field, %		Late blight infected tubers, %	
	4.*	6.	13.	20.	31.	1994	1995	1994	1995
832	<u>55.8</u> 0	<u>77.7</u> 22.1	<u>100.0</u> 77.7	<u>0</u> 100.0		72.0	38.0	40.0	2.5
834	<u>22.2</u> 0	<u>33.3</u> 11.1	<u>66.7</u> 45.0	<u>100.0</u> 100.0		88.0	38.0	17.5	0.0
836	<u>33.3</u> 1.1	<u>44.4</u> 33.3	<u>77.8</u> 55.5	<u>88.9</u> 100.0	<u>100.0</u>	53.0	10.0	10.0	2.5
839	<u>33.3</u> 0	<u>66.7</u> 22.2	<u>100.0</u> <u>78.9</u>	88.9	100.0	42.0	9.0	20.0	5.0

\* days after inoculation with suspension of *P. infestans* conidia

 Table 2. The resistance to *P. infestans* of meristem clones of variety Ants *in vitro* and in field

Clone No	Injured leaf area on 3 <sup>rd</sup> day* on <i>in vitro</i> plants, %		Injured leaf area on 26 <sup>th</sup> day* on <i>in vitro</i>		Late blight in the field, plants tubers		Yield, t ha <sup>-1</sup> (2 years average)
	A**	B	A	B	plants	tubers	
1	66.7	0	100	100	8.6	0	45.0
4	33.3	0	100	33.3	3.8	0	51.8
5	33.3	0	100	0*	1.0	0	39.5
12	33.3	66.7	100	100	1.8	0	33.9
815	0	0	100	11.1	1.4	0	38.1

\* days after inoculation with suspension of *P. infestans* conidia; \*\* A, B – *P. infestans* stems

We had a research programme designed to study the possibilities of using somaclonal variation caused by the meristem culture in potato breeding. We hoped to find genotypes which showed greater variation in agronomic traits that use the meristem culture, to the select best meristem clones with a heritable character of agronomically important properties. There were two varieties – Anti and Juku – and one breeding number J1488-88 in the tests. The results demonstrated that the highest variations in agronomic characteristics occurred in the variety Juku. The breeding number J1488-88 was characterized by the highest variation in resistance to *P. infestans* (Koppel and Rosenberg, 1998).

Special trials were conducted with the variety Eba in order to study the correlation between the yield and the characteristics of meristem clones and the correlations between the characteristics of the mother plant and the meristem clones of its offspring. The meristem clones of the variety Eba differed also in their starch content. The starch content of the seven tested clones varied from 163 to 176 g kg<sup>-1</sup>. The starch content of the meristem clone No 3373 was 170 g kg<sup>-1</sup>. The number of tubers per plant varied from 10.3 to 14.8 (3373). The plants' conditions varied according to a 5-point scale from 2.5 (3373/333) to 5.0 (3373). At the Jõgeva Plant Breeding Institute the susceptibility of tubers to *P. infestans* were tested by a one time artificial infection. The susceptibility of the four meristem clones of the variety Eba varied from 2.24 to 3.06 points, the most susceptible being the meristem clone 3373 (3.06 points). According to the same tests the susceptibility of 5 meristem clones of the variety Premiere varied from 2.53 to 3.07; the 13 meristem clones of the variety Vigri from 3.34 to 4.15 (Rosenberg, 1995).

The intensity of virus biosynthesis and the susceptibility to the viruses in the meristem clones of the same variety as well as in the meristem clones regenerated from the meristem operated from different parts, apical and lateral buds of shoot, immature flower bud of the same plant, were studied. Also the biosynthesis intensity of PVX in the 17 meristem clones of the varieties Premiere, Eba and Kondor and PVM in 27 clones Vigri, Eba, Kondor and Premiere. The highest relative resistance to PVX was found in the meristem clones Eba 3373; Kondor 1065; Premiere 804 and to PVM - in Eba 1000; Kondor 1; Premiere 356 and Vigri 918. The dependence of virus resistance of the meristem clones on the location of meristem in the plants was established. All varieties showed the highest degree of virus biosynthesis and susceptibility to PVX and PVM in the meristem clones

obtained from apical buds of soot, the lowest in the clones from a lateral bud of soot (Agur and Rosenberg, 1999).

The differences between the meristem clones in the current study can be considered as somaclonal variations caused by the meristem culture. The variability of the meristem culture is in between the intact plant culture and the callus- and cell culture. There are no lethal chromosomal or gene mutations in the meristem culture as a rule but the frequency of point-mutations can be higher than in intact plant culture. It is assumed that the meristem culture itself produces only small amounts of somaclonal variations but the variability can be increased by the other factors such as the high concentration of growth hormones in the nutrient medium, the lability of varieties, or other factors. In our disease eradication process it can be assumed that the variations may have occurred during the heat treatment, before the operation of the meristem. Special trials are being conducted in order to study the influence of high temperatures on the frequency of somaclonal variations in the meristem culture. The results should be obtained in a few years.

The somaclonal variation caused by the meristem culture is not very well studied yet. However, data exist about the somaclonal variations initiated in the callus culture and its utilization in potato breeding. In trials with plants regenerated from the callus culture of 14 varieties, differences in canopy growth, tuber yield and ripening time were observed. Most of the somaclones were with negative traits already in the early stage of development. Positive mutations were detected only on 1% of the plants. The extent of variations in the callus culture depended remarkably on variety (Thieme and Griess, 1996). Sometimes the variations that occur in the callus culture may lead to a variety or new clones resistant to late blight (Guseva and Komaletdinova, 1998).

The previous studies with many varieties evidenced show that meristem clones may differ in their yield, starch content in tubers and also in disease resistance. It is possible to improve the agronomic traits of potato varieties by selecting the meristem clones with suitable characteristics. This methodology can be and should be used in seed potato production and in plant breeding before their propagation for seed production. As it was explained above we make the first positive selection already at the first stage of meristem cultivation. We discard all abnormal chlorotic regenerants and continue trials only with the well-developed and vigorous plants. However, we have noticed that the initially abnormal plants can sometimes develop into normal ones after many subcultures done over a long period of time. So far we have not studied the characteristics of such initially abnormal plants. The necessity to select the virus-eradicated meristem clones and to study their propagation is also emphasized by Hungarian authors (Fischl *et al.*, 1988).

Our experience has shown that the variability of meristem clones serves as excellent tool in improving the yield and other agronomic properties of the potato varieties. For instance – the tubers of the variety Ants were not of uniform shape. Often the tubers were too elliptical or narrower from one end. Now the tubers of the selected meristem clone are of uniform shape and the meristem clone is propagated in seed production.

## Conclusions

Our experience has shown that the variability of meristem clones can serve as a valuable tool in improving the yield and other properties of potato varieties. We have not detected deviation from true-to-type morphological characteristics. However, some meristem clones differ in the intensity of flowering, the height of stems and the uniformity of plants.

We can conclude that before supplying the seed for production it is necessary to test the meristem clones in field trials. Good quality initial material can be obtained and it can be multiplied for seed production by using rapid multiplication methods. The studies of resistance to viruses and *P. infestans* provided a lot of interesting data but this issue needs more detailed study, and so does the possibility to use the somaclonal variation in plant breeding.

In the future detailed studies about the variations of meristem clones, their genetic pattern and the possibility to improve the tolerance of the varieties to virus, bacterial and fungal diseases are going to be developed further.

## Acknowledgements

This work was supported by the Estonian Science Foundation, grant No 6124.

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## KARTUPEĻU MERISTĒMU KLONU MAINĪBA - JAUNI FAKTI ZINĀTNEI UN PRAKSEI

**Rosenberg V., Kotkas, K., Särekanno, M., Ojarand, A., Vasar, V.**

Vīrusu samazināšanas procedūras gaitā atveseļotie augi *in vitro* tika uzturēti kā meristēmu kloni *in vitro*. Katras izdalītās meristēmas pēcnācēji bija izejas materiāls meristēmu klonam. Katra klona agronomiski nozīmīgās pazīmes tika novērtētas lauka izmēģinājumos. Meristēmu klonu izturība pret lakstu puvi tika noteikta *in vitro* līmenī, augus mākslīgi inficējot ar *Phytophthora infestans*, kā arī lauka apstākļos. Vairāk kā 600 meristēmu klonu, kas iegūti no 40 šķirnēm, tika pētīti ilgu laiku. Rezultāti parāda, ka meristēmu kloni atšķiras ražas, cietes saturā, kā arī slimību izturības ziņā. Piemēram, šķirnes 'Agrie Dzeltene' 16 meristēmu klonu trīs gadu vidējā raža variēja no 32.4 līdz 51.4 t ha<sup>-1</sup>. Šķirnes 'Bintje' viens meristēmu klons pārspēja citus ar augstu izturību pret lakstu puvi, lielāku ražu un cietes saturu. Šķirnei 'Ants' netika konstatēta sakarība starp jutīgumu pret lakstu puvi un ražu. Izturīgākā meristēmu klona raža bija 38.1 t ha<sup>-1</sup>, bet vidēji izturīga klona raža bija 51.8 t ha<sup>-1</sup>. Mēs varam secināt, ka ir iespējams uzlabot šķirnes agronomiskās īpašības atlasot meristēmu klonus, šis fenomens ir izmantojams sēklaudzēšanā un selekcijā.

## BREEDING OF WINTER WHEAT FOR END-USE QUALITY

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## Abstract

The winter wheat varieties 'Ada', 'Alma', 'Milda', 'Taurus' and 'Seda', corresponding to different groups of end-use quality, have been developed at the Lithuanian Institute of Agriculture during the last decade and included in the Lithuanian National Plant Variety List. The new varieties are of

different earliness. The earliest-ripening variety is 'Alma'. It can be harvested one week earlier than the well known variety 'Širvinta' and 2-3 weeks earlier than 'Zentos'. The variety 'Ada' is the second in earliness and has the same length of growing period as 'Širvinta'. The varieties 'Milda' and 'Taurus' ripen one week later compared to 'Širvinta', 'Seda' ripens 10 days later. The best composition of HMW subunits predicting high quality as Glu-A1 1; Glu-B1 7+8 and 7+9; Glu D1 5+10 has been determined for the varieties 'Alma', 'Milda' 'Taurus' and 'Ada'. The mean sedimentation value of the varieties tested was as follows: 'Alma'- 64 ml, 'Milda'- 62, 'Ada'- 53, 'Taurus'- 53, 'Širvinta'- 52, and 'Seda'- 22 ml. The strongest flour was produced from the variety 'Alma', excellent from 'Ada', 'Milda' and 'Taurus'. The variety 'Seda' is intended for alcohol production and confectionary. All the varieties are characterized by a high Falling Number index. The yield penalty - grain loss, associated with high quality declined for the varieties 'Ada', 'Milda' and 'Taurus'. The varieties are high yielding and are characterized by strong and elastic gluten.

**Key words:** winter wheat, breeding, bread- making quality

## Introduction

The winter wheat breeding programs of the Lithuanian Institute of Agriculture (LIA) are divided into the following groups according to their ultimate targets: early- ripening varieties with a high protein content and very strong gluten; early- medium ripening varieties with high bread-making quality; medium-ripening, high yielding varieties, with satisfactory bread- making quality; late ripening high yielding varieties not meant for bread- making purposes. For the milling industry it is necessary to have a large range of wheat varieties to produce flour mixtures because the baking industry requires raw material with specific processing characteristics. Increasing yield potential while maintaining desirable industrial quality is not an easy task because increases in grain yield usually result in decreases in protein, which is one of the major factors defining the industrial quality of wheat (Pena, 1996; Mašauskienė *et al.*, 2001).

During the last decade, 5 winter wheat varieties, corresponding to different groups of end-use quality, have been developed at the Lithuanian Institute of Agriculture. The strongest flour was produced by the variety 'Alma', excellent by 'Ada', 'Milda' and 'Taurus'. The variety 'Seda' is intended for alcohol production and confectionary (Ruzgas, 2003).

In this article we presented the results of the investigation on end use properties of the new Lithuanian winter wheat varieties, included in the National Variety List, conducted during the period 1998-2006.

## Material and Methods

The field experiments were carried out in a replicated yield test in Dotnuva during 1998- 2006. The plots were 15- 20 m<sup>2</sup> in size. The soil of the experimental site is *Endocalcari-Epihypogleic Cambisol*, of neutral acidity pH 7. It contained 1.3 g kg<sup>-1</sup> organic carbon (2.2 g kg<sup>-1</sup> humus) in the plough layer and was moderate in available phosphorus and potassium. N 90 kg ha<sup>-1</sup> fertilization was applied annually. On the basis of eight years of experiments the data of 7 varieties were processed. The varieties were characterized using the following parameters: protein content by the Kjeldahl method (LST 1523:1998), wet gluten in flour, determined according to the Lithuanian standard LST 1522:2004, sedimentation value, determined by the methods of Zeleny (LST 1498:1997) The rheological properties ( mixing tolerance in minute and other traits) were investigated and recorded by the Brabender's Farinograf (LST 1696:2001; ICC 115/1:1992 , alpha-amylase activity by the Hagberg Falling Number method (LST ISO 3093:1982). The grain quality index is a composite index including several of Brabender's pharinogram indexes: dough developing time, stability and softening. The composition of HMW subunits was analyzed using sodium dodecyl sulphate- polyacrylamide gel electrophoresis. For classification the system proposed by Payne *et al.*, was used (Blacman *et. al.*, 1987). The statistical data were processed using the software ANOVA and STAT\_ENG (Tarakanovas and Raudonius, 2003).



## Results and Discussion

The investigations of grain yield performance showed that all winter wheat varieties bred at LIA were higher yielding compared to the early ripening check variety Širvinta (Table 1). Compared to the late ripening check variety 'Zentos', 'Seda' was found to be higher yielding, which belongs to late ripening group also. The mean yield of 'Ada' was close to that of the check variety 'Zentos'. The highest gluten content was estimated in the grain of the varieties 'Alma' and 'Milda', flour output 'Milda' and 'Tauras'. All varieties were characterized by a excellent Falling Number index.

Table 1. Yield and quality characteristics of the Lithuanian winter wheat varieties, Dotnuva, 1998-2006

Variety	Grain Yield, t ha <sup>-1</sup>	Gluten content, g kg <sup>-1</sup>	Output of flour, g kg <sup>-1</sup>	Falling number, s	High molecular weight glutenins	
					Subunits	Scores
Širvinta	5.77	292	720	345	1; 7+9; 5+10	9
Zentos	7.14	254	730	375	0; 7+9; 5+10	7
Alma	6.32	312	720	366	1; 7+8; 5+10	10
Ada	7.03	278	720	371	1; 7+9; 5+10	9
Milda	6.74	306	740	361	1; 7+9; 5+10	9
Tauras	6.85	267	730	338	1; 7+9; 5+10	9
Seda	7.28	230	690	329	0; 6+8; 2+12	4
LSD <sub>0.5</sub>	0.42	21.4	11.7	33.5	-	-
LSD <sub>0.1</sub>	0.74	28.6	15.6	44.7	-	-

The dough quality is one of the most important features enabling one to predict the final bread-making value of the winter wheat variety. The elasticity of the dough was measured by Brabender's pharinograph, whose operations are based on physical methods. The diagram (pharinogram) showed direct indexes: water absorption, dough developing time, stability and other traits (Table 2, Figure 2).

Table 2. The dough rheological characteristics of the new Lithuanian winter wheat varieties

Variety	Water absorption, g kg <sup>-1</sup>	Dough developing time, min	Dough stability, min	Brabender's quality index
Ada	627	5.6	10.2	112
Alma	629	4.4	12.4	122
Milda	619	5.1	8.1	99
Tauras	597	3.7	8.2	92
Seda	565	2.1	2.9	41
Širvinta	621	3.7	6.3	77
Zentos	620	2.9	6.0	67
LSD 0.5	16	1.0	1.4	-
LSD 0.1	21	1.4	1.9	-

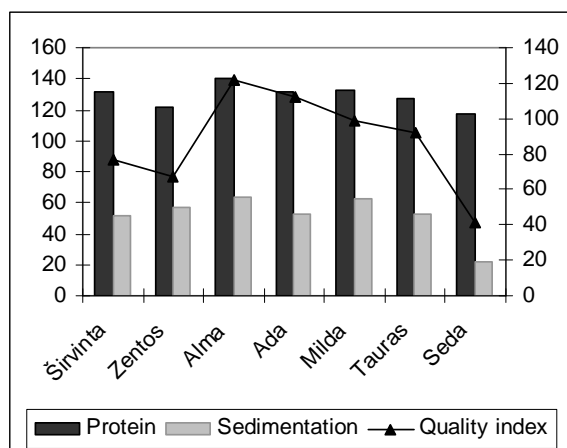


Figure 1. Protein content (g kg<sup>-1</sup>), sedimentation Zeleny index (ml) and grain quality index

It is possible to consolidate all traits in the calculated Brabender's quality index. The pharinograms precisely showed the dynamics of the dough development characteristics of the individual varieties (Figure 2).

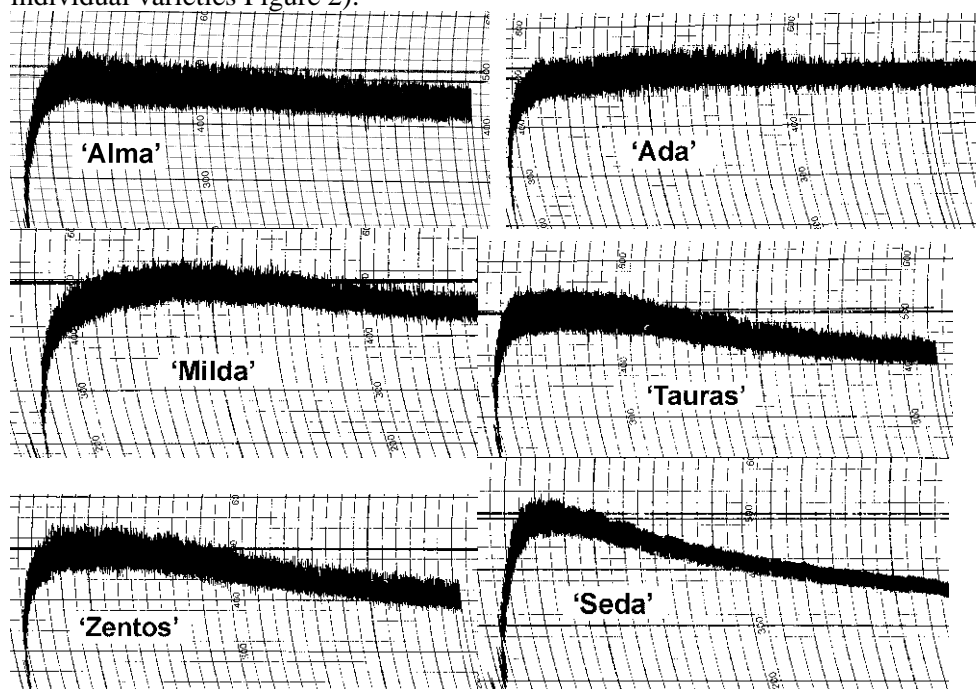


Figure 2. Dough rheology of the new Lithuanian winter wheat varieties, Brabender's pharinograms, 2006.

This study involved 5 new varieties bred at LIA and two checks, the local early ripening variety 'Širvinta' and the late ripening 'Zentos'. The new varieties are of different earliness. The earliest ripening variety is 'Alma'. It can be harvested one week earlier than the well known variety 'Širvinta' and 2-3 weeks earlier than 'Zentos'. The variety 'Ada' is the second in earliness, and has the same length of growing period as 'Širvinta'. The varieties 'Milda' and 'Tauras' ripen one week later compared to 'Širvinta'. 'Seda' ripens 10 days later. The ripening time is very important for the actual arvesting time, because the amylases of completely ripe varieties are more active compared with those of the varieties with late developing phases.

The prediction of a bread-making quality of winter wheat varieties by the analysis of HMW glutenin subunits is an important point in the conduct of breeding programs. On the another hand, the electrophoretic HMW glutenin tests are a proper method to test the genetic makeup of the new varieties, to verify if the variety is homosyous or not. The best composition of HMW subunits

predicting high quality as Glu-A1 1; Glu-B1 7+8 and 7+9; Glu D1 5+10 has been determined for the varieties 'Alma', 'Milda', 'Taurus' and 'Ada' (Paplauskienė and Ruzgas, 2002), (Table 1).

The sedimentation value indicates the protein quality. The mean sedimentation value of the varieties tested was as follows: 'Alma'- 64 ml, 'Milda'- 62, 'Ada'- 53, 'Taurus'- 53, 'Širvinta'- 52, and 'Seda'- 22 ml (Figure 1).

Varieties with sedimentation value exceeding 40 ml are considered as good, those exceeding 50 as exhibiting excellent bread-making quality. Sedimentation value below 30 ml is characteristic of wheat varieties with weak dough quality. Such varieties are intended for confectionery, alcohol production or starch separation.

Protein content is one of the more important indicators of grain quality for the bread making industry and one of the desirable traits of wheat in countries with cool climates. The Lithuanian wheat breeding programmes are focused on protein improvement and the mean protein content of the new winter wheat varieties is relatively high. It was found, that during the experimental period the mean protein value was as follows: Alma'- 140, 'Milda'- 133, 'Ada'- 131, 'Taurus'- 127, 'Širvinta'- 131, 'Seda'- 117 g kg<sup>-1</sup>. The mean protein content in the grain of the check variety 'Zentos' was 122 g kg<sup>-1</sup>.

The grain yield of the variety 'Alma' was the lowest compared with the other varieties (Table 1). It confirmed the conclusions of other authors that grain protein content is negatively correlated with grain yield (Bhatia, 1975). The equation of regression of the investigated varieties was  $y = 21.96 - 1.20x$  (x- protein content, y- grain yield);  $F = 22.56^{**}$ ; coefficient of determination  $R^2 = 0.74$ ;  $t = 3.10$ . However, this statement is true when varieties with extremely high protein content or very strong dough properties are included in the experiment. The correlation between protein content and yield, except for the variety 'Alma', was negative but not significant:  $y = 16.55 - 0.76x$ ;  $F = 5.28$ ;  $R^2 = 0.40$ ;  $t = 1.23$  because the varieties 'Ada', 'Milda' and 'Taurus' were high yielding and had strong gluten.

Investigations by Brabender's pharinograf provide information about dough properties. It is important to predict the technological features of the varieties: the water absorption of the flour, dough development time, stability and mixing tolerance, to decide the quality group of a particular winter wheat variety. The highest mean water absorption was indicated for the varieties 'Alma' and 'Ada', the lowest for 'Seda'. The highest mean dough development time was indicated for the varieties 'Ada', 'Milda' and 'Alma'. The longest dough stability time was found for the varieties 'Alma', 'Ada', 'Taurus' and 'Milda'. According to Brabender's quality index the varieties ranked in the following order: 'Alma', 'Ada', 'Milda', 'Taurus', 'Širvinta', 'Zentos', 'Seda'.

The mixing tolerance showed dough elasticity. "Strong" and "weak" flours produce dough with very different mixing properties. The difference mainly results from the quantity and quality of protein. Different food stuffs prepared from wheat require very different flour strength for their manufacture. The new Lithuanian winter wheat varieties have different elasticities of dough. The strongest flour is determined for the varieties 'Alma' and 'Ada', moderately strong for 'Milda' and 'Taurus', (Figure 2), weak for 'Seda'. The grain quality index of those varieties was 122; 112; 99; 92; 41 and dough stability time 12.4, 10.2, 8.1, 8.2 and 2.9 min., respectively.

The correlation between protein content and mixing tolerance was high, the coefficient of determination was  $R^2 = 0.58^*$ , equation  $y = -53.61 + 4.76x$  (x- protein content, y- mixing tolerance, min.),  $F = 10.84^*$ . The correlation between the sedimentation index and the mixing tolerance was lower,  $R^2 = 0.42$ , equation  $y = -0.96 + 0.15x$  (y- sedimentation index, x- mixing tolerance).  $F = 5.73^*$ . The variety 'Seda' was characterized by weak gluten. Actually, this variety was bred for alcohol, confectionery and other purposes.

One of the most important traits of winter wheat is flour output, which is the percent of flour type 550 obtained from whole grain in the milling process. The highest output was estimated for the varieties 'Milda', 'Taurus', and 'Zentos'. All the varieties were characterized by high resistance to pre-harvest sprouting. This was shown by the Hagberg Falling Number index, highly correlating with pre-harvest sprouting and alpha amylase activities (Blacman *et al.*, 1987). The highest Falling Number index was characteristic of the varieties 'Zentos', 'Ada', 'Alma' and 'Milda'. In general, all the varieties had a Falling Number index exceeding the 250 levels required for bread-making wheat and are suitable for high quality production. This trait is very important for Lithuania and other countries with high air humidity at harvesting. Varieties for confectionery

should exhibit low protein content, sedimentation value, but a high Falling Number index. The variety 'Seda' possesses the mentioned features.

### Conclusions

The winter wheat varieties 'Ada', 'Alma', 'Milda', 'Taurus' and 'Seda', corresponding to different groups of end-use quality, have been developed at the Lithuanian Institute of Agriculture during the last decade and included in the Lithuanian National Plant Variety List. The strongest flour was produced by the variety 'Alma', excellent by 'Ada', 'Milda' and 'Taurus'. The variety 'Seda' is intended for alcohol production and confectionary. All the varieties are characterized by high Falling Number index. The highest output of flour Type 550 was estimated for the varieties Milda and Taurus. The yield penalty- grain losses associated with high quality was reduced in the varieties 'Ada', 'Milda' and 'Taurus'. Those varieties are high yielding and are characterized by strong elastic gluten.

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## ZIEMAS KVIEŠŲ SELEKCIJA DAŽADIEM IZMANTOŠANAS VIRZIENIEM

### Ruzgas V., Liatukas Ž.

Pēdējā desmitgadē Lietuvas Zemkopības institūtā izveidotas piecas ziemas kviešu šķirnes – Ada, Alma, Milda, Taurus, un Seda, kas paredzētas atšķirīgiem izmantošanas virzieniem un iekļautas Lietuvas nacionālajā augu šķirņu katalogā. Jaunās šķirnes atšķiras pēc to agrinuma. Visagrīnākā ir šķirne Alma, kas nogatavojas nedēļu ātrāk, nekā labi zināmā šķirne Širvinta un divas – trīs nedēļas agrāk par šķirni Zentos. Šķirne Ada ir otra agrīnākā un nogatavojas vienlaicīgi ar šķirni Širvinta, bet šķirnes Milda un Taurus pilngatavības fāzi sasniedz nedēļu vēlāk. Visvēlīnākā ir šķirne Seda, kas nogatavojas 10 dienas vēlāk nekā šķirne Širvinta. Šķirnēm Alma, Mild, Taurus un Ada konstatēts labākais lipekļa sastāvs - Glu-A1 1; Glu-B1 7+8 un 7+9; Glu D1 5+10, kas nodrošina labu maizes kvalitāti. Šķirne Seda ir piemērota spirta ražošanai un konditorejas izstrādājumu gatavošanai. Visām šķirnēm ir augsts krišanas skaitlis. Šķirnēm Ada, Milda un Taurus nav konstatēta būtiska negatīva korelācija starp graudu ražu un kvalitāti - tām ir augsts ražas potenciāls un vienlaicīgi arī stiprs un elastīgs lipekļis.

## POTATO TESTING FOR WART RESISTANCE IN BELARUS

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### Abstract

The analysis of studying in Belarus the most dangerous and quarantine-important fungus *Synchytrium endobioticum* (Schilb.) Perc. – potato wart agent is presented in the article. The necessity of the immunological evaluation of the potato seed material for wart resistance is fortified. The conditions of preliminary and state testing of selection samples in the quarantine stationary plot of the RUC “Institute of plant protection” in accordance with the methods of potato testing for wart resistance are examined in this paper.

It is shown that in preliminary and state testing for resistance to the (D1) potato wart agent pathotype among the studied hybrid potato material from the leading breeding institutions of Belarus and Russia for the period of 2003-2007, 89,8% resistant samples were isolated.

While determining the wart disease resistance of the breeding material on susceptible sample tubers beside the usual incrustation form of potato wart affection the infestation of three non-typical forms: leaf, scab and corrugated are observed.

Considering the variations of wart-resistant potato samples injury, the necessity of carrying out not less than two years selection material state testing to wart resistance under different field conditions was undertaken in Belarus.

**Key words:** potato, potato wart disease, resistance

### Introduction

Among potato diseases the wart disease is distinguished by its harmfulness caused by the fungus *Synchytrium endobioticum* (Schilb.) Perc., and is a quarantined object in Belarus. The potato wart focus was discovered for the first time in 1939 in Belarus. In 1956 potato crops inspected for quarantine object contamination showed that the disease was spread in all six districts in private plots and on 90 collective farms and state farms.

The demand for wart resistance variety breeding was put ahead at the State level by the ex-USSR government decree dated October 20, 1947. According to this regulation the period (till 1951) was determined as a transition to the growing of wart-resistant varieties (Krishtofik, 1998). Undertaking the directed measures of wart elimination, including a set of preventive and chemical measures and also the introduction of agent-resistant varieties, gave an opportunity to remove harmful disease focuses in the rotation fields by the beginning of the 1980s. However, the solving of this problem in the private sector turned out to be significantly complicated. At present wart natural focuses are not numerous, being observed in private plots only. It demands constant monitoring of the *S. endobioticum* fungus population condition.

The history of potato wart studies shows that the most economical and ecologically safe method for overcoming losses caused by the disease is the creation of wart-resistant varieties and their application into production. The resistance trait has not lost its importance in Belarus till nowadays and it is compulsory for new variety breeding and their inclusion into the State Register of varieties and wild breeds.

One should point out that the first scientific researches in the potato studies on wart harmfulness on the territory of Belarus were carried out in the Zonal Minsk Scientific-Research Station on potato wart. It was founded in 1947 by the collective of Belarusian phytopathologists under the guidance of Academician N.A. Dorozhkin (Dorozhkin, 1948, 1953; Dorozhkin and Remneva, 1948; Gandelman, 1950, 1953; Dorozhkin and Sharikov, 1951; Sharikov, 1951; Gorlenko, 1954).

By the foundation of the Immunity Lab at the Institute of Plant Protection the main direction of potato wart research in Belarus was improvement of variety sample evaluation methods for disease resistance in the pathogen infected backgrounds, carrying out search of resistance sources to the noxious disease. By evaluating wart resistance this was the main demand according to which the infected material should display the realistic pathogen condition observed in the region.

As a result of the intra-specific diversity studies of a fungus *S. endobioticum* on the territory of the Republic of Belarus in different agro climatic zones its non-equivalence was determined with the discovering of not less than 7 geographic populations differed by pathogen activity. Though, the fact of their belonging to the same race was strictly established (Danchenko, 1974; 1978).

One of the stages of subsequent immunological evaluation improvement of selection potato samples for wart-resistance was the development and use of the infected background with wart and cyst-forming golden potato nematode at the Institute of Plant Protection. It was giving an opportunity to carry out simultaneously the evaluation of resistance to two quarantine pathogens (Tolkachiov, 1998; Portyankin, 2000) within one and the same experiment. However, it was determined that the resistance evaluation at the combined background can not give 100% of coincidence with the separate evaluation to every pathogen. That is why it is preferable to use it for preliminary testing of the first year hybrids.

At present all the public sector potato growing areas are occupied only with the wart resistant potato varieties.

For the necessity of immunological protection of growing potato varieties in relation to wart agent the objective of the present research is selection of potato varieties by testing their resistance to wart disease in preliminary and state tests. This is undertaken and should be considered as an inseparable part of the final stage of variety creation.

### Materials and Methods

Potato samples used as a material for the research were presented from breeding institutions of Belarus and Russia.

The research was carried out at potato protection lab of the RUC «Institute of Plant Protection» during the period of 2003-2007. The agro-meteorological parameters of growing seasons of the research period are presented in Table 1.

The preliminary testing was done in lysimeters in quarantine stationary plot by starting laboratory and vegetation experiments according to the methods of potato testing for wart resistance (Saltykova and Tarasova, 1979; Portyankin and Tolkachiov, 2001).

The samples were planted in lysimeters in the order of ordinal number increase. In preliminary first year testing 5 tubers were used, in the second one – 10 tubers. The tubers were located in equal rows (taking 20 pieces), tops upwards. A susceptible variety Voltman served as a control basics that followed 10 samples. The planted tubers were infected with zoosporangium powder from the calculation 0,5-0,75 g per every tuber. The amount was corrected depending on zoosporangium viability. The experiments were started in the end of April till the first decade of May. Wart infection of potato tubers was recorded at harvest after the disease expression on the sensitive control variety.

Table 1. Agrometeorological parameters of vegetation period of potato testing to wart resistance

Year	Month								
	May			June			July		
	I	II	III	I	II	III	I	II	III
Average air temperature, °C									
2003	13.4	15.1	17.9	16.3	15.0	14.9	17.5	19.4	21.2
2004	13.4	8.9	10.4	14.7	13.9	16.5	15.9	16.8	21.2
2005	9.4	10.2	17.9	14.0	16.4	16.2	18.8	20.6	19.3
2006	13.0	12.4	11.9	12.7	17.4	20.4	20.6	19.7	19.5
2007	7.0	14.0	22.0	19.3	20.7	15.9	16.1	18.9	17.8
Rainfall amount, mm									
2003	11.4	45.6	6.5	55	18.7	19.2	94.8	24.3	29.2
2004	14	15	8	6	30	38	23	4	104
2005	28	75	26	47	18	17	6	12	30
2006	0	12.1	62.5	21.5	17.1	33.9	0	53.4	24.6
2007	22.0	29.0	21.0	0.1	10.0	39.0	35.0	17.0	69.0

Note – I – the first decade, II – the second decade, III – the third decade

A year long State testing was accomplished under field conditions on the infected background by checking the hybrids showing themselves as resistant. It was based on comparison to the preliminary evaluation data. Variety samples were tested on uniform relief quarantine stationary plot, where a field infected background with the equal and strong wart agent infection was formed. The experimental plot soil was soddy-podzolic, light loam: humus content – 1.97%, soil pH -5.0. Planting of the potato samples was accomplished in the first half of May on one-row plots by the scheme 70 × 30 cm, taking 20 tubers in three replications. In every four tested samples a susceptible control – cv Voltman was planted. During the planting water moistened tubers were additionally powdered. Zoosporangium powder was used, mixed with sand in a ratio 1:1 from the calculation 1–1.5 g per one tuber.

The infected variety samples were considered the ones the plants of which have got the typical symptoms for the disease - knots on tubers, stolons, root neck or outside parts. The susceptible one considered to be hybrids having infection on one tuber, at least, the resistant one – if not a single sample tuber was infected.

Meteorological data of the Republican Meteorological Centre were used for the analysis of hydrothermal resources of vegetation period favorable for potato wart development.

### Results and Discussion

By the results of the research done in preliminary testing for resistance to common (D1) potato wart pathotype among the hybrid material in 2003-2007 there were isolated from 82.3 to 100% resistant samples depending on year (Table 2).

Table 2. Resistance of breeding samples to common potato wart pathotype (quarantine stationary plot, p. Priluki Minsk region)

Type of testing	Year	Resistant samples observed, %
Preliminary	2003	86.4
	2004	85.6
	2005	85.7
	2006	100
	2007	82.3
	2003	91.3
State	2004	92.5
	2005	93.8
	2006	94.1
	2007	86.6
Preliminary and State	2003-2007	89.8

For the period 2003 - 2007 more than 90 potato variety samples from the leading breeding institutions of Belarus and Russia were under State testing to the common wart pathotype. As one can see in Table 2, 86.6-94.1% among the varieties turned out to be resistant to quarantine disease. One should point out that a significant amount of valuable selection material was presented from the Scientific and Practical Centre of the NAS of Belarus on Potato, Fruit and Vegetable Growing.

So, for years of research in preliminary and State trials nearly 89.8% of potato varieties have determined their resistance to the disease.

It should point out that under lysimeter conditions on tubers besides usual knot form of wart infection, three non-typical forms of wart infection were detected: leaf, scab-like and corrugated. Such forms of disease expression were observed on the tested hybrid material.

There was also a control checking of wart-resistance stability among the regionalized Belarus potato varieties in 2006-2007. By such a symptom on the infectious background in lysimeters and following the methods of preliminary testing they were evaluated from the early group - Axamit, Dolphin; semi-early – Archids, Dina, Odissey, Yavar; semi-ripening Zhivitsa, Krinitsa, Rosinka, Scarb, Talisman; semi-late – Zarnitsa, Vetrax, Lasunak and late ripening – Alpinist, Orbit, Zdabytak, Atlant, Temp, Vytok. There were no typical signs

for the disease in the form of wart knots formation in the radical stem zone and in formed tuber yield on plants of all tested variety samples. This proves the expression of resistance sign to the quarantine object in the varieties belonging to wart-resistant ones in relation to the common pathotype of potato wart agent.

The analysis of detected resistance to wart among potato variety samples in State testing shows the variation of the given index from 86.6 to 94.1% against the expected 100% as only the samples having resistance in preliminary tests had been under testing. Meanwhile, by testing potato variety samples to wart resistance one should evaluate the conditions it has been carried out, as well as, the physiological condition of plants being analyzed. This can be determined by many factors: temperature and moisture parameters, planting time, seed material size and reproduction, its phytosanitary condition and etc. The possible influence of such factors on plant and the process of its infection by obligate parasite were noticed by English scientist S.Tarr (Tarr, 1975). The importance of scientific studies on this question was proved by the research carried out in the Ukraine (Melnik, 2005). It showed that under the influence of different conditions of the environment not only the expression of the disease but also the parasitic activity of potato wart agent has been changed as well. This includes expression of aggressive races and also its specialization, both, the biological one and the genotype specific.

The results of our research have proved the benefits of above-mentioned positions. Considering weather changes analysis based on the average air temperature data and rainfall amount in July, soil temperature at the depth of 10 cm during vegetation it follows (Figure 1), their parameters were not always suitable for potato wart development (the literature data optimums are averaged) and varied by years. As it is known, the influence of environmental conditions may stimulate the differences in potato sample reaction to wart infection, the varieties both from resistant and susceptible group. An important role in pathogens development can be played by average air temperature, especially the one higher than 18°C what is of optimal for the fungus development. Browning and Darling (2005) indicate a change of host-plant susceptibility to *S. endobioticum* infection at different temperatures of inoculation (10°C, 15°C and 20°C). So, a year's field testing of potato samples to wart-resistance is not enough for the final evaluation based on the given trait as it is accepted in the Republic in State testing practices at present. It stipulates the necessity of introduction potato selection samples testing for potato wart resistance under field conditions in different by hydrothermal resources vegetation periods (Sereda and Zhukova, 2006) in State testing in Belarus for the period of not less than two years.

Moreover, as a result of presence the ecological and biological prerequisites for new pathotypes of wart can be observed in the Republic of Belarus. This regards a possible change of potato varieties reaction to contamination by phytopathogen due to unfavorable for the disease physiological conditions inside host plant cell what perhaps stimulate the expression of three more non-typical forms apart the common knot form of wart infection – leaf, scab-like and corrugated. A systemic control of wart resistance among the varieties cultivated in the Republic of Belarus will possibly become an outstanding and pending matter in the near future.



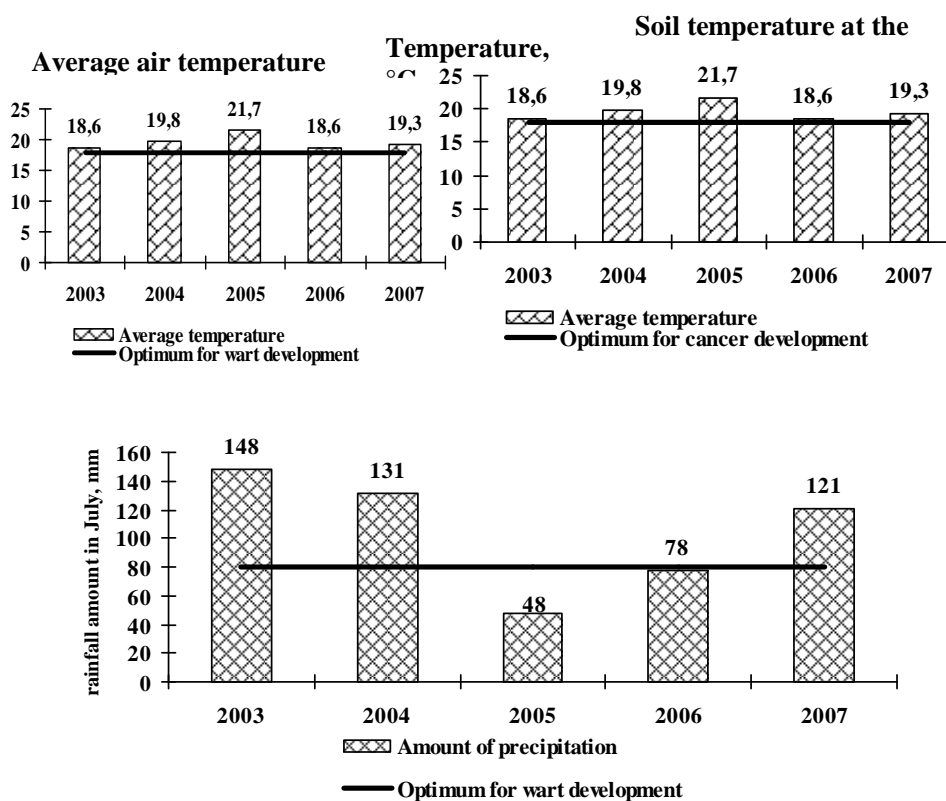


Figure 1 Variation of weather conditions during testing breeding samples to wart resistance

## Conclusions

Concerning the results of testing of breeding material to wart-resistance for the period 2003-2007 in preliminary and state testing, 89.8% wart-resistant potato variety samples were detected. Finding out wart resistant varieties among the variety samples in State testing has varied by years. This stipulates a necessity of introduction of not less than two years State testing of potato breeding samples under different hydrothermal field conditions vegetation periods in Belarus.

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## **KARTUPEĻU PĀRBAUDES IZTURĪBAI PRET VĒZI BALTKRIEVIJĀ**

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Rakstā atspoguļota Baltkrievijā bīstamākā patogēna - *Synchytrium endobioticum* (Schilb.) Perc. – kartupeļu vēža izraisītāja - pētījumu analīze, pamatojot nepieciešamību pēc kartupeļu sēklas materiāla izturības izvērtēšanas. Rakstā aprakstīti sagatavošanas un valsts pārbaudu apstākļi paraugu atlasei karantīnas stacionāra laukā RUC „Augu aizsardzības institūtā” saskaņā ar kartupeļu izturības pret vēzi metodēm. Sagatavošanas un valsts pārbaudēs, kurās nosaka izturību pret kartupeļu vēža izplatītāko patotipu (D1), izvērtējot no vadošajiem Baltkrievijas un Krievijas selekcijas institūtiem saņemto hibrīdo kartupeļu materiālu, laika posmā no 2003. līdz 2007. gadam tika atlasīti 89.8 % izturīgu paraugu. Selekcijas materiāla izturības izvērtēšanas laikā uz paraugu bumbuļiem, kuri uzrādīja jutību pret kartupeļu vēzi, bez zināmiem infekcijas bojājumiem tika novēroti vēl trīs netipiski bojājumu veidi: plēksnes, kārpveida izaugumi un rievājumi. Pamatojoties uz kartupeļu paraugu izturības pret vēzi mainību, kartupeļu selekcijas materiāla izturības pret vēzi pārbaudes Baltkrievijā jāveic ne mazāk kā divus gadus lauka apstākļos, vēlams dažādos audzēšanas apstākļos pēc mitruma nodrošinājuma un temperatūras režīma.

## COMPARISON OF POTATO CLONES DEVELOPED AND TESTED IN ORGANIC AND CONVENTIONAL GROWING CONDITIONS

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### **Abstract**

The development of organic farming needs suitable organic growing conditions. A part of demanded conditions are included in conventional potato breeding programmes, but some of the characteristics are particularly significant for organic growing conditions. Breeding for organic agriculture could take an advantage that the expression of many traits is highly correlated between conventional agriculture and organic agriculture. In 2006 and 2007 potato clones (4<sup>th</sup> and 5<sup>th</sup> field generation) were evaluated in conventional and organic fields. The clones had been selected according to the assessment of leaf coverage, maturity, and resistance to the late blight of foliage under conventional growing conditions. The resistance to pests in field conditions, the length of the growing period, yield, starch content and others factors were evaluated and compared in both growing conditions. The breeding of new varieties for organic farming could be done in conventional conditions as part of the existing breeding programme. The differences between traits, mostly determined by genotype, were relatively similar in different growing conditions, so a genotype with an acceptable trait could be selected in a conventional breeding programme. Several assessments of traits, determined mainly by the environment, had no significant correlation. Those traits could be evaluated directly in organic growing conditions. Results of this trial prove that particular selection for organic agriculture has to be done in an organic field, as selected potentially suitable genotypes with acceptable traits in a conventional field did not fit organic conditions as expected. There has to be additional traits' assessment included in an organic breeding programme.

**Key words:** potato breeding, organic farming, breeding for organic farming.

### **Introduction**

Organic farming in Europe is developing very widely. Among other crops, the potato is one of the most demanded on the market. Organic potato production depends on growing conditions, nutritional support, pest control, especially late blight. Those circumstances are partially overcome in conventional agriculture by fertilising and spraying pesticides. Flexible and robust varieties are required for such specific organic growing conditions. The evaluation of potato varieties in organic fields determined the most important traits for organic potato production. The most important trait is resistance to different pathogens (late blight, black scurf, virus diseases, rhizoctonia etc.), (Zimnoch-Guzovska, 2003; Tiemens-Hulscher *et al.*, 2003; Vogt-Kaute, 2001). The desired traits for organic potato breeding are adaptability to organic fertilization (adequate root system, rapid juvenal root and plant development, good growth vigour, efficient mineral uptake and use), the ability to produce a good yield in a short growing period (early bulking and ripening, yield stability, acceptable quality, good storability) (Tiemens-Hulscher *et al.*, 2003) and meeting market needs. A part of the above mentioned traits are included in conventional potato breeding programmes, but some of the characteristics are particularly significant for organic growing conditions. As conventional and organic growing conditions are different, requirements for varieties are different, also; (Colon *et al.*, 2003). Breeding for organic agriculture could take an advantage that the expression of many traits is highly correlated between conventional and organic agriculture (Loschenberger, 2007). One of the ways is to start breeding for organic farming in a conventional programme, using so called indirect selection, and at a defined generation evaluate potentially acceptable clones in organic conditions. Because of the expected large plant – environment – management interactions under organic conditions the most efficient way is to start selection in the organic field as early as possible (Lammerts van Bueren, 2002). The selection in the organic environment will include emphasis on rapid establishment, good ground cover, early

bulking yield potential and tolerance to changeable humidity and fertility conditions through the better root system (Bradshaw, 2007). But in some cases, for instance – onion breeding, the selection differential differed for some traits between organic and conventional selection (Thiemens-Hulscher *et al.*, 2007).

The aim of the study was to compare the evaluation and selection of selected clones from the conventional breeding programme in conventional and organic growing conditions, with a purpose to estimate the possibility of carrying out part of the breeding programme for organic farming in a conventional breeding programme, including selection in conventional growing conditions.

### Materials and Methods

Nine potato clones (4<sup>th</sup> and 5<sup>th</sup> field generation) were evaluated in conventional and organic fields for two years - 2006 and 2007. The clones were selected from the existing potato breeding programme according to the assessment of leaf coverage, foliage resistance to late blight and maturity under conventional growing conditions. The variety 'Brasla' was used as a standard variety. The leaf coverage of five clones was assessed as having moderate density, but four clones – tight density. The leaf coverage of the standard variety 'Brasla' was of moderate density. Leaf coverage density is a possibly significant trait in organic field weed control – dense foliage successfully covers the ground and does not allow weed development. But at the same time wide leaf areas could possibly be a breeding ground for leaf diseases. The four selected clones and 'Brasla' have a low resistance to late blight (*Phytophthora infestans*(Mont.) de Bary). One clone's resistance was assessed as moderately low and the other four clone's resistance - as moderate.

The maturity time of selected clones was predicted taking into account their parents' maturity. Two clones were obtained from crosses of two early varieties, so it was expected that the clones could be of early maturity. Two others were obtained from medium early parent crosses, and the next two from a cross of early and medium early varieties. These clones could be early to medium early. The other two clones were obtained from medium late maturity parent varieties, so these would be medium late. One clone's parent varieties were medium late and early, the maturity of this clone would range widely. The maturity of standard variety 'Brasla' is medium late.

The soil characteristics of both environments is described in Table 1.

Table 1. Characterisation of soil in conventional and organic growing conditions

Year	2006		2007		
Growing conditions	organic	conventional	organic	conventional	
Soil type	sandy loam	loamy sand	sandy loam	loamy sand	
pH <sub>KCl</sub>	6.5	5.3	5.9	5.7	
Humus g kg <sup>-1</sup>	2.7	1.2	2.3	2.5	
N mg kg <sup>-1</sup>	N mg kg <sup>-1</sup> (in soil)		60.4		
	fertilised mg kg <sup>-1</sup>	65	-	65	
	Total	38	60.4	65	
P mg kg <sup>-1</sup>	mg kg <sup>-1</sup> (in soil)	61	54	69	
	fertilised mg kg <sup>-1</sup>	-	64	64	
	Total	61	179	54	133
K mg kg <sup>-1</sup>	K <sub>2</sub> O mg kg <sup>-1</sup> (in soil)	121	168	77	111
	fertilised mg kg <sup>-1</sup>	-	75	-	75
	Total	121	242	77	186
Precrop	winter wheat	winter cereals	winter wheat	winter cereals	

The soil acidity in the organic field was lower than in the conventional one. The amount of organic matter (humus) in organic field was nearly two times more than in the conventional field in 2006. The humus content in the the soil of the conventional field exceeded by a slighty margin the humus content of the organic field in 2007. Nitrogen content was not detected in conventional fields, so only the added nitrogen was taken into account. The estimated amount of nitrogen content in the organic field before potato planting was 1.7 times less than the conventional field on wich nitrogen

was applied. The amount of nitrogen added to the potato crop was low in the organic field, but during growing period it was naturally improved due to the decomposition of the organic matter in the soil. The nitrogen supply was higher in the organic growing conditions of 2007 than in the previous year. The coverage of phosphorus and potassium was higher in conventional conditions due to better soil parameters and fertilising. The supply with nutrients determined obtaining higher yield in conventional field in both years.

The potato clones were planted in 10 m<sup>2</sup> plots in 4 replications in both environments. The seed material planted in the organic field was presprouted with the purpose to shorten the time the crop is in field conditions. Potatoes were planted in the second decade of May and harvested in the last decade of August or the 1<sup>st</sup> decade of September. In both years fungicide Tatu 550 was applied twice in the conventional field after potato flowering to limit foliage diseases.

The rainfall during the potato growing period in 2006 was very low (Figure 1.). It rained quite heavily in June: 23 – 75% of the decade's long term average data. Later in July and August rainfall varied from 0% to 26% of the normal amount, so the moisture in the soil depended only on rainfall so the potato crop suffered from drought and lack of nutrients. The crop roots could not be supplied with nutrients due to insufficient soil moisture. This was the reason for a noticeably low tuber yield in 2006. The rainfall did not differ a lot from normal amounts (Figure 1) and, consequently, soil moisture was acceptable for potato growing in 2007.

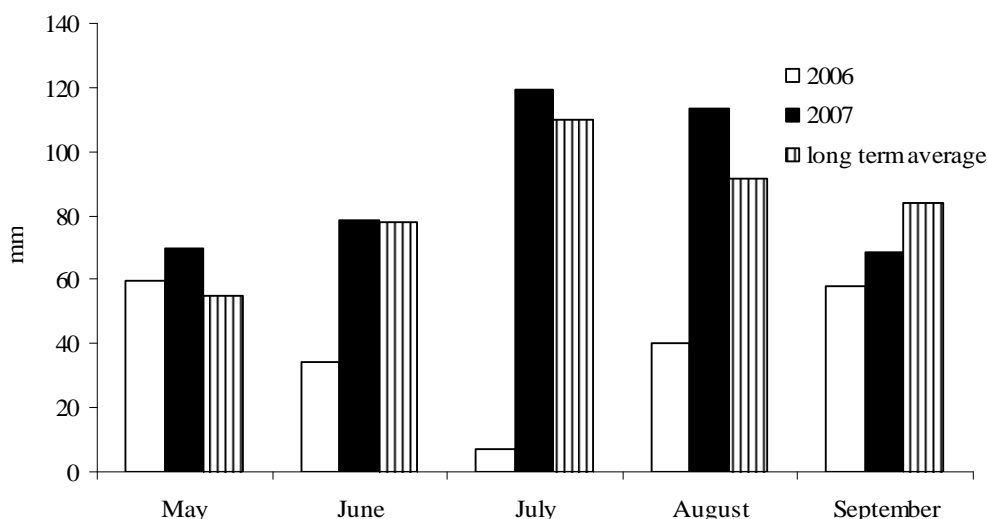


Figure 1. The rainfall during potato growing period of 2006 and 2007

The significant temperature differences during the potato growing period of both trial years was observed at the end of May and the beginning of June. During the last decade of May and the first decade of June the average day temperature was only 10.4 °C and 11.5 °C in 2006. It was 8.7 °C and 6.7 °C lower than the temperature in 2007. Owing to chilly weather, the sprouting of the potato crop was delayed in 2006. The average day temperature later in the potato growing period was not noticeably different in both trial years. In general the temperature in both years was higher than normal. The average day temperature from May to August exceeded the norm for 2.03 °C in 2006 and for 2.3 °C in 2007. The quite hot and dry weather conditions in 2006 were unfavourable for late blight (*Phytophthora infestans* (Mont.) de Bary) development and this disease was not found on potato leaves. An other disease – early blight (*Alternaria solani* Ellis and Martin) was found on potato foliage and the weather conditions were favourable for its development. The following year sufficient moisture advanced late blight development, the first damage caused by disease was observed in the first decade of August 2007. Starting in the middle of June 2007 Colorado beetles began to infest the fields. Insecticide was used in the conventional field.

The evaluation of potato clones was done during the growing period and after harvesting. Leaf resistance to pests was assessed visually (% of the damaged leaves area) during disease development in the field. The length of the growing period was determined counting days from

when 80% of the planted tubers had sprouted to the end of the vegetation period. After harvesting yield and tuber size distribution were measured. The starch content in tubers was determined indirectly via specific gravity as a percentage of the fresh weight. The boiled tuber taste was assessed by a expert panel using a 9 point scale (9 – very tasty, 1- very nasty). The results of the trait assessments in both environments were compared. The main stress was on the selection of clones suitable to the organic growing conditions.

The obtained data were analysed using descriptive statistics and the Pearson correlation coefficient. The significance of the differences between the samples was assessed using the T-test (Liepa, 1974).

## Results and Discussions

Resistance to diseases. Foliage resistance to early blight was assessed in 2006 as late blight did not appear in the field. The evaluation of the damage on leaves was started on August 1<sup>st</sup>, when early blight spots covered 0-5% of the leaf area depending on the genotype. After a week damages did not exceed 10% in both environments. One potato clone had no early blight damage in both conditions. One clone's damage was only 1% of the leaf area in both environments on August 8th. For two genotypes, including the standard variety 'Brasla', the disease damage was larger in the conventional field than in the organic. But for the other two clones the damage in organic field exceeded the early blight damage experienced in the conventional field. It seems that fungicide spraying was not effective in the conventional field, perhaps due to hot and dry weather conditions, and only genotype resistance protected the crop in both environments. But a significant correlation was not found between assessments in both growing environments ( $r = 0.21 < r_{0.05,10} = 0.63$ ). Some other circumstances – nutrition uptake, resistance to drought, foliage cover and others affected clone resistance to early blight.

The distribution of late blight on potato clone foliage was assessed only in 2007. The first spots in the organic field were observed on July 30<sup>th</sup> but only on the leaves of one clone. A week later the damage was in the range from 0 – 30% in the organic environment. The foliage was damaged almost completely for all clones in the organic field on August 13<sup>th</sup>. Late blight distribution in the conventional field started later – the first spots were observed on August 13<sup>th</sup>. After a week late blight damage reached 60 – 100% of the leaf area depending on genotype, but less than in the organic field. The application of fungicide delayed late blight development in the conventional field and saved crop vegetation for a longer time. The amount of damage in the conventional field was less than in the organic field where spraying was not used. The correlation between late blight damage assessments in both fields was not significant ( $r = 0.42 < r_{0.05,10} = 0.63$ ). It means that genotype assessment in the conventional field which had fungicide application does not tell us about genotype resistance to late blight in organic conditions where fungicide was not applied. The evaluation to disease resistance has to be done in the organic field to get reliable data.

**Growing period.** The growing period was shorter in both environments during 2006 than in 2007, because of extremely dry and quite hot weather, which quickened plant development and limited nutrient availability in 2006.

The potato plants emerged 2 - 3 days earlier in organic the field than the conventional field both years due to tuber seed presprouting. But the growing period for clones in the organic field was shorter by about ten days than in the conventional field (Table 2). One reason was that presprouting quickened plant development. Another, was especially in 2007, was that late blight damaged foliage and interrupted vegetation in the organic field earlier than in the conventional field. The length of the growing period differed between genotypes according to predicted maturity time. Clones obtained from both early maturity parents and one early maturity cross with later maturity parents mostly showed faster development, as was expected. The growing period was from 57 to 63 days in 2006, from 65 to 80 days in 2007 in the organic field, and from 54 to 77 days in 2006, about 80 days in 2007 in the conventional field. The growing period of potato clones obtained from both medium late maturity parents lasted from 6 to 22 days longer in both environments. But clones expected to be more early than late maturing was (both parents medium early) had a longer growing period (59-86 days in organic and 65-97 days in conventional fields in 2006 and 2007 respectively). The difference between the average data of both environments in both years was significant (Table 3.). The correlation between the lengths of the growing period in both

environments was not significant (Figure 2). Significant relationship was not found between assessments in the organic field both years, but a correlation between the assessments of both years in the conventional field was significant. The growing period in the organic environment was more affected by weather conditions and late blight distribution than in conventional environment.

Table 2. The characteristics of the traits of potato clones in organic and conventional fields in 2006 and 2007

Traits	Organic field				Conventional field			
	2006		2007		2006		2007	
	min-max	average	min-max	average	min-max	average	min-max	average
Growing period, days from emergence to the end of vegetation	57-71	63.7	54-90	74.8	65-83	77	80-102	87.9
Tuber yield, t ha <sup>-1</sup>	10.1-28.4	16.1	17.0-35.1	24.6	4.7-41.8	23.1	33.7-65.0	44.8
Tubers > 50mm in yield, %	2-35	20	4-64	41	9-43	25	32-78	62
Starch content, %	13.9-21.4	17.2	14.9-19.4	16.2	11.5-19.1	15.0	12.2-19.3	15.6
Taste of boiled tuber, points	6-7.5	6.8	6-7.8	7.1	6.3-7.7	7.0	6.1-7.8	6.9

Yield. The potato tuber yield in 2006 was about twice as low in both environments than in 2007 (Table 2). The influence of different weather conditions, affecting soil moisture was observed during the trial. Tuber yield in organic growing conditions was lower than in conventional growing conditions during both years. The growing period in conventional growing conditions was longer and nutrition supply was better than in the organic growing conditions. This was the reason for a larger yield in the conventional field. Four potato clones significantly exceeded the yield of the standard variety 'Brasla' in the conventional environment in 2006 ( $p = 0.05$ ), but only one of them significantly exceeded the yield of the standard variety in the organic environment. A year later the yield of three clones was significantly higher than the yield of the standard variety in the conventional field and the yield of the same clone was higher in organic field ( $p = 0.05$ ). The length of the growing period for this clone was assessed as medium late and the resistance to late blight in 2007 was assessed as moderate in organic conditions. There were no late blight damages on the leaves of the clone in the conventional field in 2007. This clone possibly has some features better adapted to organic growing conditions, which were not tested in the trial: for example, a better root system that is more tolerant to changeable moisture and nutrient uptake. If selection was done only in the conventional field, more clones could be accepted as suitable for organic farming. But in organic conditions part of them turned out unsuitable. Comparing the average data of the potato yield in the organic and conventional field a significant difference was found in both trial years (Table 3).

Table 3. The significance level between trait assessments in organic and conventional fields (2006-2007)

Trait	$t_{\text{fact}}$ between means in organic and conventional fields	
Growing period	2.63	3.02
Tuber yield	6.22	6.97
Tubers > 50mm in yield	1.39*	4.14
Starch content	2.88	2.72
Boiled tuber taste	0.87*	0.92*

$t_{0.05} = 2.26$ ; \* - not significant

It means that genotype yield is significantly differently in different environments. The correlation between yield assessments in both environments was significant in both trial years (Figure 2.), so the relative difference between yields was similar in different environments. A significant relationship was found between tuber yields both years in the same environment. The selection of potentially high yielding potato clones for organic growing conditions could be done in the conventional breeding field, but promising clones should be tested in the organic field because predictions do not always turn out to be true.

The amount of large tubers (> 50 mm) in the yield. The genotype's ability to form bigger size tubers was assessed evaluating the percentage of large tubers (> 50 mm) in the yield. The growing conditions, probably mainly a lack of moisture in the soil, restricted large tuber formation in both environments in 2006 (Table 2). The greater amount of large tubers in the yield was obtained in the conventional field in 2007. The difference between the means of trait assessment in both environments was not significant in 2006, but there were differences in 2007 (Table 3). There was a significant correlation between the amount of large tubers in the clones' yield in the organic and conventional field in 2006. Not significant, but quite high correlation existed in 2007 (Figure 2). The correlation was not significant between trait assessments when analysing the yield in the same environment for two years. The amount of large tubers mostly depends on genotype environment a interaction in particular growing conditions.

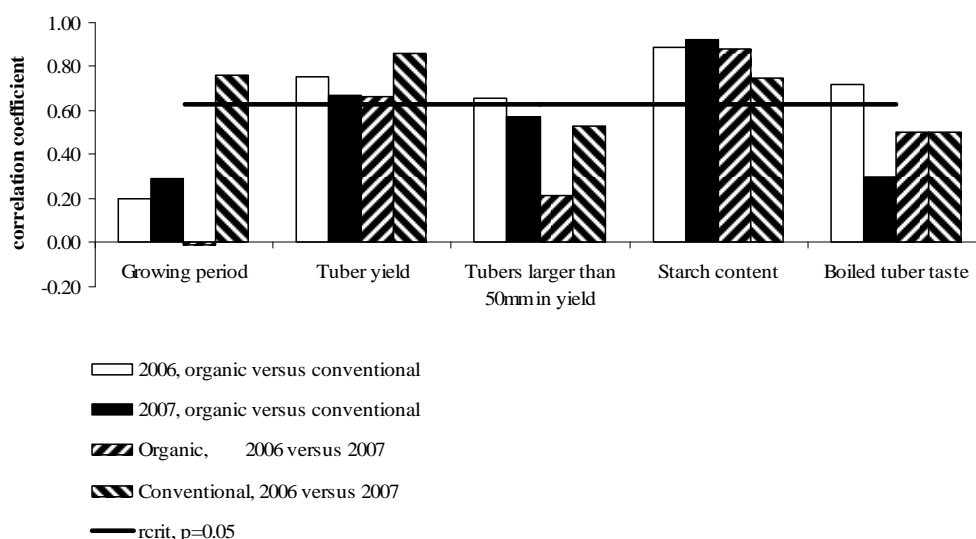


Figure 2. Correlation coefficient between trait assessment in organic and conventional growing conditions, 2006-2007

Starch content. The starch content of potato clone tubers in the organic field was higher than in the conventional field in 2006 (Table 2). Next year the starch content of the clones grown in the conventional field exceeded starch content of the clones grown in the organic field. The reason for this difference was the late blight damage which provoked the end of the growing period approximately a week earlier in the organic field than in the conventional one. This extra growing period week in the conventional field was a chance to accumulate more starch in the tubers utilising sun energy for a longer time. The starch content in tubers is mainly determined by genotype. Comparing starch content in both environments, the difference was significant in both trial years (Table 3). A significant correlation was found between the results in different environments and years (Figure 2). The genotype's ability to accumulate a comparatively higher amount of starch could be assessed in any environment because of the potato clones ability to accumulate starch in tubers was relatively similar in different growing conditions and environments.

Taste of boiled tubers. The assessment of the taste of boiled tubers did not differ much depending on the growing conditions of both years. The assessment of the clone tubers from the organic field was a little lower in 2006 and a little higher in 2007 than from the conventional field (Table 2.). There was a significant difference between the taste assessments of the clones from both



environments during the two trial years (Table 3). A significant correlation between the taste assessment of the clones grown in the organic and in the conventional field was found in 2006 (Figure 2). The next year this relationship was not significant. There was no significant correlation between two-year trait assessments in the same environment. The taste trait depends mostly on how genotypes react to different growing conditions.

### Conclusions

The assessment of traits - starch content, tuber yield and partly boiled tuber taste – in the organic field significantly correlated with the assessment in the conventional field. The expression of traits in the particular environment depended on the genotype. The impact of different growing conditions on the trait expression of genotypes was similar, so differences between genotypes in each environment were relatively similar. Part of the selection for breeding potato varieties for organic farming could be done in the existing conventional breeding programme.

A significant relationship between assessments of traits like the length of the growing period and the ability to form large size tubers in both environments was not found. The traits more determined by environmental conditions preferably could be assessed and selected in particular (organic) growing conditions.

The resistance assessment of clone leaves to diseases has to be continued, the first conclusions verify that evaluation in particular growing conditions for selection would be preferable.

It is advisable to include the new trait assessments that are particularly significant for organic growing conditions in the breeding programme for organic farming. Some of these traits are the root system, plant rapid establishment and nutrient uptake. The development of specific assessment methodology is recommended for these traits.

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## KARTUPEĻU KLONU VĒRTĒŠANAS SALĪDZINĀJUMS BILOĢISKAJOS UN KONVENCIŅĀLAJOS AUGŠANAS APSTĀKĻOS

### Skrabule I.

Bioloģiskās lauksaimniecības attīstība rada pieprasījumu pēc bioloģiskās audzēšanas apstākļiem piemērotām laukaugu šķirnēm. Daļa no šķirņu īpašībām, kuras būtu vēlamas šādiem audzēšanas apstākļiem, tiek izvērtētas jau pastāvošajās konvencionālajās selekcijas programmās, bet atsevišķas pazīmes ir nozīmīgas tieši bioloģiskajos laukos. Selekcija bioloģiskajai lauksaimniecībai varētu

izmantot sakarību, ka pastāv ļoti cieša korelācija starp daudzu pazīmju izpausmi bioloģiskajos un konvencionālajos audzēšanas apstākļos. Kartupeļu klonu 4. un 5. gada pavairojums 2006. un 2007. gadā tika izvērtēti bioloģiskajos un konvencionālajos augšanas apstākļos. Klonu izvēle pamatojās uz iepriekš konvencionālajos augšanas apstākļos noteiktajām pazīmēm un to iespējamās atbilstības bioloģiskajiem audzēšanas apstākļiem: aplapojuma, veģetācijas perioda ilguma, lapu izturības pret lakstu puvi. Kartupeļu klonu izturība pret lapu slimībām lauka apstākļos, veģetācijas perioda garums, raža, cietes saturs un citas pazīmes tika novērtētas un salīdzinātas abos audzēšanas apstākļos. Jaunu šķirņu selekcija bioloģiskajai lauksaimniecībai varētu tikt daļēji veikta jau esošās konvencionālās selekcijas programmas ietvaros. Izmēģinājuma rezultāti pierāda, ka bioloģiskajos laukos noteikti jāveic īpaša klonu atlase, jo konvencionālajā laukā selekcionētie labākie kloni ar bioloģiskajiem apstākļiem it kā piemērotām pazīmēm ne vienmēr izrādījās piemēroti bioloģiskajiem audzēšanas apstākļiem.

## EVALUATION OF MAIN TRAITS AND THEIR RELATIONSHIPS OF SPRING WHEAT

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### Abstract

Study was carried out at the State Stende Cereal Breeding Institute in 2005-2007. There were included 10 spring wheat varieties registered in Latvian Catalogue of Plant Varieties. The morphological and agronomical traits of the varieties and influence of weather conditions on yield and quality were evaluated. Yield, grain quality and correlation between traits were significantly influenced by meteorological conditions of year. On evaluation data the yield potential was on the level 5-8 t ha<sup>-1</sup>, but there was a great influence on grain yield and quality traits of year. The grain yield was influenced by variety – 20.64%, but by meteorological conditions 54.02%, interaction of both factors was 16.38%. Significant positive correlation between the yield and the 1000 kernel weight was found, but the significant negative- between the 1000 kernel weight and the volume weight; the starch content and the Zeleny index; the starch content and the protein content; the protein content and the 1000 kernel weight. New variety 'Uffo' was created at State Stende Cereals Breeding institute during 1992-2004, included in the Latvian Catalogue of Plant Varieties from 2008. The variety 'Uffo' is characterizing with high yield potential (6-8 t ha<sup>-1</sup>), grain quality is suitable for bread making.

**Key words:** spring wheat, varieties, yield, grain quality

### Introduction

In Latvia spring wheat became more popular crop by the last decade of 20th Century. Growing area ranged between 50-60000 ha in each year, but there is a tendency to increase the sowing area and the production. Traditionally the spring wheat has a lower grain yield, but better grain quality (Blakman and Payne, 1987). Spring wheat is used mostly for food, less for feed or bioethanol production. The most popular spring wheat is used for conventional farming, but there are varieties suitable for organic farming too (Belederok *et al.*, 2000).

Spring wheat breeding programme was renewing in State Stende Cereals Breeding Institute in 1990, starting with renovation and evolution of the genetic resources. The main goals of breeding program were to create new varieties, characterizing with the high yield, grain quality conform with the requirements of producers, resistance to lodging and main diseases.

The aim of this study was to evaluate the main morphological and agronomical traits of spring wheat varieties and to find their relationships as well as to establish influence of weather conditions on yield and quality, and to select promising genotypes recommending for farmers.

## Materials and Methods

The trials of 10 spring wheat varieties were set up at the State Stende Cereals Breeding Institute during 2005–2007. There were included varieties registered in Latvian Catalogue of Plant Varieties. Trials were carried out in conventional cereal breeding crop rotation field. The soil at the experimental site was sod-podzolic sandy loam, soil pH<sub>KCl</sub> –5.6, humus content – 15 mg kg<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> –203 mg kg<sup>-1</sup>, K<sub>2</sub>O– 194 mg kg<sup>-1</sup>. The precrop was winter rapeseed, NPK 18:9:9 500 kg ha<sup>-1</sup> was applied. Check variety – ‘Vinjett’. The plot size was 10 m<sup>2</sup> in 4 replications.

The agronomical and economical traits were evaluated in the trial: period of maturity, plant height, ear length, grain yield, 1000 kernel weight, volume weight, resistance to lodging and to diseases, grain quality (protein, starch, gluten content, Zeleny index, Falling number).

The grain quality determined by grain analyser ‘Infratec 1241’.

Statistical analyses were performed using Agrobases 4 software package. Analysis of variance (ANOVA).

The average of temperatures and amount of precipitation was different during 2005, 2006, and 2007. In 2005 meteorological conditions were suitable for spring wheat sowing and growing. In August amount of precipitation was very high and the sprouting of kernel was recorded. For this reason the grain quality was lower as usually.

In 2006, meteorological conditions were suitable for spring wheat sowing, but during the growing period amount of precipitations was lower than norm. In the first decade of July the mean air temperature was 20° C, but amount of precipitation was 0.7 mm. In July the total amount of precipitations was 42.4 %. For this reason the size of spring wheat kernels were smaller than usually.

In 2007 meteorological conditions were particularly suitable for obtaining the high spring wheat yield with good grain quality. In June the average of air temperature was 2,2° C above long term and amount of precipitation to compare with long term was less (72%). Sufficient soil humidity and air temperature was favourable for plant development. Spring wheat heading and anthesis was recorded 10 days earlier than in 2005 and 2006. In July the air temperature was –0,1°C below long term, but amount of precipitation was higher than long term (120,1%). In August total amount of precipitation was 129,4% comparing with long term, for this reason the spring wheat yield harvesting was delayed and started in the third decade of August.

## Results and Discussion

Grain yield. In 2005 the grain yield was from 4.02 to 5.45 t ha<sup>-1</sup>. The highest grain yield had varieties ‘Triso’, ‘Fasan’ and ‘Eta’ (5.45, 5.36, 5.21 t ha<sup>-1</sup>), but it was on the standard ‘Vinjett’ (5.23 t ha<sup>-1</sup>) yield level. In the 2006 the grain yield was from 5.87 to 7.20 t ha<sup>-1</sup>. Significant higher yield had varieties ‘Eta’ (+0.62 t ha<sup>-1</sup>), ‘Uffo’ (+0.36 t ha<sup>-1</sup>) and ‘Picolo’ (+0.34 t ha<sup>-1</sup>) (LSD<sub>0,05</sub>=0,31). The highest yield level was reached in 2007; grain yield was from 5.57 to 8.83 t ha<sup>-1</sup>. Significant higher yield had variety ‘Uffo’ (+1.09 t ha<sup>-1</sup>), (LSD<sub>0,05</sub>=0,49).

In years 2005-2007 on average grain yield of spring wheat varieties ranged from 5.19 t ha<sup>-1</sup> to 6.96 t ha<sup>-1</sup>. Among varieties the highest yield had varieties ‘Uffo’ and ‘Eta’, but difference compared with standard was not significant. Significant lower yield showed variety ‘Aniniina’ (-1.41 t ha<sup>-1</sup>), which characterized with short vegetation period and varieties ‘Picolo’ and ‘Munk’ (-0,50 t ha<sup>-1</sup>) (Table 1).

Table 1. Grain yield of spring wheat varieties

Variety	Origin	Grain yield, t ha <sup>-1</sup>							
		2005	+/- t ha <sup>-1</sup>	2006	+/- t ha <sup>-1</sup>	2007	+/- t ha <sup>-1</sup>	Average	+/- t ha <sup>-1</sup>
Vinjett	SE	5.23	-	6.84	-	7.74	-	6.60	-
Zebra	SE	4.69	-0.54	6.83	-0.01	7.35	-0.39	6.29	-0.31
Triso	DE	5.45	+0.22	6.21	-0.63	7.86	+0.12	6.50	-0.10
Fasan	DE	5.36	+0.13	6.75	-0.09	7.48	-0.26	6.53	-0.07
Piccolo	DE	4.97	-0.26	7.18	+0.34	6.15	-1.59	6.10	-0.50
Munk	DE	4.81	-0.42	5.87	-0.97	7.63	-0.11	6.10	-0.50
Eta	PL	5.21	-0.02	7.46	+0.62	8.06	+0.32	6.91	+0.31
Jasna	PL	4.37	-0.86	6.70	-0.14	8.19	+0.45	6.42	-0.18
Aniniina	FI	4.02	-1.21	5.97	-0.87	5.57	-2.17	5.19	-1.41
Uffo	LV	4.86	-0.37	7.20	+0.36	8.83	+1.09	6.96	+0.36
LSD <sub>05</sub>		0.43	0.31	0.49	0.37				

Statistical analysis showed, that the grain yield was influenced by variety – 20,64 %, but by meteorological conditions 54,02 %. Interaction of both factors was 16,38 %. It confirms the results of other authors (Sofield *et al.*, 1977; Lapinski *et al.*, 1988).

Relationship of morphological and agronomical traits. Genes controlling the plant height, ear shape and length or period of vegetation may be of interest for their direct and indirect effects on the varieties yield potential (Worland, *et al.*, 1987).

Spring wheat varieties suitable for growing in the Latvian agroclimatical conditions should be characterized with early or middle-early growing period. Varieties with long period of vegetation prevent the sowing time of winter crops; also meteorological conditions in harvest time often are not suitable for obtaining high grain quality.

In our trials the mean vegetation period was 133 days in 2005, 119 days in 2006 and 135 days in 2007. The growing period of standard variety ‘Vinjett’ varied from 116 to 130 days during 2005-2007. Variety ‘Aninnina’ had shorter vegetation period, but varieties ‘Eta’ and ‘Munk’ characterized with longer growing period.

Lelley, 1976, noted that correlation between growing period and wheat grain yield potential is not proven. In our investigations the correlation between grain yield and growing period was positive in the all years, but significant in 2007 ( $r_{0,05}=0.87$ ). In this year the duration of period from the anthesis to the maturity or grain filling period was longer compared to 2005 and 2006 and due to the yield was higher than in previous years. There were the significant correlation between yield and period of anthesis in 2006 ( $r_{0,05}=0.81$ ) and correlation between period of anthesis and 1000 kernel weight in 2007 ( $r_{0,05}=0.61$ ).

Worland, *et al.*, 1987, noted that many genetic factors, which regulate development and morphology, would have effects on final plant height. Reducing plant height also frequently reduce grain yield (Law, *et al.*, 1978). The positive correlation was found between yield and plant height, but it was significant only in 2005 ( $r_{0,05}=0.68$ ). Plant optimal height (90-100 cm) is very important trait for varieties used for organic farming. There was not significant correlation between the growing period and the plant height, and the growing period and ear size. Ear size determines mostly genotype but less affects by environment (Rajaram, 1994b). There was not found correlation between the yield and the ear size.

Grain size is one of the most important traits and had direct effect on grain yield. In our study the significant coefficient of correlation was found between yield and 1000 kernel weight in 2005 ( $r_{0,05}=0.78$ ), in 2006 ( $r_{0,05}=0.77$ ) and in 2007 ( $r_{0,05}=0.76$ ). There was no significant correlation between volume weight and yield.

Significant negative correlation between the volume weight and the 1000 kernel weight was found in all years in 2005 ( $r_{0,05}=-0.58$ ), in 2006 ( $r_{0,05}=-0.61$ ) and in 2007 ( $r_{0,05}=-0.61$ ).

It is very important to unite high yield with high grain quality. Usually negative correlations between the protein content and the grain yield was found (Worland, *et al.* 1987). All genes affecting yield influence protein levels in the grain. Both genotype and environment have influence

on the grain quality of wheat. Problem in breeding is to produce cultivars with good an even quality from year to year (Johanson and Svenson, 1998; Johanson *et al.*, 2001).

In our investigations the average protein content of spring wheat varieties ranged from 107 g kg<sup>-1</sup> (variety 'Eta') to 158 g kg<sup>-1</sup> (variety 'Aniniina'). Each year more even protein content had varieties 'Zebra', 'Triso', 'Fasan', 'Picolo' and 'Aniniina'. Standard variety 'Vinjett' protein content varied from 119 g kg<sup>-1</sup> to 146 g kg<sup>-1</sup>.

The significant negative correlation between yield and protein content was in 2005 ( $r_{0,05} = -0.78$ ) and in 2007 ( $r_{0,05} = -0.84$ ), also negative correlation in all years was between protein and 1000 kernel weight in 2005 ( $r_{0,05} = -0.85$ ), in 2006 ( $r_{0,05} = -0.81$ ) in 2007 ( $r_{0,05} = -0.81$ ). Positive correlation was between yield and starch content, significant in 2007 ( $r_{0,05} = 0.71$ ).

There was a significant negative correlation between protein content and starch content in 2005 ( $r_{0,05} = -0.80$ ) in 2006 ( $r_{0,05} = -0.59$ ) and starch content and Zeleny index in 2005 ( $r_{0,05} = -0.66$ ), in 2006 ( $r_{0,05} = -0.61$ ) (Table 2).

Table 2. Coefficients of correlation of spring wheat traits

Traits	2005	2006	2007
Yield-growing period	0.45	0.42	0.87**
Yield- period of anthesis	0.09	0.29	0.81**
Yield- plant height	0.80**	0.41	0.41
Yield-ear size	0.28	-0.003	-0.08
Growing period-plant height	0.06	0.26	0.27
Growing period-ear size	0.45	0.56	0.21
Period of anthesis-1000 kernel weight	0.34	0.37	0.61*
Yield- 1000 kernel weight	0.78**	0.77**	0.76**
Yield-volume weight	0.20	-0.17	0.41
Yield-protein content	-0.78**	-0.48	-0.84**
Yield-starch content	0.44	0.52	0.71**
Volume weight-1000 kernel weight	-0.58*	-0.61*	-0.61*
Protein content-1000 kernel weight	-0.85**	-0.81**	-0.81**
Protein content - starch content	-0.80**	-0.59*	-0.52
Starch content- Zeleny index	-0.66*	-0.61*	-0.54
Starch content-1000 kernel weight	0.67*	0.20	0.52
Volume weight-starch content	0.46	0.29	0.24

Significant \* at  $r_{0,05} = 0.576$ ; \*\*  $r_{0,01} = 0,708$ ; n=10

New variety 'Uffo'. Variety 'Uffo' (Eta/Sigma) was developed at the State Stende Cereals Breeding Institute during 1992-2004. Variety is a middle-early (three days later comparing with standard 'Vinjett'), characterizing with high yield potential (6-8 t ha<sup>-1</sup>) moderate resistance to lodging (5-7), plant height – 93-100 cm. Also have moderate resistance to *Blumeria graminis f.sp. tritici*, *Puccinia triticina*, *Septoria tritici*, susceptible to *Drechslera tritici-repentis*. Grain quality is suitable for bread making. Variety 'Uffo' is registered in the Latvian Catalogue of Plant Varieties from 2008.

Table 3. Characteristic of spring wheat variety 'Uffo', 2005 –2007

Variety	Grain yield, t ha <sup>-1</sup>	+/- standard	1000 kernel weight, g	Volume weight, g l <sup>-1</sup>	Protein content, g kg <sup>-1</sup>	Zeleny index, ml	Gluten content, %
Standard 'Vinjett'	6.60	-	39.03	778	130	47	28
'Uffo'	6.96	+ 0.36	40.1	782	128	43	25
LSD <sub>0,05</sub>	0,37						

### Conclusion

On evaluation data of 10 spring wheat varieties in 2005-2007 the yield potential was on the level 5-8 t ha<sup>-1</sup>, but there was a great influence on grain yield and quality traits of year. The grain yield was

influenced by variety – 20.64%, but by meteorological conditions-54.02%, interaction of both factors was 16.38%. Significant positive correlation between the yield and the 1000 kernel weight was found, but the significant negative- between the 1000 kernel weight and the volume weight; the starch content and the Zeleny index; the starch content and the protein content; the protein content and the 1000 kernel weight.

New variety 'Uffo' was created at the State Stende Cereals Breeding Institute during 1992-2004, included in the Latvian Catalogue of Plant Varieties from 2008. The variety 'Uffo' is characterizing with high yield potential (6-8 t ha<sup>-1</sup>), grain quality is suitable for bread making.

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## VASARAS KVIEŠU AGRONOMISKĀS UN MORFOLOĢISKĀS PAZĪMES UN TO MIJIEDARBĪBA

### Strazdiņa V.

Izmēģinājumi ar desmit Latvijas Augu katalogā reģistrētajām vasaras kviešu šķirnēm bija iekārtoti Valsts Stendes graudaugu selekcijas institūtā. Laikā no 2005.-2007.gadam tika izvērtētas šķirņu morfoloģiskās un agronomiskās īpašības (veģetācijas perioda ilgums, augu un vārpa garums, 1000 graudu masa, tilpummasa, graudu kvalitātes rādītāji- proteīna saturs, lipekļa saturs un kvalitāte, cietes saturs, krišanas skaitlis), kā arī noskaidrota šo īpašību savstarpēja mijiedarbība. Konstatēts, ka meteoroloģiskie apstākļi būtiski ietekmē graudu ražu un tās kvalitāti (54.2%), šķirnes ietekme bija 20.64%, bet abu faktoru savstarpējā mijiedarbība bija 16.38%.

Būtiski pozitīva korelācija bija starp graudu ražu un 1000 graudu masu, bet būtiski negatīva korelācija starp 1000 graudu masu un tilpummasu, kā arī starp cietes saturu un proteīna saturu, cietes saturu un Zeleny indeksu, kā arī proteīna saturu un 1000 graudu masu.

Vasaras kviešu selekcijas darba rezultātā no 1992.-2004.g. izveidota jauna šķirne 'Uffo', kas raksturojas ar augstu graudu ražu (vidēji 3 gados 6,96 t ha<sup>-1</sup>), vidēju izturību pret veldri un bīstamākajām kviešu lapu slimībām, graudu kvalitāte piemērota maizes cepšanai. Šķirne reģistrēta Latvijas Augu šķirņu katalogā no 2008.gada.

## THE NEW POTATO VARIETY 'REET'

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### Abstract

The characteristics of the new nematode (Ro1) and wart (D 1) the resistant to early and to medium ripening potato variety Reet (Mats x Gitte) developed at the Jõgeva Plant Breeding Institute (Jõgeva PBI) is discussed in this paper. The variety Reet was included in the Estonian and European Variety List in 2007.

The breeding goal was to obtain a variety, of potato which meets the requirements of food markets and processing factories. Reet passed the preliminary and final trials in 2000-2004 at the Jõgeva PBI, where it was compared with the standard varieties Berber (early), Piret (medium) and Ants (medium late). The tuber yield of Reet was equal to Ants and Berber but lower than Piret. The yield of the marketable tubers of Reet was equal to the variety Berber in a dynamics trial performed at different harvest times. Reet exceeded the standard varieties by tuber weight, although the number of tubers per plant was low. There was a low appearance of black scurf, common scab and potato virus infection on the plants of the new variety. It had a relatively good resistance to late foliage blight, exceeding all standard varieties. Therefore the new variety is suitable for organic farming. Reet has good quality characteristics (shallow eyes, regular shape, no darkening).

The peeling test with an abrasive peeler showed that Reet had relatively small peeling remains, approximately 100.0-150.0 g kg<sup>-1</sup>. The content of reducing sugars was low. This makes refers suitable for making chips and French fries.

**Key words:** potato variety, yield, disease resistance, cooking quality.

### Introduction

Due to different conditions and requirements in various locations, many potato varieties exist all over the world. These varieties correspond with the different purposes for which the crop is grown, with the different tastes and preferences of the people and with the different environments in which the crops are grown. Some varieties can be grown in many places and have a wide range of usability; others are meant for very specific purposes or for specific environmental conditions. Apart from production capacity, an important varietal characteristic is the resistance to pests and diseases. Breeding work is constantly going on to develop new varieties, combining high yield and other favourable characteristics with new or improved resistance to all kinds of pathogens. Every potato producer knows that locally bred varieties are most suitable for local growing conditions. The main goal of potato breeding at the Jõgeva PBI is developing medium and medium late, disease (especially late blight resistant), pest and mechanical damage resistant, high yielding and quality table and industrial potato varieties. The yielding capacity, agronomic characteristics, tuber quality and disease resistance of new varieties should be equal or better than these of foreign varieties. Quality characteristics should correspond to contemporary requirements for table and industrial varieties.

### Materials and Methods

The new nematode and wart resistant early to medium potato variety Reet (breed J 649-94) was developed at the Jõgeva PBI, Estonia. Reet was selected from the cross between the Estonian variety Mats and the German nematode resistant variety Gitte. This paper gives information about the economical and biological characteristics of the new variety Reet in 2000-2004, when it was tested in preliminary, final and dynamic trials. The official trials in Estonia and the technical examination (DUS test) in Czech Republic were carried out in 2005-2006.

Reet was compared with the standard varieties Berber (early Dutch variety), Piret (medium Estonian variety) and Ants (medium late Estonian variety).

Conventional breeding methods, crossing with different hybrid varieties or hybrids and repeated selection of hybrid tuber generations were used in breeding. The crossing and growing of seedlings was carried out in the greenhouse and the next generations were tested in the field. Experimental fields of the Jõgeva PBI are located on sandy loam *Calcaric Luvisol* soil by FAO/UNESCO classification. The fields were deeply shredded, cultivated and complete chlorine free mineral fertilizer (containing 80 g kg<sup>-1</sup> N, 50 g kg<sup>-1</sup> P and 190 g kg<sup>-1</sup> K) by 650 kg ha<sup>-1</sup> was used in the spring. Chemical control of weeds was carried out with mixture of the herbicides *Sencor* (250 g ha<sup>-1</sup>) and *Titus* (25 g ha<sup>-1</sup>). To avoid an early late blight infection of foliage and thus suffer a decrease of yield, chemical control was done with *Ridomil Gold MZ 68* (2.5 kg ha<sup>-1</sup>). During the growth period the plants were hilled up three times and harrowed once. The preliminary, final and dynamic trials were planted in 5 replications, designed and analysed by the NNA (Nearest Neighbour Analysis) method using the AGROBASE 20 computer package. To assess the probability of differences between traits, the least significant differences (LSD<sub>05</sub>) were calculated. In this paper all experimental data are presented as an average of the years 2000-2004.

The yield was weighed, the analysis of the yield structure was carried out and the starch content was estimated with Reimann scales (on the basis of special weight). The yield structure contains tubers per plant, tuber weight and damaged tubers. The dynamics of the tuber yield was determined three times at intervals of 7 days. Each sample consisted of 10 plants harvested by hand from the test plots. The planting material was pre-sprouted.

*Phytophthora infestans* DB (late blight) was visually estimated as a percentage of the infected foliage surface. The estimate of late blight was carried out three times and the first estimate was based on the beginning of the infection. Resistance to tuber diseases (tuber rots, black scurf, common scab) was estimated on a 0-5 point scale and potato viruses visually on a 0-9 point scale, where 0 was the most resistant.

Cooking estimats of the cooking and other quality traits tests were carried out by the workers of the potato breeding section of the Jõgeva PBI in autumn and spring (Tsahkna, 2004<sup>b</sup>). The taste was estimated on a 9-point scale where 9 was - excellent and 1 - unsuitable, with strong flavour. Mealiness was estimated on a 5-point scale, where 1 was watery, mellow and moist, 5 dry and very mealy. Disintegration (destruction or disintegration during boiling) was also estimated on a 5-point scale, where 1- stood for tuber that remained whole and 5 for a tuber that had disintegrated into pieces. Enzymatic darkening as well as after cooking darkening was estimated after 1,5 and 24 hours on a 9-point scale, where 1 showed no darkening and 9 a strong darkening of the tuber (Tsahkna, 2000). Two methods for making an estimate for the suitability of potato varieties for chips have been used: the colour test after frying the tuber slices (1-9 points, where 8 and 9 points are preferred) and the determination of the content of reducing sugars by the colorimetric method. Varieties with high (200-220 g kg<sup>-1</sup>) dry matter and low (2.0-2.5 g kg<sup>-1</sup> in raw mass) reducing sugars content are preferred for chips. The suitability for chips was estimated both in autumn and spring. In order to estimate chip quality in the spring, the samples were kept for 4 weeks in +10°C temperature (Tsahkna, 1995).

For the peeling test five tubers were peeled with an abrasive peeler. The tubers were weighed before and after the peeling and the peeling remains were calculated (Tsahkna, 2004<sup>a</sup>).

## Results and Discussion

The morphological description of the variety Reet is given in accordance with UPOV characteristics. Plant height is medium to tall and growth habit erect. The leaf is small, with a medium intensity of green colour. The thickness of the main stem is medium to thick and with medium extension of antocyanin coloration. The flower corolla is small, blue-violet and the intensity of the antocyanin coloration of the inner side is medium to strong. Lightsprout is medium, spherical and blue-violet. The skin colour of tuber is yellow, the colour of the flesh is yellow and the depth of the eyes is shallow.

The most important yield characteristics (yield of tubers, number of tubers per plant, weight of tuber, damaged tubers) and starch content of new potato variety Reet are compared with the standard varieties (Berber, Piret and Ants) and presented in Table 1.

Table 1. Yield characteristics of the potato variety Reet at the Jõgeva PBI in 2000–2004

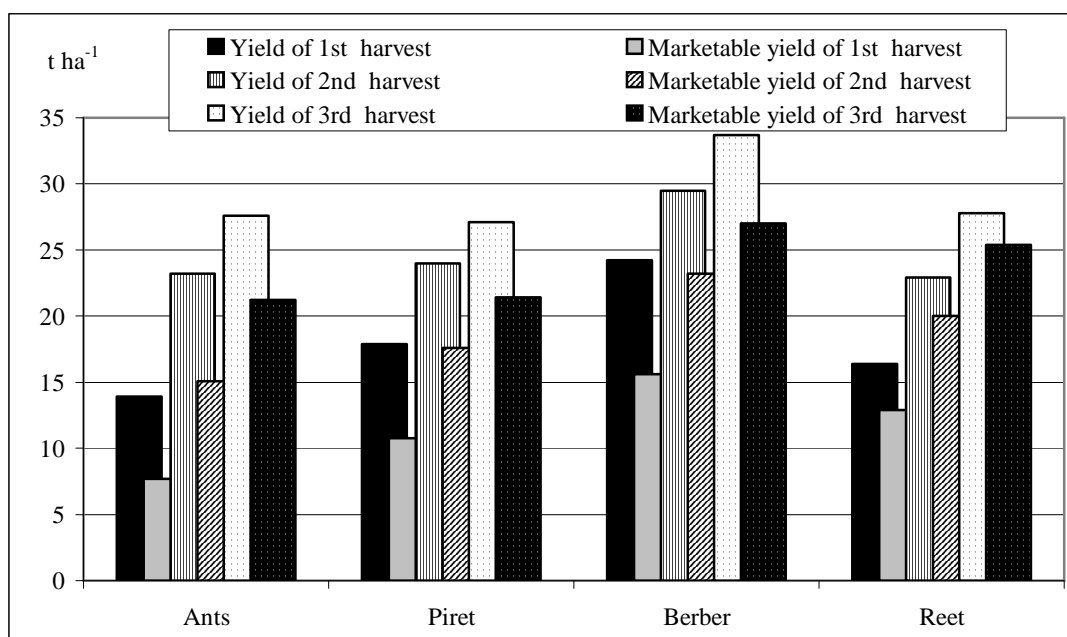


Character	Unit	LSD <sub>05</sub> <sup>1</sup>	Ants	Piret	Berber	Reet
Tuber yield	t ha <sup>-1</sup>	3.4	37.8	41.6	34.2	36.6
Tubers per plant	number	1.4	9.0	8.9	8.5	6.9
Tuber weight	G	10.0	75.0	84.0	74.0	100.0
Starch content	g kg <sup>-1</sup>	7.0	141.0	155.0	118.0	159.0
Damaged tubers	g kg <sup>-1</sup>	6.0	26.0	25.0	20.0	38.0

<sup>1</sup>LSD<sub>05</sub> – least significant differences at P = 0.05

The results of the dynamic trials demonstrated the earliness of the variety Reet. The marketable yield of tubers harvested at three different times is shown in Figure 1.

Disease resistance to the most important diseases (late blight, tuber rots, black scurf, common scab, potato viruses X-Y-PRLV-M) of the new variety is given in Table 2. The early variety Berber is very susceptible to foliage late blight (*Phytophthora infestance*), Ants and Piret are moderately resistant to late blight.



LSD<sub>05</sub> of tuber yield of 1st harvest –3,4 t ha<sup>-1</sup>; LSD<sub>05</sub> of tuber yield of 2nd harvest –2,6 t ha<sup>-1</sup>; LSD<sub>05</sub> of tuber yield of 3rd harvest –2,9 t ha<sup>-1</sup>.

LSD<sub>05</sub> of marketable tuber yield of 1st harvest –3,1 t ha<sup>-1</sup>; LSD<sub>05</sub> of marketable tuber yield of 2nd harvest –2,6 t ha<sup>-1</sup>; LSD<sub>05</sub> of marketable tuber yield of 3rd harvest –1,9 t ha<sup>-1</sup>.

Figure 1. Yield of tubers and the marketable yield of the variety Reet in the dynamic trial at the Jõgeva PBI in 2000–2004

The cooking quality traits and the peeling quantity of Reet are very important because it was bred as a table and industrial variety. Table 3 presents the data of taste, mealiness, disintegration, darkening, chips colour and the content of reducing sugar of Reet compared with standard varieties. The results of peeling quantity of some listed varieties tested in 2005-2006 are compared in Figure 2. The vegetation period of the pre-sprouted variety Reet is 90-100 days. The initial development is medium rapid in Estonian climatic conditions.

Yield characteristic. The tuber yield of Reet (36.6 t ha<sup>-1</sup>) was equal to Ants and Berber but significantly lower 5.0 t ha<sup>-1</sup> than that of Piret (LSD<sub>05</sub> 3.4 t ha<sup>-1</sup>). The tuber weight and the number of tubers per plant are closely related. All standard varieties exceeded the new variety Reet by number of tubers per plant, but the average tuber weight of Reet was the biggest (100 g) compared with other varieties. The largest tubers of Reet tended to be damaged more during the harvest (Table 1).

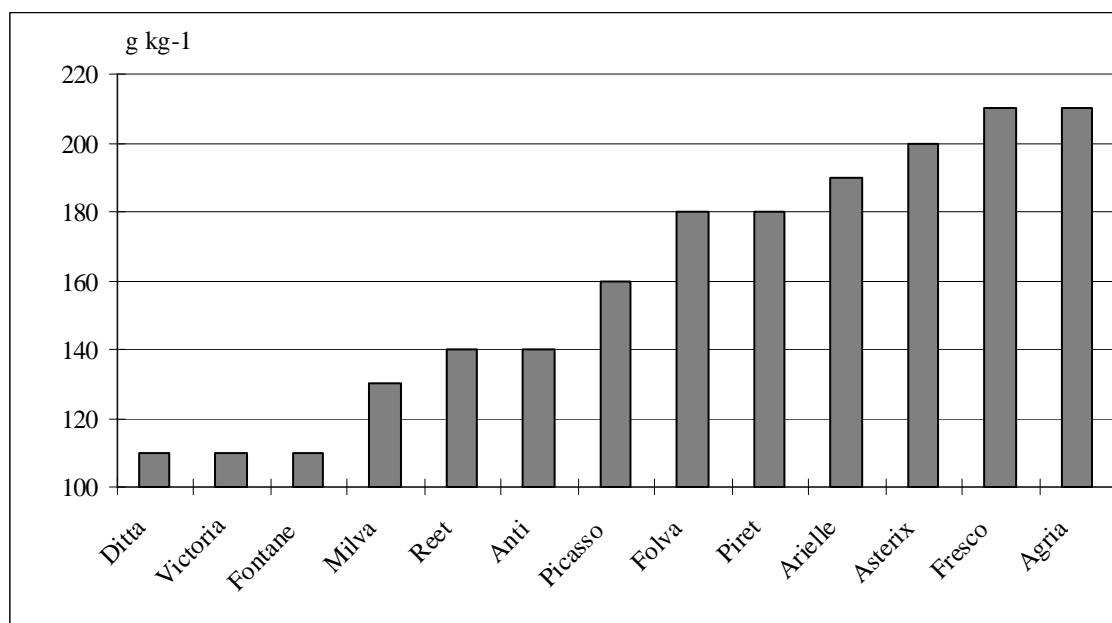
Table 2. Disease resistance of the potato variety Reet at the Jõgeva PBI in 2000–2004

Character	Unit	Ants	Piret	Berber	Reet
Late blight: 1 <sup>st</sup> estimation	% <sup>2</sup>	4.5	3.1	54.1	2.0
2 <sup>nd</sup> estimation	% <sup>2</sup>	34.1	23.0	82.8	18.0
3 <sup>rd</sup> estimation	% <sup>2</sup>	60.6	54.7	100.0	45.1
Tuber rots	g kg <sup>-1</sup>	0.6	0.5	1.0	1.1
Black scurf	0-5 points <sup>3</sup>	0.4	1.0	0.8	0.4
Common scab	0-5 points <sup>3</sup>	0.4	0.4	1.0	0.6
Potato viruses X-Y-PRLV-M	0-9 points <sup>4</sup>	3-1-0-0	3-0-1-1	9-5-7-2	4-0-0-3

<sup>2</sup> – percentage of infected foliage surface

<sup>3</sup> – tuber disease resistance 0–5 points (0–resistant)

<sup>4</sup> – plants infected by potato viruses X-Y-PRLV-M in scale 0–9 (0–not infected)



LSD<sub>05-15</sub> g kg<sup>-1</sup>

Figure 2. Peeling remains of some varieties in the Estonian Variety List tested at the Jõgeva PBI in 2005–2006,

The content of dry matter, including starch content, is influenced by the variety, the physiological age of tuber at harvesting, the intensity of light, the water supply in the soil, soil conditions, etc (van der Zaag, 1992; Tsahkna, Tähtjärvi, 2007). As an average for the years 2000-2004 the starch content of the variety Reet was equal to the variety Piret, but higher than that of the varieties Berber and Ants.

The dynamics of potato yield formation is significantly influenced by weather conditions. Therefore the average of 5 experimental years gives an objective view of the yield formation and earliness of the new variety (Figure 1). The early variety Berber exceeded the variety Reet significantly by tuber yield in all three harvests in the dynamic trials but in terms of marketable tuber yield the numbers were not significant. By marketable tuber yield Reet exceeded the variety Ants in all harvests respectively 5.2, 4.9 and 4.2 t ha<sup>-1</sup> and the variety Piret in the 3<sup>rd</sup> harvest. From these results we have concluded that because of the higher total tuber yield for the variety Reet we can also get higher marketable tuber yield compared to variety Berber.

Disease resistance. Reet is a nematode (Ro1) and wart (D 1) resistant variety. The importance of resistance breeding has not changed but the importance of external and internal tuber quality is becoming more and more important. External tuber disorders, like coverage with common scab (*Streptomyces scabies* Waksman et Henrici) and black scurf (*Rhizoctonia solani* Kühn) reduces their marketability. According to the trial data presented in Table 2 the appearance of black scurf

and common scab in the variety Reet was low, as were the amount of plants infected by potato viruses. The X-virus was the most destructive among potato viruses (4 points).

Different strategies have been used at the Jõgeva PBI to breed late blight resistant varieties. Two different resistance forms to late blight exist in potato plants. Major genes give full protection against the different physiological races of *Phytophthora infestans*, but from the emergence of new races resistance is lost. Increases in pathotype complexity and diversity create additional difficulties in potato breeding for late blight resistance. Only varieties with a high level of field resistance are able to resist severe late blight epidemics. New sources of disease resistance were used to create more resistant varieties (Koppel, Tsahkna, 2003). The variety Reet exceeded the standard variety Berber its low first infection rate and the slower speed of foliage late blight infection in all 3 estimates (Table 2). Compared to standard varieties the new variety had a higher resistance to foliage late blight. This is a very important trait of the variety that may increase tuber yield.

Cooking quality. The taste of potato is not a very important trait in the breeding programme at the moment, but since our consumers use the potato as a table potato we will still estimate this feature. In addition to taste disintegration, mealiness after cooking the darkening of cooked potatoes and enzymatic darkening are the most important traits to determine cooking quality. Reet has been bred as an industrial variety but its cooking quality also shows that it is suitable as a table potato (Table 3). The taste of Reet is considered not as good as standard varieties. Disintegration is also low and mealiness is at the same level as the variety Ants. That the new variety can be used for salads and as a table (cooking type AB). The high content of chlorogenic acid is one of the main reasons for the darkening of raw potato tubers and is caused mainly by genotype, but also by weather conditions (drought, water stress). The trials data has shown that a low content of chlorogenic acid in the varieties correlates with low after cooking (non-enzymatic) darkening (Koppel, Tsahkna, 2003). Table 3 shows that enzymatic darkening of Reet is absent or is minimal and after cooking darkening is also less compared with the standard varieties. It is very important in industrial use. The contents of dry matter and reducing sugars are also very important traits for industrial use. The results of the trials show that the variety Reet has a low content of reducing sugars in autumn and a slightly higher content in the spring resulting in light yellow (8 points) even coloured. The results have proved that the suitability of potato genotypes for making chips can be estimated a visual color test after frying.

Table 3. Cooking quality of the potato variety Reet at the Jõgeva PBI in 2000–2004

Character	Unit	Ants	Piret	Berber	Reet
Taste	1-9 points <sup>1</sup>	7.3	7.5	6.9	6.2
Mealiness	1-5 points <sup>2</sup>	2.5	2.8	1.6	2.3
Disintegration	1-5 points <sup>2</sup>	1.1	1.6	1.0	1.0
Darkening: after cooking	1-9 points <sup>1</sup>	1.6	1.2	1.3	1.3
Enzymatic	1-9 points <sup>1</sup>	4.0	1.2	1.7	1.7
Chips colour: in autumn	1-9 points <sup>1</sup>	7.6	8.0	6.5	8.0
in spring	1-9 points <sup>1</sup>	5.5	7.3	5.0	8.0
Content of reducing sugars: in autumn	g kg <sup>-1</sup>	2.5	2.0	5.0	2.0
in spring	g kg <sup>-1</sup>	6.0	2.5	10.0	3.2

<sup>1</sup> – 9–the excellent taste or max darkening or preferred chips colour; <sup>2</sup> – 5–max mealiness or crumbliness

As the suitability of the tubers for mechanical processing cannot be estimated solely on the basis of the external quality traits of tuber, peeling tests with an abrasive peeler were carried out. The results of the peeling remains of the tested varieties of the Estonian Variety List are given in Figure 2. The variety Reet had no significant differences in peeling remains compared with the varieties Milva and Anti. The varieties Ditta, Victoria and Fontane had less peeling remains. Had significantly more peeling remains the varieties Picasso, Folva, Piret, Arielle, Asterix, Fresco and Agria.

## Conclusions

The nematode (Ro1) and wart (D 1) resistant early to medium ripening potato variety Reet (breed J 649-94) has been developed at the Jõgeva PBI and included in the Estonian and European Variety List in 2007. Reet is a selection from the cross between Mats and GitteN.

The tubers of the new variety have regular shape, shallow depth eyes, yellow skin and flesh colour and a blue-violet flower corolla. Reet has medium tuber size and a high marketable tuber yield. The number of tubers per plant is medium but the average tuber weight is large. The new variety is relatively resistant to potato virus diseases, foliage late blight, black scurf and common scab. Because the foliage late blight infecting starts slower and spreads less the new variety is suitable for use in organic farming.

The new variety is free from darkening after cooking and enzymatic discoloration, it has a low content of reducing sugars and rather small peeling remains. Reet can be used as a table and industrial potato, including making chips and French fries.

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## JAUNA KARTUPEĻU ŠĶIRNE ‘REET’

**Tsahkna A., Tähtjärv, T.**

Rakstā izklāstīts jaunas, pret nematodi (Ro1) un vēzi (D 1) izturīgas, agras līdz vidēji agras, Jegevas selekcijas institūtā (Jõgeva PBI) izaudzētas kartupeļu šķirnes ‘Reet’ (‘Mats x ‘Gitte’) raksturojums. Šķirne ‘Reet’ 2007. gadā iekļauta Igaunijas un Eiropas Augu Šķirņu Kataloģā. Selekcijas mērķis bija izveidot šķirnes, kas atbilst pārtikas tirgus un pārstrādes uzņēmumu prasībām. Šķirnei ‘Reet’ 2000. – 2004. veikta iepriekšējās un gala pārbaudes Jõgeva PBI, salīdzinot ar standartšķirnēm ‘Berber’ (agra), Piret (vidēji agra) un Ants (vidēji vēla). Kartupeļu bumbuļu raža šķirnei ‘Reet’ bija līdzvērtīga šķirņu ‘Ants’ un ‘Berber’ ražai, bet zemāka kā šķirnei ‘Piret’. Dinamikas izmēģinājumos dažādos ražas vākšanas termiņos bumbuļu preču produkcijas raža šķirnei ‘Reet’ bija vienāda ar šķirnes ‘Berber’ ražu. Kartupeļu bumbuļa svara ziņā šķirne ‘Reet’ pārspēja standartšķirnes, lai gan bumbuļu skaits vienā cerā bija zems. Šķirnes augiem bija ļoti maz melnā kraupja, parastā kraupja un vīrusslimību infekcijas pazīmju. Tai bija relatīvi laba izturība pret lakstu puvi uz lapām, pārsniedzot visu standartšķirņu izturību. Tātad jaunā šķirne ir piemērota bioloģiskajai lauksaimniecībai. Šķirnei ‘Reet’ ir labas kvalitatīvās īpašības (seklas acis, izlīdzināta forma, netumšojas). Mizošanas testa ar abraziwo mizotāju laikā konstatēts, ka šķirnes bumbuļiem ir relatīvi maz mizošanas atlieku, apmēram 100.0-150.0 g kg<sup>-1</sup>. Reducējošo cukuru saturs bija zems. Tas liecina par piemērotību čipsu un frī gatavošanai.

## YIELD AND QUALITY OF WINTER RYE IN TRIALS AT THE JÕGEVA PBI

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### Abstract

Rye bread has been a staple food for centuries in Estonia, Latvia, and Lithuania. The aim of the study was to compare the yield and quality of winter rye varieties and breeds from all the three countries in Estonian climatic and soil conditions at the Jõgeva Plant Breeding Institute (PBI). The varieties Elvi (Estonia), Kaupo (Latvia), and Joniai (Lithuania) and the breeds J 92-5 (Estonia), LAT 9504 (Latvia), and LIA 426 (Lithuania) were sown using conventional cultivation in three series of trials (2005–2007). Weather conditions of the trial years were relatively similar. No significant winter damage was observed. All the varieties and the breeds demonstrated high yielding potential. Comparing the three-year averages, the variety Elvi and the breed J 92-5 had the highest yields. The thousand-kernel weight (TKW) exceeded 30 g in all the trial years and the average was the highest in 2005. The falling number (FN) was generally suitable for bread baking on average, Joniai and breed LIA 426 had the lowest FN in all the years. In wet conditions, the harvest of this variety and breed must be completed fast.

**Key words:** winter hardiness, yield components, yield, thousand-kernel weight, quality

### Introduction

Rye bread is a valuable component in the human diet, and the consumption of the bread in physiologically reasonable quantities may make us feel good as well as protect us against health problems (Kann, 2002). In 2005, the area under winter rye in Estonia was rather small: only 7.4 thousand hectares. By 2007 it had increased to 16.2 thousand hectares ([www.stat.ee](http://www.stat.ee)). The climatic conditions of Estonia are suitable for the cultivation of winter rye but it is important to find an appropriate variety. The success of rye cultivation depends on the climatic conditions and the adaptation of varieties to variable growing conditions (Tupits and Kukk, 2000). Experiments with different rye varieties, investigating development, winter hardiness, yield formation and quality in Finland, showed that both the yield and the quality depended mostly on local climatic conditions (Pahkala *et al.*, 2004).

Chmielewski (1992) admits relying on his long-term study, that even relatively small climate change can affect crop yields. Climate change towards warming can also be observed in Estonia (Jaagus, 1999). Because of climate change, there is a need for new rye varieties that would be suitable for cultivation in changeable conditions, have good yielding capacity, are resistant to plant diseases, and have a high bread baking quality (Hakala and Pahkala, 2003). The aim of this study was to compare the yield and the quality of the new local breeding material with the breeding material from neighbouring countries and with the best varieties from Estonia, Latvia and Lithuania. Foreign winter rye varieties suitable for the Estonian climatic and soil conditions are also a good source of initial material for rye breeding.

### Material and Methods

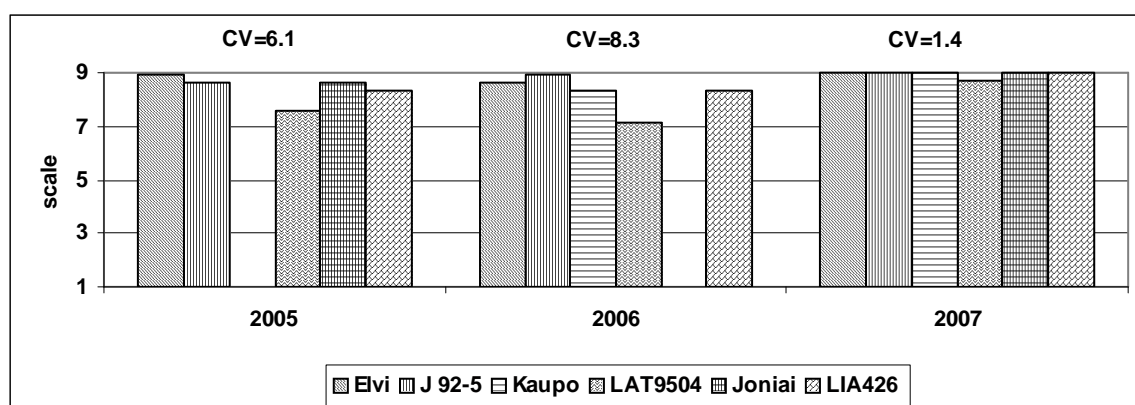
The study involves three growing seasons (2005–2007). By FAO-UNESCO classification, the Jõgeva PBI trial area is located on podzolized soddy-calsareous soil (Kask, 1995). Based on soil analyses ( $\text{pH}_{\text{KCl}}$  6.5), 290 kg ha<sup>-1</sup> of the complex fertilizer Kemira Skalsa (N<sub>0</sub>P<sub>12</sub>K<sub>24</sub>) was applied before sowing. In spring, after the onset of plant growth, 150 kg ha<sup>-1</sup> of ammonium nitrate (N<sub>51</sub>) was added. Non-treated seeds were sown on black fallow in three replications, using a completely randomized block design according to the Nearest Neighbour Analyses method (NNA). The trial plots were 5 square meters each. The sowing rate was 500 germinating seeds per square meter and the sowing time was the first decade of September. The optimal sowing time of winter rye in Estonia is from August 25 to September 10. The following varieties and breeds were included in the trials: Elvi and J 92-5 (Estonia), Kaupo and LAT 9504 (Latvia), Joniai and LIA 426

(Lithuania). The determined grain yields ( $\text{kg ha}^{-1}$ ) describe the weight of dried and cleaned seeds calculated to 14% moisture content. Winter hardiness was assessed on a 1–9 scale (1–poor winter hardiness, 9–winter hardy) by the visual inspection of all the trial plots. The yield components were determined from samples, compiled from 25 randomly picked plants, by counting the tillers per plant and the kernels per head, and weighing the kernels. The falling numbers (FN) were determined according to the standard method of AACC 56-81A with the Falling Number 1800 equipment. The yield data were statistically analysed using the database management and analysis system Agrobases (Agrobases<sup>TM</sup> 20, 1999) ( $\text{LSD}_{0.05}$ ). The coefficient of variation (CV) was used as a basis for the comparison between different trial years.

In Estonia, the vegetation period of rye usually begins in the middle of April and the harvest at the end of July or in early August. Meteorological data from the beginning of the vegetation period to the harvest were recorded by the field meteorological weather station Metos Compact. The long-term (1922–2007) average sum of precipitation of the vegetation period of rye at Jõgeva is 255 mm, and the long-term average cumulative sum of effective (over  $+5^\circ\text{C}$ ) air temperatures is 994 degrees. The vegetation periods of 2005, 2006 and 2007 were considerably drier and warmer than the long-term average. For yield and for quality in general, not only the amount, but also the distribution of precipitation during the vegetation period is important. The beginning of the 2005 vegetation period was cool but from the end of May to the end of July the air temperature was above the long-term average (sum – 1024 degrees). In May it was wet, in June and July there was drought and rain started again just before harvesting. The sum of precipitation of the 2005 vegetation period was 287 mm. The vegetation periods of 2006 and 2007 were dry (precipitation 111 and 214 mm respectively) and warm (1006 and 1010 degrees respectively) from the beginning and the rye seeds reached the hard dough development stage about two weeks earlier than usual. In 2007 it started to rain shortly before harvesting.

## Results and Discussion

In general, winter hardiness in the trials was high (Figure 1). The highest winter hardiness during all the trials was assessed in 2007 (CV 1.4). In 2005 and 2006, the average winter hardiness was lower than in 2007 (CV 6.1 and 8.3 respectively). In all the trial years, the Estonian variety and breed were the most winter hardy, followed by the breed from Lithuania. The Latvian breed had the lowest winter hardiness.

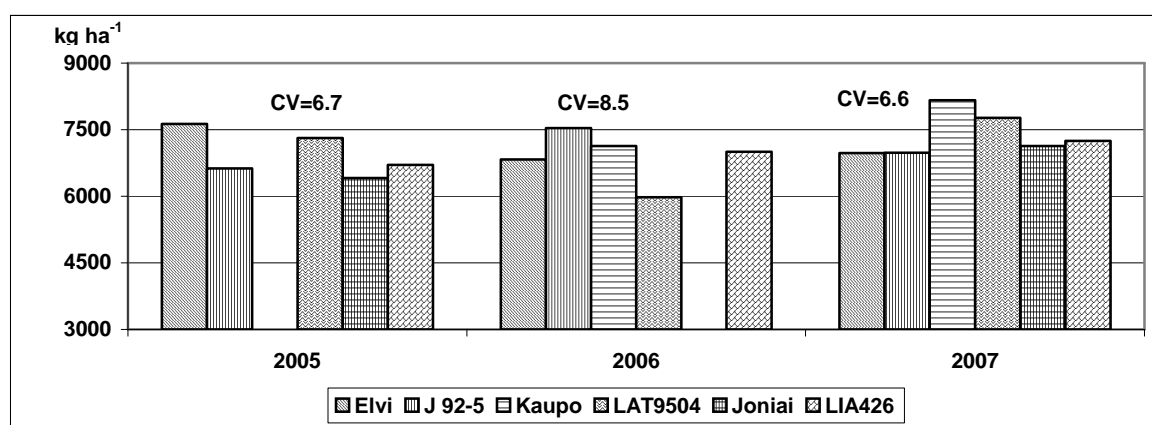


( $\text{LSD}_{0.05}$  for 2005=0.8; 2006=0.65; 2007=0.2)

Figure 1. Winter hardiness of the rye varieties and breeds in the trials at the Jõgeva PBI

The highest average yield of the trial years was in 2007 –  $7,377 \text{ kg ha}^{-1}$  (CV 6.6), in 2006  $6,977 \text{ kg ha}^{-1}$  (CV 8.5) and in 2005  $6,940 \text{ kg ha}^{-1}$  (CV 6.7) (Figure 2). Elvi had the highest three-year average yield:  $7,280 \text{ kg ha}^{-1}$ , followed by the yield of J 92-5 ( $7,050 \text{ kg ha}^{-1}$ ), LAT 9504 ( $7,020 \text{ kg ha}^{-1}$ ), and LIA 426 ( $6,980 \text{ kg ha}^{-1}$ ). The lowest single yield was produced by LAT 9504 ( $5,980 \text{ kg ha}^{-1}$ ) in 2006. Kaupo and Joniai were included in the trials in 2006 and 2007, and in 2005 and 2007, respectively. In the two-years average the yield of Kaupo was  $7,645 \text{ kg ha}^{-1}$  and that of Joniai  $6,770 \text{ kg ha}^{-1}$ . In 2007, Kaupo produced the highest yield of the whole trial period ( $8,160 \text{ kg ha}^{-1}$ ). The two-year average yield of LAT 9504 was lower in comparison with Kaupo. The two-

year average yield of Joniai was lower than that of LIA 426, which showed a good yielding capacity. The three-year average yield of Elvi exceeded that of J 92-5. The yield of LAT 9504 varied significantly (CV 18.0) during the trial period, followed by the yield of Kaupo (CV 9.5), Joniai (CV 7.5), J 92-5 (CV 5.6), LIA 426 (CV 3.9) and Elvi (CV 2.6).



(LSD<sub>0.05</sub> for 2005=996; 2006=1006; 2007=1080)

Figure 2. Yield of the winter rye varieties and breeds in trials at the Jõgeva PBI

The analysis of the yield components of the breeds showed, that on the average LAT 9504 had 6.5 productive tillers per plant, 56 kernels per head and the weight of kernels in 2005 was 2.4 g. LIA 426 had 5.4 tillers, 52 kernels and the weight of kernels was 2.2 g. J 92-5 had 4.5 tillers, 59 kernels per head and in 2005, the kernels weighed 2.2 g in 2005 (Table 1). In 2006, when the plant density on the plots of LAT 9504 was low, the number of tillers was high – 11.7, the number of kernels per head was 57, and the kernels were heavy – 2.3 g. There were only small differences in the yield components of Elvi, J 92-5 and Kaupo.

Table 1. Yield components in the trials at the Jõgeva PBI

Variety/ Breed	Tillers/plant			Kernels/head			Kernel weight,g		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
Elvi	4.1	6.6	4.9	53	56	64	2.2	1.7	2.2
J 92-5	4.5	6.6	4.2	59	53	56	2.2	1.7	1.7
Kaupo		6.9	5.5		54	57		1.8	2.0
LAT 9504	6.5	11.7	5.1	56	57	59	2.4	2.3	1.9
Joniai	4.6		4.2	50		58	2.0		2.3
LIA 426	5.4	4.8	4.1	52	50	60	2.2	1.9	2.2

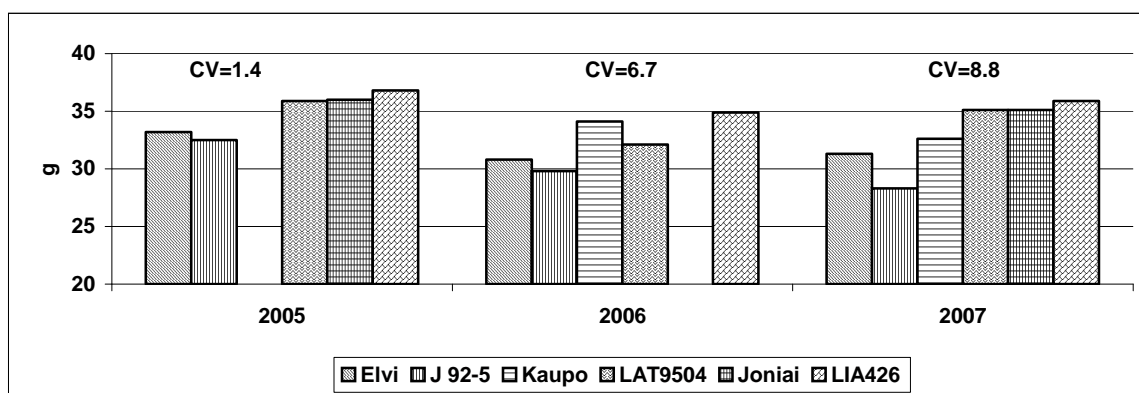
LIA 426 had 4.8 tillers per plant, 50 kernels per head and the weight of kernels was 1.9 g on the average. In 2007, Kaupo had the highest number of tillers per plant (5.5) whereas LIA 426 had the smallest (4.1). Elvi had the highest number of kernels per plant (64) and J 92-5, the smallest (58). On the average Joniai had the heaviest kernels (2.3 g) and J 92-5 the most and lightweight kernels (1.7 g). On the average, the thousand-kernel weight (TKW) was the highest in 2005 – 35 g (CV 1.4), in 2007 – 33 g (CV 8.8) and in 2006 – 32 g (CV 6.7) (Figure 3).

The highest three-year average TKW was demonstrated by Joniai – 35.6 g and LIA 426 – 35.9 g, followed by the TKW of LAT 9504 – 34.4 g, Kaupo – 33.3 g, and the lowest TKW-s of Elvi – 31.8 g and J 92-5 – 30.3 g.

The two-year average thousand-kernel weights of Kaupo and LAT 9504 were equal. Comparing the TKW of Joniai and LIA 426, the kernels of LIA 426 were larger and heavier.

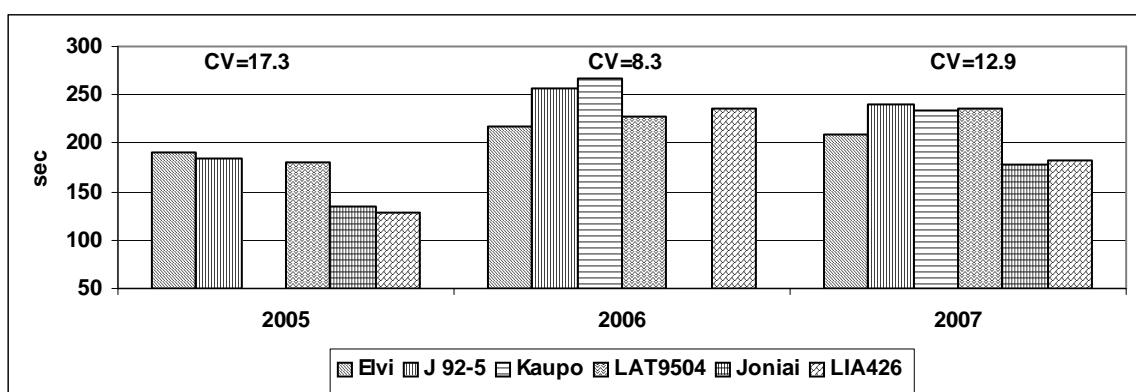
In 2005, the falling numbers were the lowest, the average of the trial was 164 sec (CV 17.3), followed by 213 sec (CV 12.9) in 2007, and the highest 241 sec (CV 8.3) in 2006 (Figure 4). The

highest single FN of all the trials was demonstrated by Kaupo by 266 sec, followed by J 92-5 by 257 sec, both in 2006. The lowest FN occurred in 2005, LIA 426 by 129 sec and Joniai by 135 sec. The three-year average FN of the mentioned breed was 182 seconds and it varied the most (CV 29.4) during the years. The two-year average FN of Joniai was the lowest –156 seconds (CV 19.5).



(LSD<sub>0.05</sub> for 2005=1.66; 2006=1.0; 2007=2.27)

Figure 3. Thousand-kernel weight of the winter rye varieties and breeds in the trials at the Jõgeva PBI



(LSD<sub>0.05</sub> for 2005=32.0; 2006=28.7; 2007=36.8)

Figure 4. Falling number of the winter rye varieties and breeds in the trials at the Jõgeva PBI

The long-term yield variation is associated with climate changes, which occurred from years to decades and influenced vegetation (Chmielewski, 1992). Estonian climate data testify that the average air temperature is increasing, especially in spring. For example, the average air temperature in March is 1° C higher than in mid-sixties and the snow melts about a week earlier. At the same time, the amount of precipitation has increased by 50 mm, mostly in autumn and early winter (Jaagus, 1999). During the described trial years, the snow cover of the fields was scanty and the plants of winter cereals were exposed to rain, ice cover and cold (Keppart, 2007).

The most essential characteristic of winter rye is winter hardiness. For winter hardiness, the duration of the vegetation period in autumn is important. When the autumn growing season is short, plant development will be insufficient and rye may be killed off during the winter (Chmielewski, 1992). In long-term trials in Germany, it was found that warm and sunny autumn weather had a positive influence on the density of winter rye plants and the number of kernels per head (Chmielewski and Köhn, 2000). The data on cumulative air temperature in autumn demonstrates an increase in our region, which means that the growing season is more than two weeks longer than in the 1990-ies (Keppart, 2008).

In all the trial years, the autumns were warm and air temperatures dropped and persisted below zero in the middle of January. In the trials, all the varieties and breeds developed well in autumn, five out of six showed high winter hardiness, and only the breed LAT 9504 had lower resistance to



cold. In 2005 and 2007, winters were mild after a prolonged autumn. In February 2006, the winter was harsh and a number of killed off LAT 9504 plants were seen in spring. There was no snow mould in the trials.

Grain yield is the sum of the three yield components, number of heads per m<sup>2</sup>, kernels per head and weight of kernels (Fowler, 2002). In 2005, visual assessment suggested no big differences in plant density but LAT 9504 had the highest yield among breeds, followed by LIA 426 and J 92-5. The yield of Elvi exceeded the yield of Joniai. Next year, the plant density on plots of LAT 9504 was low, and although the number of kernels and the weight of kernels were the highest, the yield was small. At the same time, the number of kernels per head and the weight of kernels of J 92-5 were the lowest. The average yield of this breed was the highest of all the trial years. In 2007, the plant density on plots was equal and number of tillers per plants was analogous but there were differences between the numbers of kernels per head and kernel weights, which resulted in differences in the yields.

Winter rye needs about 20-30% less water for seed formation compared to wheat (Starzycki, 1976, Кобылянский, 1982). In drought years, winter rye yields are usually bigger than those of spring cultivars (Häusler, 1996) because rye has a bigger and deeper root system (Kutschera, 1960). In all three years, the yields of winter rye exceeded the yields of spring cereals at Jõgeva (Keppart and Tupits, 2008). During trials in the Czech Republic it was found that high air temperature and precipitation before heading had a good influence on the yield formation of rye varieties, but after heading, high air temperature decreased the yield (Petr *et al.*, 1985). Chmielewski and Köhn (2000) have also found that high temperatures and drought during the ripening stage may have a negative influence on the kernel weight. The thousand-kernel weights of Elvi and J 92-5 were lower than those of the Latvian and Lithuanian varieties and breeds, which were probably more resistant to drought.

Rye is very sensitive to pre-harvest sprouting (Drews *et al.*, 1976; Salmenkallio-Marttila *et al.*, 1998). Due to unsuitable weather conditions during grain maturation and harvesting, sprouting may reduce the end-use quality of winter rye. The years of the trials were dry and warm, but just before the 2005 and 2007 harvestings, heavy showers occurred and high humidity influenced the activity of ferments that dissolve starch. According to the quality requirements of the Tartu Grain Mill Ltd., the main purchaser of rye grain in Estonia ([www.tartuveski.ee](http://www.tartuveski.ee)), the minimum falling number of rye for bread baking is 160 sec for the first bread quality category, and 120 sec for the second category. The falling numbers of the trials fulfilled these requirements. The lowest falling numbers of the trial period were in 2005, however the Estonian variety and breed and the Latvian breed had much higher falling numbers than those from Lithuania. The same situation recurred in 2007, although the values were higher than in 2005. In Estonian conditions, not only the high value of the falling number, but also long-term stability is important. A special test in artificial conditions in the moisture chamber was arranged in 2000–2006 (Tupits, 2007). The results concerning the germination starting time of the 63 breeds was follows the first – high, but quickly starting germination, the second – high and moderately starting germination, and the third – high and slowly starting germination. The second and third categories included varieties from Germany, Sweden, Finland, Latvia and Estonia, the first, different breeds and the variety Joniai from Lithuania. The falling numbers of ripe grain remained high enough for bread baking just for one day in highly humid conditions.

### Conclusions

All the breeds included in the trials had high yielding potential, medium to good winter hardiness, and medium to good quality. The plants of LAT 9504 were more sensitive to cold and a number of plants were killed off during the harsh winter. The plants on sparser plots had twice as many tillers as on dense plots, the size and the weight of the kernels of these plants were bigger, but the total yield was small all in all.

In comparison with Latvian and Lithuanian varieties and breeds, J 92-5 had bigger-size kernels, but thousand-kernel weights and kernel weights per head were smaller. These traits need improvement during breeding in coming years.

In rainy or foggy conditions before and during harvest, the sprouting of kernels may occur often. Joniai and LIA 426 had high falling numbers in droughty year, but if rain occurred before harvesting, the values dropped quicker than these of the other varieties or breeds.

### Acknowledgement

Author is grateful to Aina Kokare from the State Priekuli Plant Breeding Station and Vida Plyčevaitiene from the Lithuanian Institute of Agriculture for distributing seed material and good collaboration in winter rye testing.

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## ZIEMAS RUDZU RAŽA UN KVALITĀTE IZMĒĢINĀJUMOS JŌGEVA PBI

### Tupits I.

Rudzu maize gadsimtiem bijis pamatēdiens Igaunijā, Latvijā un Lietuvā. Pētījuma mērķis bija salīdzināt visās trijās valstīs izveidotu ziemas rudzu šķirņu un hibrīdu ražu un kvalitāti Igaunijas klimatiskajos un augsnes apstākļos Jegevas selekcijas institūtā (Jogeva PBI). Trīs gadu izmēģinājumā (2005.-2007.) konvencionāli saimniekojot tika sētas šādas šķirnes un hibrīdi: 'Elvi' (Igaunija), 'Kaupo' (Latvija), 'Joniai' (Lietuva), J 92-5 (Igaunija), LAT 9504 (Latvija) un LIA 426 (Lietuva). Laika apstākļi izmēģinājumu gados bija līdzīgi. Netika novēroti būtiski ziemošanas bojājumi. Visas šķirnes un hibrīdi uzrādīja augstu ražas potenciālu. Salīdzinot trīs gadu rezultātus, augstākās ražas bija šķirnei Elvi un hibrīdam J 92-5. Tūkstoš graudu masa (TKW) pārsniedza 30 g visos pētījuma gados, vidēji augstākais tika novērots 2005. gadā. Krišanas skaitlis (FN) kopumā bija atbilstošs maizes cepšanai, zemākais FN visos pētījuma gados bija 'Joniai' un LIA 426. Mītros apstākļos šīs šķirnes un hibrīda raža vāksana jāveic ātri.

## CHARACTERIZATION OF LATVIAN POTATO GENETIC RESOURCES BY DNA FINGERPRINTING WITH SSR MARKERS

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### Abstract

SSR (Simple Sequence Repeats) markers have been broadly applied in plant material identification, genetic diversity evaluation, in various gene banks for collections maintenance and in breeding programs for the monitoring of elite alleles and material exchange, as well as in ploidy level prediction, construction of genetic maps, evolutionary and population studies. The high polymorphism level and the co-dominance of SSR markers allow for efficient cultivar characterization and can discriminate even closely related cultivars. However, several factors must be considered when applying SSR markers to polyploid plant species.

After initial optimization and the pre-screening of 15 SSR markers, all potato (*Solanum tuberosum* L. subsp. *tuberosum*) cultivars listed in the Latvian Plant Genetic Resource database were analyzed using eight SSR markers that were found to be most polymorphic. Cultivar fingerprinting, genetic distance evaluation and cluster analyses were performed. Two pairs of the tested cultivars were identical in all screened loci and couldn't be discriminated; the remaining potato cultivars could be discriminated using a minimum of 4 SSR markers. Similar genetic relationships were observed in the potato cultivar collection when analysed with different phylogenetic methods. An increase in the genetic diversity of the newly bred potato cultivars was identified when compared to the older cultivars.

**Key words:** genotyping, potato, SSR, cultivar identification

### Introduction

Potato cultivars are vegetatively reproduced every year for distribution and breeding purposes. Currently the identification of potato cultivars is based on phenotypic characteristics, which are difficult to distinguish, time-consuming and can be affected by environmental factors all of which results in a high risk of misclassification. The autotetraploid genome of cultivated potato ( $2n=4x=48$ ) and their outcrossing nature makes them a difficult candidate for genetic studies. A narrow genetic base has led to a high genetic similarity in European cultivated potatoes (Gebhardt *et al.*, 2004; Simko *et al.*, 2006).

Several molecular markers technologies have been applied to different potato genetic materials and compared to evaluate the most efficient method of potato germplasm identification and the evaluation of genetic variation (Milbourne *et al.*, 1996; McGregor *et al.*, 2000; Braun and Wenzel, 2005). Simple Sequence Repeat (SSR) markers are highly polymorphic, abundant in the non-coding regions of the genome, co-dominant, and many have been placed on genetic maps. The use of the polymerase chain reaction (PCR) and fragment length detection with fluorescent labels makes the use of SSR genotyping simple, fast and robust, and the obtained data comparable and reproducible. SSR markers have been used to successfully characterize potato germplasm collections in several countries and SSR fingerprints have been proposed as one of the units in the cultivar certification process (Moisan-Thiery *et al.*, 2005; Reid and Kerr, 2007). Genetic fingerprints can be used for plant cultivar registration, the protection of the plant breeder's rights and for tests of purity for plant producers.

The evaluation of genetic distances for the tetraploid potato is complex due to the inability to interpret the dosage of alleles based on microsatellite markers data (Provan *et al.*, 1996; Milbourne *et al.*, 1996; Braun and Wenzel, 2005). However, the high and stable heterozygosity of the potato cultivars that results from vegetative reproduction by tubers makes them highly discernible and suitable for genetic fingerprinting using SSRs. Recent studies demonstrate that five or six SSR markers pairs were sufficient to distinguish a large amount of potato cultivars (Moisan-Thiery *et al.*, 2005; Reid and Kerr, 2007; Ghislain *et al.*, 2000; Provan *et al.*, 1996; McGregor *et al.*, 2000; Coombs *et al.*, 2004). Pedigree information about potato cultivars is often scanty, and the use of the hybrids of local cultivars as parents is common in the Latvian potato breeding program. Therefore the second important task of using DNA markers is to help investigate the degree of relatedness in the potato germplasm and to improve parental line selection for breeding programs. In the study of Braun and Wenzel (2005), 26 SSR markers identified 37 German cultivars and 10 advanced clones with known pedigree including sibs and half sibs, but no clear relationships were proven in the case of several clones. Nevertheless, most relationships were confirmed using the pooled binary data of allele presence/absence and the inability to interpret the dosage of allele was considered to be minor as the alleles might be in balance between genotypes. Several methods have been suggested for evaluating the allele dosage in polyploid cultivars. For example, measuring the signal strength for each allele peak obtained (Provan *et al.*, 1996; Esselink *et al.*, 2004; Nybom *et al.*, 2004 – by Moisan-Thiery *et al.*, 2005), or using a computational algorithm for estimating the allele frequencies under certain pattern of inheritance (De Silva *et al.*, 2005). However, these methods are still under development and have not been widely applied. SSR marker fingerprints are not publicly available, therefore it is impossible to compare our data with other studies and genebanks. In order to be able to directly compare genetic diversity and the relationships between potato collections, and also for breeder rights protection, it would be useful to determine a common SSR fingerprinting marker set and to standardize this fingerprinting method.

The aims of this study were to evaluate the discriminatory power of SSR markers within Latvian potato genetic resources, fingerprint all potato cultivars listed in the Latvian plant genetic resource database and to characterize the amount and partitioning of genetic diversity within this collection.

## Materials and Methods

61 potato cultivars were obtained from the State Priekuli Plant Breeding Institute collection. This included 45 Latvian bred cultivars and 14 locally adapted cultivars with an unknown pedigree ("local cultivars") and two foreign cultivars "Felicitas" (Germany) and "Kuras" (Netherlands) (<http://tor.ngb.se/sesto/> - accessed on 18.03.2008). Genomic DNA was extracted from fresh leaves or sprout material with the Genomic DNA Purification Kit K0512 (Fermentas). To verify the results, DNA extraction was repeated for cultivars with identical genetic fingerprints.

Fourteen SSR markers were selected from markers previously developed by Milbourne *et al.* (1998) and one marker "STPATP1" by Provan *et al.* (1996). Previous studies found that five or six SSR marker pairs were sufficient to distinguish a large amount of potato cultivars (Moisan-Thiery *et al.*, 2005; Reid and Kerr, 2007). Five of these markers were present also in our study ("STM2005", "STM1097", "Lemalx", "STM1024" and "STM2020"). Initially, the 15 marker set was tested on ten Latvian potato cultivars and based on the criteria of good amplification quality, the number of alleles and the chromosomal location eight markers were selected for the final

fingerprinting of the remaining cultivars (Table 1). The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow for the visualisation of the amplified products. SSR locus amplification was carried out using the following PCR conditions: 95 °C for 3 min, 35 cycles of 95 °C for 20 sec, 50 °C – 20 sec, 72 °C – 20 sec; 72 °C - 10 min; in a total volume of reaction 20 µl containing 50 ng template DNA, 1x PCR buffer, 2 mM MgCl<sub>2</sub>, 0,2 mM dNTP mix, 0,5 U *Taq* polymerase (*Fermentas*), 0,5 mM of forward (labeled) and reverse primers (*Applied Biosystems*). Amplification fragments were separated on an ABI Prism 3130xl Avant Genetic Analyzer (*Applied Biosystems*) and analysed with the GeneMapper 3.5. The diversity index (DI) was calculated using the formula “ $DI=1-\sum p_g^2$ ”, where “ $p_g$ ” is the frequency of an individual genotype. Phylogenetic analyses were conducted using MEGA 4 (Tamura *et al.*, 2007) on the combined binary allelic data of all markers. Population analyses were performed with the GenAlEx 6 version (Peakall and Smouse, 2006). Information on the pedigree of potato cultivars was supplied by the State Priekuli Plant Breeding Institute (SPPBI) or obtained from the online potato pedigree database resource (Berloo *et al.*, 2007).

### Results and Discussion

A total of 63 SSR alleles were observed within the cultivars analyzed, with a maximum of 12 alleles generated by the marker “STM1004” (Table 1). Altogether 168 unique genotypes were generated; from which 15 genotypes were monoallelic, 53 – diallelic, 66- triallelic and 34 – quadriallelic. The marker “STM1021” generated the most quadriallelic genotypes. The remaining markers detected more two or three- allelic variants (average frequency (fq)=0,36), but fully monoallelic or quadriallelic genotypes were rare (average fq=0,14).

The obtained average marker Diversity Index (DI) in Latvian potato cultivars (0,82) is comparable with that estimated for potato cultivars by Milbourne *et al.*, 1996 (0,73), Moisan-Thierry *et al.*, 2005 (0,84); Braun and Wenzel, 2005 (0,98). The highest discriminative power was observed for markers the “STM1021”, “STM1004” and “STM0037” (Table 1).

Table 1. Information on microsatellite markers used for potato cultivar fingerprinting.

Marker	Repeat	Product size, bp	Location	DI	Number of alleles	Number of genotypes
STM1021	(C) <sub>17</sub> ...(CT) <sub>8</sub> (AT) <sub>9</sub>	161 - 213	IX	0.93	12	32
STM0037	(TC) <sub>5</sub> (AC) <sub>6</sub> AA...(AC) <sub>7</sub> (AT) <sub>4</sub>	64 - 82	XI	0.94	8	26
STM2020	(TAA) <sub>6</sub>	140 - 158	I	0.90	7	19
Lemalx	(ATT) <sub>6</sub>	119 - 146	V	0.73	5	10
STM1049	(ATA) <sub>6</sub>	177 - 198	I	0.69	4	9
STM1004	(AAG) <sub>7</sub>	151 - 259	VII	0.96	12	37
STM0007	(AC) <sub>9</sub>	187 - 237	XII	0.84	10	20
STM3016	(GA) <sub>27</sub>	97- 109	IV	0.87	5	15

We clearly distinguished 55 cultivars, but two pairs of cultivars showed completely identical fingerprint patterns. All the procedures, including sample acquirement, DNA extraction and fingerprinting were repeated. However, the same fingerprints were obtained for the cultivars “Laima” and “Priekulu Baltie”; and for cultivars the “Spidola” and “SPO-11”. In both cases the identical cultivars have one maintainer. Cultivars “Laima” and “Priekulu Baltie” belong to the old generation of Latvian potato cultivars and have a common ancestor – “Irish Cobbler” (Figure 1).

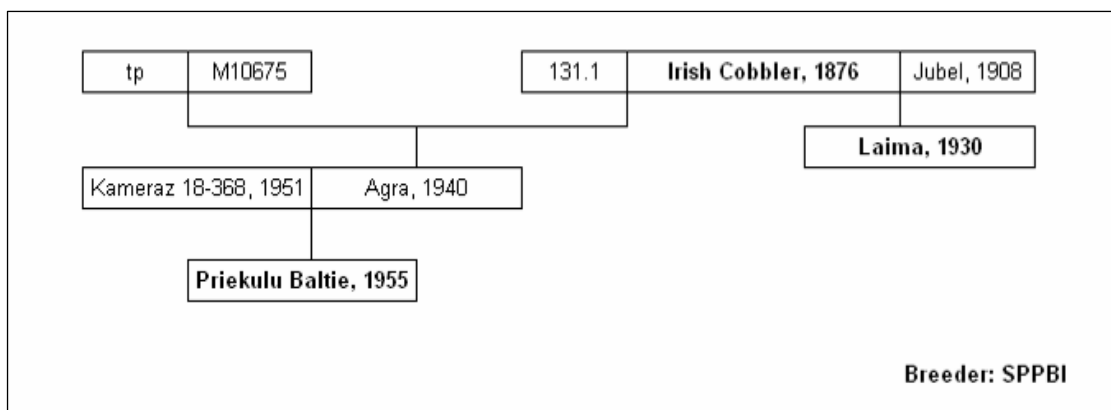


Figure 1. Pedigree of the potato cv. ‘Priekulu Baltie’ and ‘Laima’

The other two cultivars – “Spidola” and “SPO-11”- have an unknown parentage. These cultivars were developed by the breeder Alberts Saulitis; and it is possible that these two cultivars are irradiation induced somaclonal variants, where the cultivars have different phenotypic traits, but little genetic changes. In these cases SSR markers may not distinguish the two variants as in the study of Reid and Kerr (2007). Several reasons could be suggested for this result, such as the low genetic variability of the material, errors in plant reproduction or in marker selection. Careful comparison of the cultivars’ phenotypic traits will be undertaken by the maintainer (SPPBI).

Phylogenetic analyses were performed with the binary data of eight SSR markers scored as presence/absence of allele. The data sets were analyzed with the UPGMA (Unweighted Pair Group Method with Arithmetic mean) - Sneath and Sokal, 1973 – by Tamura *et al.*, 2007) combined with the bootstrapping analysis (Felsenstein, 1985 – by Tamura *et al.*, 2007), the Maximum Parsimony method that is based on the minimal information principle with consensus tree uilding and the bootstrapping test and the Neighbour-Joining (NJ) method.

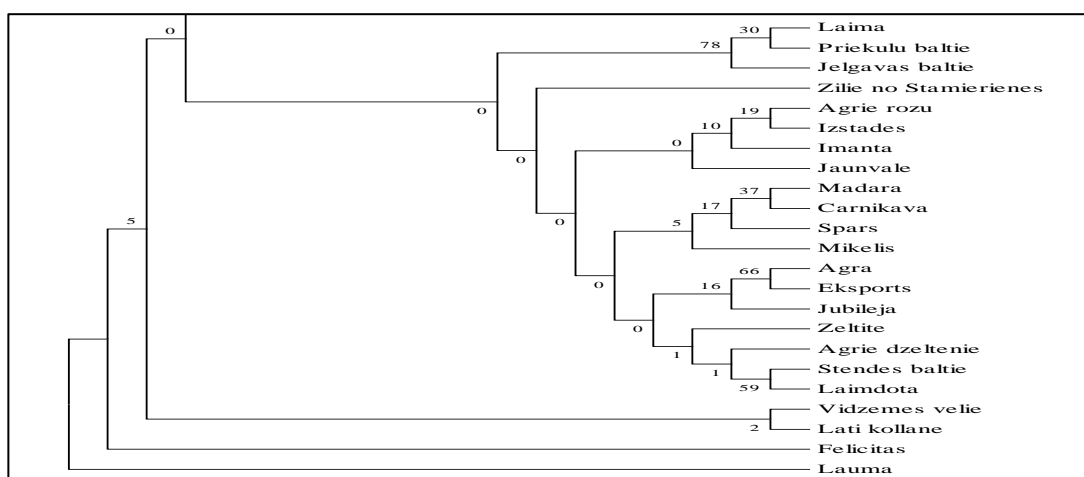


Figure 2. Cluster of old and locally cultivated potato cultivars divided in Maximum Parsimony analysis with bootstrapping based on allele presence/absence

UPGMA phylogenetic clustering was not robust as demonstrated by the generally low bootstrap inference values (data not shown). However, some of the cultivars clustered together with high inference values in likelihood, parsimony and NJ analyses: “Stendes baltie” clustered with “Laimdota”, “Jelgavas baltie” with “Priekulu baltie” and “Laima”; “Agra” with “Eksports”; “Mutagenagrie” with “Bertas”, “Astra” with “Sniegoga”; “Vita” and “KPAX-1”..

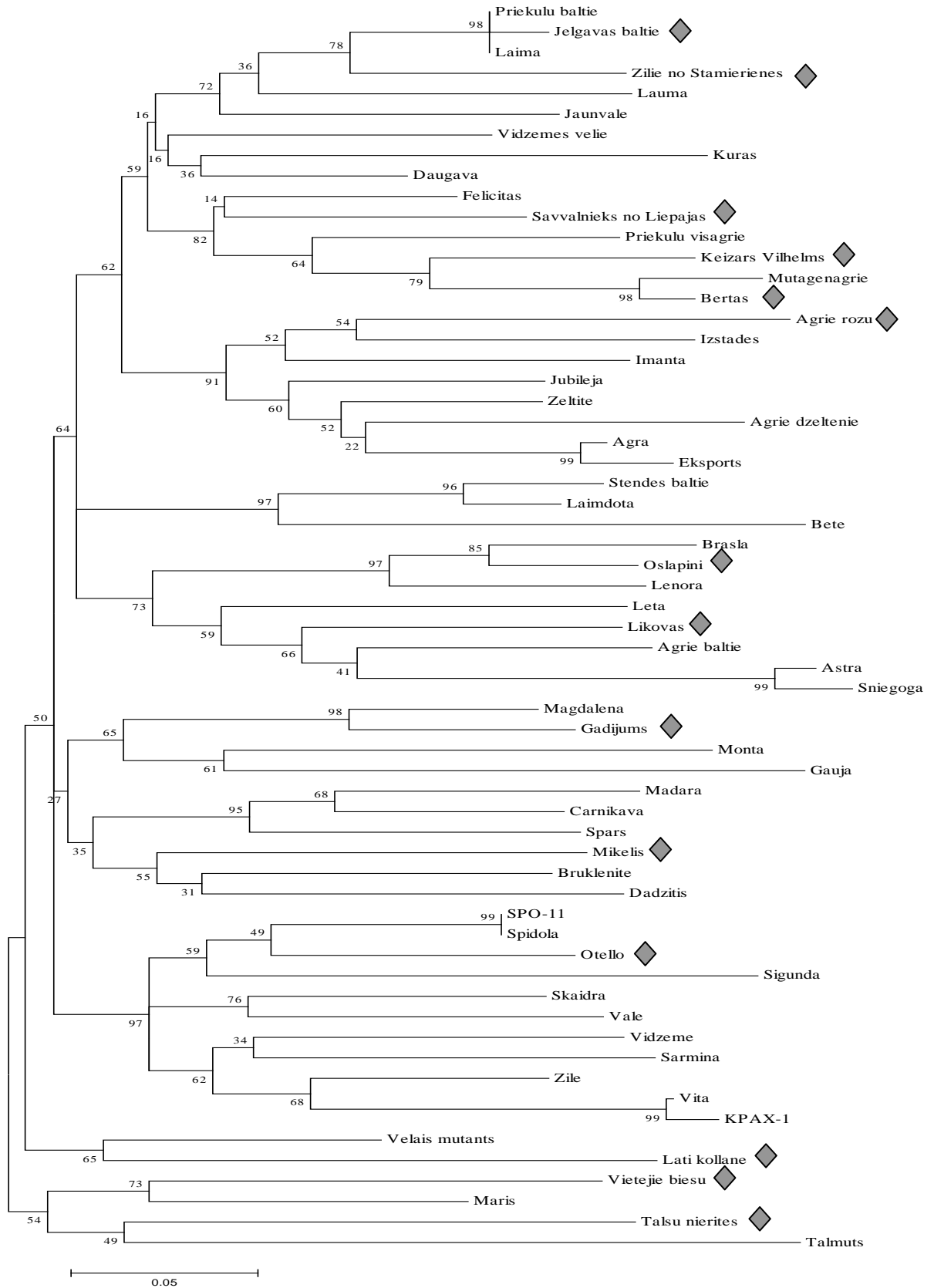


Figure 3. Genetic relationships of the potato cultivars studied. The phylogenetic tree is constructed based on the computation of allele presence/absence using Neighbour-Joining cluster analysis followed with the Interior-Branch test. Local cultivars are marked

We noticed that Maximum Parsimony divided cultivars bred in the beginning the 20<sup>th</sup> century and some of the local cultivars formed into a separate group (Figure 2) with a few exceptions such as the cultivars “Imanta” (2008) and “Madara” (1984). The German cultivar “Felicita” and the Latvian cultivar “Lauma” clustered separately from other cultivars. NJ phylogenetic tree branching shows higher confidence values using the Interior-Branch test (Figure 3).

As information on the parentage of the cultivated potato is scarce and hybrid lines occur in the parentage of many cultivars, in most cases we were unable to compare the constructed phylogenetic trees with cultivar pedigree information

Clustering analysis performed with the same data pools using different methods (UPGMA, Maximum Parsimony, NJ) demonstrate similarity of clustering in terminal branches and some groups of cultivars. Often, each cluster contains a local cultivar along with the bred cultivars, which could reflect the origin of the breeding material. However because the pedigree records of local cultivars are not available, this can not be confirmed. The suggestion that the allele dosage estimation is not required (Braun and Wenzel, 2005) was partly supported by our data as many stable clusters of particular cultivars are present in all phylogenetic trees obtained. Maximum Parsimony separates potato cultivars with higher bootstrap inference values than UPGMA and groups older cultivars into one cluster (Figure 2). NJ phylogenetic trees show higher confidence values, therefore these were used for more detailed comparisons. Still, differences between analytic methods are present and combined with the lack of pedigree information about cultivars; results in the situation that definitive conclusions about of phylogenetic relationships are difficult. To better elucidate the genetic relationships between Latvian potato cultivars, additional genotype data is required. To explore, changes in the genetic diversity of Latvian potato germplasm over time, the data was divided into three populations: old cultivars, which were released before 1978; modern cultivars, which were bred in 1978 or later; and local cultivars, which have been continuously cultivated in different regions of Latvia and for which there is no pedigree information.

The foreign cultivars “Felicita” and “Kuras” were excluded from this analysis. Cultivars with an unknown year of release (bred by A.Saulitis), were classified as modern cultivars.

The number of alleles in each population was 54 (modern), 52 (local) and 48 (old). New cultivars have six unique alleles, local cultivars – five unique alleles, but old cultivars only two unique alleles. In modern cultivars private alleles were generated by the markers: “STM1021” (213 bp), “STM0037” (66 bp), “STM1004” (154 bp), “STM0007” (215 bp, 235 bp), “STM3016” (97 bp). The most frequent of these were 154 bp (0,154) and 97 bp alleles (0,115). In local cultivars private alleles were found with the same frequency (0,071) and were generated with the markers “STM0037” (64 bp), “STM1049” (198 bp), “STM1004” (187 bp, 190 bp, 226 bp). In old cultivars private alleles were generated by the markers “STM2020” (140 bp, fq=0,167) and “STM0007” (237 bp, fq=0,053). The percentage of polymorphic loci in old cultivars is lower (74,6%) than in new cultivars (85,7 %), and local cultivars (82,5 %). Nei's Genetic Distance between new and local cultivars was 0,030; between new and old cultivars 0,061, and between local cultivars and old cultivars - 0,027. AMOVA (Analysis of Molecular Variance) analysis found 6 % ( $p < 0,001$ ) polymorphism among these populations. This data shows a higher level of genetic diversity in the modern cultivars.

The distribution of alleles with a frequency higher than 0,34 in each population was compared. 16 alleles were common to all the populations. The most distinct high frequency alleles were found in the modern cultivars (163, 70, 191 and 82); in old cultivars only two such alleles were found (125 and 109), but all alleles found in local cultivars were shared either with modern or old cultivars. A high similarity of microsatellite alleles between all Latvian potato cultivars was found. Local cultivars contain common alleles with both old cultivars and modern cultivars and are more polymorphic than the old cultivars. That may reflect a tendency to use local cultivars as material for breeding with the aim of increasing genetic diversity, while maintaining local adaptive traits. Some new alleles were introduced into the modern potato cultivars bred after 1978. This increase of genetic diversity could be a result of the use of new gene pools in modern potato breeding. The potato breeding program in Latvia was expanded with the development of the infrastructure after 1973 and the ability to acquire germplasm with new meristematic methods after 1978 (Bebre, 2003).



## Conclusions

All 59 potato cultivars listed in the Latvian genetic resources database were fingerprinted and discriminated from each other with eight SSR markers, with the exception of two pairs of cultivars. If these non-discriminated cultivars are excluded, all remaining potato cultivars could be discriminated using a minimum of four SSR markers: STM1021, STM1004, STM3016 or STM0037 (to the separate cultivars “Lenora” and “Oslapini”), and STM1049 (to the separate cultivars “Vita” and “KPAX-1”). The obtained fingerprints will be routinely used in the future for potato cultivar identification for various purposes.

There are no distinct groupings or divisions within Latvian potato cultivars, however the genetic diversity of potato cultivars bred after the 1978 has increased. These modern cultivars contain newly introduced unique alleles as well as alleles from local cultivars and from old cultivars. This reflects the combination of older, locally adapted material with the increased genetic diversity of modern European potato cultivars. For a more detailed investigation into the phylogeny and genetic relationships of Latvian potato cultivars additional SSR or different DNA marker data is required.

## Acknowledgements:

The Genetic Resource Centre and the Latvian Gene Bank are financed by the Latvian Ministry of Agriculture.

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## LATVIJAS KARTUPEĻU ĢENĒTISKO RESURSU RAKSTUROJUMS AR DNS MOLEKULĀRO MARĶIERU PASPORTIZĀCIJAS METODI

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Molekulārus marķierus, tai skaitā vienkāršus DNS sekvences atkārtojumus (SSR, no angl. val. - Simple Sequence Repeat), plaši izmanto dažāda augu materiāla identifikācijai, ģenētiskās daudzveidības noskaidrošanai, gēnu banku kolekciju uzturēšanai, augu selekcijas programmās elitāro alēļu skrīningam un materiāla apmaiņas kontrolēšanai, kā arī ploīditātes pakāpes paredzēšanai, ģenētisko karšu konstruēšanai, evolucionāros un populāciju pētījumos. SSR marķieru augsts polimorfisma līmenis ļauj atšķirt pat tuvi radniecīgas šķirnes.

Kartupeļu (*Solanum tuberosum* L. subsp. *tuberosum*) šķirnes (59) reģistrētās Latvijas Augu Ģenētisko Resursu katalogā tika izpētītas ar SSR molekulāriem marķieriem. Tika identificēti astoņi īpaši polimorfī marķieri no 15 marķieru grupas un tie izmantoti visas kartupeļu šķirņu kolekcijas raksturošanai. Tika veikta katras šķirnes raksturojošo molekulāro nospiedumu noteikšana (molekulārā pasportizācija), ģenētisko distanču aprēķināšana un filoģenētiskās analīzes. Divi šķirņu pāri tika identificēti vienādi pēc visiem lokusiem, pārējās šķirnes var atšķirt ar četrus marķieru molekulāriem nospiedumiem. Tika analizēta līdzīgu šķirņu klasteru veidošanās, izmantojot dažādus datu apvienošanas principus un analīzes metodes. Ģenētiskās daudzveidības palielināšanās atrasta nesen selekcionēto kartupeļu šķirņu grupā, salīdzinot tās ar pagājušā gadsimta sākumā veidotajām šķirnēm. Efektīvo molekulāro marķieru metožu izmantošana Latvijas šķirņu identifikācijai un radniecības pakāpes noskaidrošanai uzlabos turpmāku kartupeļu šķirņu kolekciju uzturēšanu, izplatīšanas kontroli, kā arī selekcijas materiāla atlasī.

## POSSIBILITIES TO GROW OATS FOR FOOD IN LATVIA

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### Abstract

In order to improve the nutrient content of oat food products, there is a need for good quality grains. In our study, 15 oat varieties grown in a field trial (2005-2007) at the State Stende Cereals breeding Institute and 78 oat samples collected from different farms in Latvia were analysed for volume weight, 1000 grain weight, husk content, crude protein and fat. The grain processing enterprise *Rīgas Dzirnāvnīks* Ltd has determined the criteria of oats for food. The grains have to have a volume weight higher than 480 g l<sup>-1</sup> and husk content below 250 g kg<sup>-1</sup>. Results of the experimental trial showed that these demands are accessible. The grain volume weight ranged from 480 to 518 g l<sup>-1</sup>, 1000 grain weight from 33.7 to 38.4 g, the husk amount from 206 to 312 g kg<sup>-1</sup>. However, only four of 15 analysed oat varieties met the standards requested for food quality. The analyses of oat samples collected from farms showed generally lower grain quality than the analysed grain samples from experimental fields. Thus, to obtain oat quality under farming conditions is more difficult than under experimental conditions.

**Key words:** oats, grain quality, volume weight, husk amount

## Introduction

Grain products are among healthy foodstuffs needed for humans. Oats, due to their particular biochemical composition, are considered ideally suited for the production of dietary products. Oat grains are characterized by a balanced composition of essential amino acids, high unsaturated fat content, the presence of  $\beta$ -glucane, vitamins and antioxidants (Beķeris *et.al.*, 2001). The production of high quality foodstuffs is possible due to high quality raw material – grain. In oats, not only the biochemical but also the economic indices of grain, such as volume weight, TKW and husk content are important as the quality of oat products obtained from each ton of purchased grain is dependent on them (Richards, 1990). The optimal values of these indices are fixed by each grain processing enterprise. Thus, e.g., in Germany grain processing enterprises have declared the following requirements for oat grain: volume weight  $>530 \text{ g l}^{-1}$ , 1000 grain weight  $>27 \text{ g}$ , husk content  $<26\%$  (Grasmann *et.al.*, 1995). In Latvia as well the oat grain processors have minimal requirements regarding purchased grain quality indices. They are: volume weight  $>480 \text{ g l}^{-1}$  and husk content  $<25\%$  (<http://www.rigas-dzirnavnieks.lv>). The results of different studies have confirmed that yield and quality indices in grain vary with the season, location and varieties (Meyer *et al.*, 1996.) Obtaining higher grain yields per hectare is in the farmers' interests. To reach the goal, improved oat varieties and optimal growing technologies are searched for in each region. Research results show that correlative relationships between the productivity of crop varieties and grain quality indices are low as estimated by grain processors (Meyer *et al.*, 1996; Zute *et al.*, 2002). Oat varieties which are plastic in changing climatic conditions and capable of ensuring both stable yields and corresponding grain quality from year to year are most popular among oat breeders. In 2005, researchers from the State Stende Cereals Breeding Institute in collaboration with the grain processing enterprise *Rigas Dzirnāvnieks* Ltd started a joint research project to clarify which oat varieties should be recommended for growing in Latvia and which technological elements ought to be applied in oat cultivation to satisfy both the farmers and the processors of oats.

The information presented in the paper was obtained in the following way: firstly, analysing the quality indices of the oat varieties registered in the Latvian Plant Variety List and, secondly, inspecting oat production farms and estimating the quality of the produced grain.

## Materials and Methods

The results obtained in oat variety trials were analysed. These trials were conducted in selection plant rotation of the State Stende Cereals Breeding Institute in 2005, 2006 and 2007. The following 15 oat varieties were evaluated: the Latvian oat varieties 'Laima', 'Stmara', 'Stendes Liva', 'Arta' and 'Stendes Dartā', the Swedish oat varieties 'Freja', 'Belinda', 'Selma', 'Margaret', the German 'Vendela', 'Hecht', 'Jumbo' and 'Aragon', and the Russian oat variety 'Kirovec'. Variants – test varieties were laid out in four replications according to the standard method. 'Laima' (most widely cultivated in the production sowings in Latvia) was the standard variety. Trial plots were established as follows: standard variety plot after five test varieties' plots. The recorded plot area was  $10 \text{ m}^2$ .

Growing conditions: sod-podzolic soils (WRB – Eutric Podzovisols), well cultivated moraine loamy sand soil with  $\text{pH}_{\text{KCl}}$  of 5.4 – 6.6 in arable layer, and 16 – 25  $\text{g kg}^{-1}$  organic matter, previous crop – potatoes.

Fertilization was carried out before sowing. The mineral fertilizer *Kemira NPK 18:9:9* was incorporated into the soil; nitrogen active ingredients  $60 \text{ kg ha}^{-1} \text{ N}$  were applied. Weed control was done by harrowing twice (prior to the seed germination stage and at the tillering stage of plant development) and spraying with herbicide *granstar* –  $12 \text{ g ha}^{-1}$ . Sowing was performed in optimal conditions. Oats were harvested using the grain harvester Sampo – 130. Phenological observations were done on the field. On plots the harvested grain yield was weighted and expressed at 14% moisture. After harvesting the oat grain was dried and cleaned using the cleaning machine *Mini Petkus* (size of the sieve mesh:  $12.0 \times 1.9 \text{ mm}$ ). During the winter period, at the laboratory of Grain technology and agro-chemistry of the Institute the economic value and technological properties of the grain were analysed: husk content ( $\text{g kg}^{-1}$ ), volume weight ( $\text{g l}^{-1}$ ), 1000 grain weight ( $\text{g}$ ), crude protein ( $\text{g kg}^{-1}$ ), crude fat ( $\text{g kg}^{-1}$ ) and starch content ( $\text{g kg}^{-1}$ ).

In 2007, 78 samples of oat grain were collected from crop production farms. These crop production farms were chosen from different regions of Latvia. Oat breeders presented information regarding

the main indices characterizing crop management: the name of the oat variety, the timing of sowing and harvesting, the previous crop, applied fertilizer rates and plant protection measures, etc. Grain was sampled after harvesting and free from first treatment transported to the Institute. At the laboratory of Grain technology and agro-chemistry of the Institute the economic value and technological properties of the grain were analysed.

*Ms Excel* ANOVA and correlation analysis was used for data processing.

### Results and Discussion

Oat variety trial seeds of the oat varieties developed in Latvia and registered in the Latvian Plant Variety List as well as promising lines offered by oat breeders from other countries were seeded. The goal of this trial was to observe the stability of the productivity level and oat grain quality indices in test varieties from year to year. The test varieties significantly differ in their productivity level, the biochemical composition of the grain and the value of their agronomic traits. In Latvia, the agro-climatic conditions in the vegetation period of plants significantly differ from year to year. This is demonstrated by productivity fluctuations in oat varieties over a period of years. In this trial from 2005 till 2007, when applied crop management measures provided maximum and equal growing conditions there resulted a productivity variation in oat varieties ranging from 2.88 ('Vendela') to 17.38% ('Kirovec') (Table 1). A medium-high coefficient of variation (> 10%) was shown by the oat varieties 'Arta', 'Freja', 'Jumbo' and 'Aragon'. Compared to the standard variety 'Laima', all these oat varieties matured by 3 to 5 days earlier. Oat breeders' long-term observations indicate that oat varieties having a shorter vegetation period are more sensitive to different stress factors during the growing period than late-maturing oat varieties.

In variety testing, higher 3-year average yields of oat grain were obtained from the varieties 'Margaret', 'Belinda', 'Laima' and 'Stendes Darta'. The average productivity of all these test varieties exceeded 6 t ha<sup>-1</sup>. This corresponds to the yield level showed by the standard variety 'Laima'. The lowest grain yields were recorded for the oat varieties 'Arta', 'Stendes Liva' and 'Kirovec', respectively 3.84, 4.57, and 5.35 t ha<sup>-1</sup>. Such results are correspond to the yield potential of these oat varieties. When estimating the average productivity level reached in each trial year, it was observed that the lowest oat grain yield was attained in 2007 – on the average 5.24 t ha<sup>-1</sup> or respectively by 0.71 and 0.59 t ha<sup>-1</sup> lower than in the years 2005 and 2006 (Figure 1).

Table 1. Grain yield of oat in variety trials, 2005 – 2007

Varieties	Grain yield, t ha <sup>-1</sup> , average for 2005-2007			CV* between years
	Min-max	Mean	+/- Laima	
Laima	5.86 – 6.56	6.27	0.00	5.82
Arta	3.38 – 4.16	3.84	-2.43	10.60
Stendes Līva	4.46 – 4.72	4.57	-1.70	2,91
Stmara	5.48 – 5.91	5.62	-0.65	4,41
Stendes Dārta	5.71 – 6.72	6.08	-0.15	8.71
Kirovec	4.33 – 6.15	5.35	-0.92	17.38
Freja	5.18 – 6.16	5.87	-0.40	10,17
Belinda	5.88 – 6.28	6.27	0.00	6.07
Selma	5.22 – 5.8	5.52	-0.75	5,26
Hecht	5.30 – 6.15	5.87	-0.40	8.46
Margaret	5.71 – 6.66	6.29	0.02	8,12
Vendela	5.76 – 6.07	5.88	-0.39	2.83
Triton	5.15 – 6.08	5.71	-0.56	6.31
Jumbo	5.08 – 6.32	5.69	-0.58	10,91
Aragon	5.52 – 6.75	6.04	-0.23	10,51
LSD <sub>0,05</sub>		0.222		

\*CV – coefficient of variation

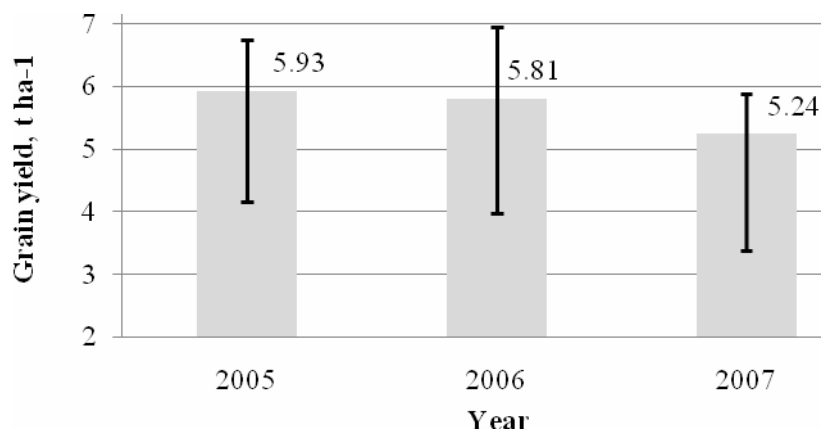


Figure 1. Grain yield of oat, min, max and average in the trial, t ha<sup>-1</sup>, 2005-2007

In 2007 warm and dry weather conditions in the last part of April – at the tillering stage of oats – were favourable for frit fly (*Oscinella frit*) infestation. This insect caused serious damage to 40 - 75% of the oat plants. Though sufficient moisture in the subsequent period of vegetation contributed to the tillering and regrowth of oat plants, nevertheless this stress resulted in smaller grains, lower volume weight, and a higher proportion of husk in the yield of grain.

In the trial, the 3-year average volume weight of the oat grain corresponded to the minimal requirements of the grain processors – 480 g l<sup>-1</sup> (Table 2). The grain processors prefer to purchase grain of a high volume weight. The trial results confirmed that the oat grain having the highest volume weight (on the average > 510 g l<sup>-1</sup>) could be obtained from the test varieties ‘Aragon’, ‘Triton’, ‘Arta’ and ‘Kirovec’. However, it should be noted that the yield characterizing quality indices varied from year to year. Though for the volume weight from year to year the coefficient of variation was low – from 2.4 (‘Freja’) up to 7.3 (‘Stendes Līva’), in 2007 the grain volume weight of six oat test varieties was below 480 g l<sup>-1</sup>. They included the oat varieties ‘Margaret’ and ‘Belinda’ which were most productive that year.

Husk content in grain yield is also a trait the variation coefficient which was comparatively low from year to year. For oat varieties included in the trial it ranged from 1.1 (‘Jumbo’) up to 7.4% (‘Stmara’). The value of this trait was largely due to variety genotypes. In the trial, during all the test years, the oat varieties ‘Arta’, ‘Freja’, ‘Margaret’, ‘Vendela’ and ‘Jumbo’ were characterized by the lowest husk content in the grain yield, i.e. < 250 g kg<sup>-1</sup> or 25% from grain the yield.

In Latvia, the 1000 grain weight is not estimated when purchasing food grain, however a higher product outcome is obtained with coarse-grained oats. Research results show that frequently oat varieties characterized by the highest grain volume weight had a medium- high 1000 grain weight (e.g., ‘Arta’, ‘Kirovec’, ‘Laima’, ‘Stendes Dārta’), but for the most coarse-grained oat variety ‘Hecht’ the 1000 grain weight was 41.31g on a 3-year average. A positive and significant correlation was established between the grain husk content and the 1000 grain weight (correlation coefficients respectively  $r=0.594$ ,  $0.539$  and  $0.584$ ,  $r_{crit}=0.486$ ) for all oat varieties included in the trial in the years 2005, 2007 and on the average for all the three trial years. In this trial significant correlative coherence was not established between grain volume weight and 1000 grain weight, grain volume weight and husk content in yield as well as between the above mentioned quality parameters and grain yield. It shows that indices of traits were more dependent on the peculiarities of genotype of each variety and not on mutual trends in the trial. Out of all test varieties included in this trial, ‘Arta’, ‘Vendela’, ‘Freja’ and ‘Jumbo’ were most suitable to meet the requirements of grain processors. In these oat varieties comparatively low husk content and high grain volume weight were concurrently found. In the most productive oat varieties ‘Laima’ and ‘Aragon’ the 3-year average husk content somewhat exceeded the minimum defined by grain processors.

Table 2. Testing results of oat grain quality, 2005 – 2007

Oat varieties	Volume weight, g l <sup>-1</sup> ± sd		1000 grain weight, g ± sd		Husk, g kg <sup>-1</sup> ± sd	
	Average	2007	Average	2007	Average	2007
	2005 - 2007		2005 - 2007		2005 - 2007	
Laima	503.7 ± 1.56	487.0	35.41 ± 2.56	38.32	253.7 ± 7.2	262.0
Arta	513.3 ± 2.06	490.5	35.10 ± 0.59	35.77	211.5 ± 12.6	221.0
Stendes Līva	489.0 ± 3.61	450.0	36.78 ± 1.36	38.18	246.7 ± 10.7	259.0
Stmara	492.0 ± 1.91	47.20	35.37 ± 1.49	36.72	263.6 ± 20.2	254.0
Stendes Dārta	506.7 ± 1.21	484.0	34.91 ± 2.67	37.29	261.50 ± 16.9	257.0
Kirovec	511.0 ± 1.87	494.0	36.05 ± 1.37	37.24	251.4 ± 11.1	261.0
Freja	504.0 ± 1.22	498.0	35.95 ± 2.76	39.00	243.8 ± 9.4	240.0
Belinda	486.3 ± 2.31	463.0	38.93 ± 1.64	40.46	270.2 ± 22.0	295.0
Selma	508.3 ± 2.08	490.0	38.08 ± 1.60	37.48	263.1 ± 10.0	273.0
Hecht	495.3 ± 1.45	481.0	41.31 ± 2.21	43.86	330.6 ± 5.7	337.0
Margaret	479.3 ± 3.24	443.0	37.34 ± 1.23	38.59	236.2 ± 9.7	247.0
Vendela	499.7 ± 2.04	477.0	37.56 ± 1.64	39.34	227.0 ± 9.8	230.0
Triton	516.3 ± 1.91	509.0	39.49 ± 3.51	43.53	255.4 ± 11.2	259.0
Jumbo	496.0 ± 3.21	475.0	38.88 ± 1.66	38.81	247.2 ± 2.9	244.0
Aragon	518.0 ± 2.15	500.0	38.69 ± 3.08	42.10	269.0 ± 9.0	269.0
Average	-	480.8	-	39.11	-	259.3

In the inspected grain production farms the total area sown to oat occupied 1934 hectares out of which 79 samples of grain were collected. The greatest number of grain samples was obtained for the oat variety 'Laima' (48 samples or 60.7%), 'Kirovec' and 'Vendela' (6 samples for each variety), 'Selma' (5 samples), and 'Freja' (4 samples). The rest of the varieties, such as 'Līva', 'Aragon', 'Ivory', and 'Cval' were represented by one or two samples of grain. The quality indices of the cultivated oat grain varied widely. This was seen best when analysing grain samples of one oat variety – that of 'Laima' (Table 3). Overall the grain volume weight in 90% of the oat grain samples was above 480 g l<sup>-1</sup>. However, it should be observed that this parameter was determined after fine grain separation. The outcome of grain ware production (above 1.9 mm sieve) varied from 43.8 to 90.3%. Grain husk content below 250 g kg<sup>-1</sup> was determined only in 36 samples of grain or 46% of the samples. As for 1000 grain weight, great differences were also observed between grain samples, i.e. from 31.1 to 43.7 g. The results of the trial lead to the conclusion that growing conditions (meteorological, soil, etc.) and crop cultivation technology applied in the farms had played a decisive role in the assurance of grain quality indices.

Table 3. Quality characteristics of oat grain samples collected in farms, 2007

Varieties	Number of samples	Values	Volume weight, g l <sup>-1</sup>	Husk, g kg <sup>-1</sup>	1000 grain weight, g
Laima	48	Mean	518.8	252.5	36.71
		Min-max	441 - 566	231 - 307	31.09 – 40.40
Kirovec	6	Mean	518.7	255.2	37.47
		Min-max	490 - 551	234 - 272	35.16 – 39.39
Vendela	6	Mean	513.0	225.0	40.83
		Min-max	477 - 534	208 – 259	38.76 – 43.74
Selma	5	Mean	518.6	252.0	37.45
		Min-max	499 - 543	241 - 270	34.67 – 39.98
On average in samples	79	Mean	517.6	249.3	37.51
		Min-max	441 - 566	208 - 307	31.09 – 43.74

Estimating the mutual interaction of traits, the correlative relationship was studied choosing the results of the analysis of the oat variety 'Laima' because it had the greatest number of collected grain samples – 48. First of all, the correlative relationship was found to be between the grain yield of oats and economically significant indices: volume weight, husk content and 1000 grain weight.

Calculations showed that in 2007, in the production sowings of the oat variety 'Laima', significant and positive correlation was established between the grain yield and volume weight, but significant and negative correlation was established between the total grain yield and the husk content, respectively  $r = 0.385$  and  $r = -0.317$ , if  $n = 48$  and  $r_{0.05} = 0.292$ . Each of these traits affected the productivity indices of the oat variety respectively by 15 and 10% ( $R^2 = 0.149$  and  $R^2 = 0.101$ ). Though the obtained correlation coefficient was medium-low, yet it showed a tendency, that providing higher productivity levels, improvement in grain quality indices – husk content and volume weight would be possible thus meeting the requirements of the grain processors. When delivering grain for analysis, oat producers furnished information regarding crop management measures in farms. Analysing the effects of sowing time, the previous crop and the total rates of the nitrogen fertilizer on the values of previously analysed indices, several significant correlative relationships were established: the later in spring sowing was performed (from the first part of April till the first part in May), the lower the yield, and with this the lower resultant grain volume weight and higher husk content. This correlative relationship is affirmed by the calculated correlation coefficients as seen in Table 4.

Table 4. The correlative relationship between agronomic traits and the components of growing technology in the oat variety 'Laima'

Correlative relationship	n	r	$r_{0.005}$	$R^2$
Grain yield : Husk amount	43	-0.317	0.301	0.100
Grain yield : Volume weight	43	0.386	0.301	0.149
Grain yield : Sowing time	41	-0.329	0.310	0.108
Grain yield : Nitrogen rate	37	0.513	0.321	0.263
Grain yield : Previous crop	39	0.333	0.315	0.111
Husk amount : Volume weight	48	- 0.321	0.284	0.103
Husk amount : 1000 grain weight	-48	-0.265	0.284	0.071
Husk amount : Sowing time	41	0.353	0.310	0.125
Volume weight : Previous crop	39	0.345	0.315	0.119
Volume weight : Sowing time	41	0.297	0.310	0.088
Volume weight : 1000 grain weight	48	-0.187	0.284	0.035
Volume weight : Nitrogen rate	37	0.299	0.321	0.089

The oat productivity level in farms was closely related to the applied nitrogen fertilizer rate ( $r = 0.513$ ). Grouping of previous crops chosen by farms also showed that a medium-close but significant correlative relationship was established between the oat yield and the previous crop ( $r = 0.333$ ). Higher yields of grain were attained from fields with grasses, potatoes, buckwheat and rape as the previous crops, but lower yields from fields with winter wheat, rye and barley as the previous crops. An analogous correlative relationship was also established between the grain volume weight and the previous crop.

## Conclusions

The results of oat variety trials conducted in Stende from 2005 to 2007 suggest that the grain quality indices in the 'Arta', 'Freja', 'Jumbo' and 'Vendela' oat varieties most closely correspond to the requirements of the grain processors, i.e., the volume weight of oat grains in all the three test years exceeded  $480 \text{ g l}^{-1}$  and the husk content in the yield did not exceed  $250 \text{ g kg}^{-1}$ .

Stable and high grain yields during the trial years were provided by the tested oat varieties 'Laima', 'Aragon', 'Vendela' and 'Margaret', i.e., the 3-year average productivity of these oat varieties exceeded  $6 \text{ t ha}^{-1}$ .

The 3-year investigation with 15 oat varieties show, that observing unified growing technologies significant and stable correlative relationships were not established between grain volume weight, husk content and the total grain yield. It shows that the indices of the traits were more dependent on each genotypic expression in a definite year and not on common tendencies in the trial.

Analysis of the collected grain samples of the oat variety 'Laima' showed that grain higher in volume weight and lower in husk (correlation coefficients respectively  $r = 0.386$  and  $r = - 0.317$  ( $r_{0.05} = 0.301$ )) were obtained in the fields with the highest grain yield. Hence, also the measures used in crop management which had a significant effect on the grain yield of oats are valued as being important to produce grain high in volume weight and low in husk. Farm inspection in 2007

showed that these economically significant indices were affected by sowing time, the previous crop and the total nitrogen fertilizer rate.

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## IESPĒJAS IZAUDZĒT PĀRTIKAS AUZAS LATVIJĀ

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Kvalitatīvu pārtikas produktu ražošanai ir nepieciešamas labas kvalitātes izejvielas – graudi. Ražošanas procesā svarīgi ir ne tikai graudu bioķīmiskie, bet arī saimnieciskie rādītāji – ražība, tilpummasa, TKW, plēkšņainība. Pārtikas produktu ražotāji ir norādījuši, ka iepērkot graudu minimālai tilpummasai jābūt lielākai par 480 g l<sup>-1</sup>, plēkšņainība zemāka par 250 g kg<sup>-1</sup> graudu. Sadarbībā ar A/S *Rīgas Dzirnāvnīks* Stendē veic pētījumus par pārtikas produktu ražošanai svarīgu rādītāju uzlabošanas iespējām Latvijā audzētām auzām. Pētījumā analizē Latvijas augu šķirņu katalogā reģistrēto auzu šķirņu kvalitātes rādītājus institūtā iekārtotos izmēģinājumos un novērtē ražošanas saimniecībās izaudzēto graudu kvalitāti.

No 2005. līdz 2007. gadam Stendē šķirņu salīdzinājumā vērtēja 15 Latvijā audzētas auzu šķirnes. Izmēģinājumos vidējais auzu šķirņu ražības līmenis variēja no 4.2 līdz 6.8 t ha<sup>-1</sup>, graudu tilpummasa – no 480 līdz 518 g l<sup>-1</sup>, 1000 graudu masa – no 33.7 līdz 38.4g, plēkšņainība – 206 līdz 312 g kg<sup>-1</sup>. Tikai četrām no pārbaudē iekļautajām auzu šķirnēm graudi atbilda pārtikas produktu ražošanas standartprasībām, t.i., ‘Arta’, ‘Freja’, ‘Jumbo’, ‘Vendela’.

Saimniecībās ievāktos graudu paraugus kvalitātes rādītāji bija ievērojami zemāki nekā izmēģinājumos. Graudu ražības un kvalitātes rādītāji variēja atkarībā no saimniecībā pielietotās agrotehnikas. Pēc 2007. gada apsekojuma rezultātiem korelatīvās sakarības parādīja, ka graudu raža, tilpummasa un plēkšņainība ir rādītāji, kas ir ne tikai savstarpēji saistīti, bet arī būtiski atkarīgi no sējas laika, priekšauga un slāpekļa mēslojuma normas.

## RESULTS FROM EVALUATION OF THE OAT VARIETY ‘STENDES DARTA’

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## Abstract

The new oat variety ‘Stendes Darta’ (line No. L 28650) has been developed at the State Stende Cereals Breeding Institute. The variety is registered by the Plant Protection Service of Latvia (No. A-8 since 27.12.2004) and included in a catalogue of Plant varieties both of Latvia and EU in 2005. It is recommended for food and feed production.

The oat variety is a result of hybridisation and repeated selection work. During five consecutive years (1999 – 2003), this variety gave a significantly greater yield of grain than the standard variety ‘Laima’ (+ 0.44 t ha<sup>-1</sup>,  $RS_{0.05} = 0.21$  t ha<sup>-1</sup>). On the average, line L 28650 was one to two days earlier, had higher lodging resistance (+ 2 points), and was more resistant to loose smut (on



artificial infection background), oat leaf crown rust and mildew (on natural infection background) than the standard variety 'Laima'. Oil content of the variety 'Stendes Darta' was high (on the average + 0.46% more than for the standard variety 'Laima'). The oat line showed stable yields and good competition with weeds under organic farming. Evaluation of the variety 'Stendes Darta' during test period (1999 – 2006) showed that the variety was plastic and might provide rather suitable yields under unfavourable growing conditions as in 2006.

The results of evaluation confirm that the oat variety 'Stendes Darta' may provide stable yields and good grain quality. Consequently, the variety is suitable for the growth in conventional and organic farming systems.

**Key words:** grain quality, oat variety, yield

## Introduction

The goal of the Latvian oat breeding program is to develop high-yielding oat varieties resistant to lodging and diseases with corresponding grain quality suitable to local conditions. In the last ten years Latvian oat breeders have been offered a lot of high-yielding oat varieties of the intensive type developed in European countries, and several oat varieties developed in Stende among which most popular is the oat variety 'Laima', which excels in plasticity and capacity to provide medium high yields of grain under different growing conditions. Proceeding with the selection of the most promising lines, oat breeders wished to develop varieties higher in lodging resistance and quality of grain. Partly these wishes are realized in the oat variety 'Stendes Darta'.

In the Plant Variety Testing department of the State Plant Protection Service (VAAD) the new oat variety 'Stendes Darta' was registered to test value for cultivation and use (VCU tests) in autumn 2002. The author's right to the State Stende Plant Breeding Station was confirmed by VAAD in 27 December 2004, and the oat variety 'Stendes Darta' received the registration number A-8. This oat variety has been included in a catalogue of Plant varieties of Latvia since 1.01.2005. The authors of the oat variety 'Stendes Darta' – M. Gruntiņa, S. Zute, P. Buļbiks, S. Maļeckā, J. Briedis.

## Materials and Methods

The oat variety 'Stendes Darta' was developed at the State Stende Cereals Breeding Institute (till 2006 – at the State Stende Plant Breeding Station). In 1993, under field conditions using the hybridisation method a cross combination P4175: PCU – 32 / L 24156 was obtained. PCU – 32 is an oat line of Polish origin, L 24156 is a line of Stende obtained from cross combination *Pantker // Bug / Parsival*. The oat varieties 'Pantker' and 'Parsival' are developed by German oat breeders, but the oat 'Bug' is of Byelorussian origin.

Stages of selection work in breeding the oat variety 'Stendes Darta':

1993 – hybridisation under field conditions, five hybrid seeds were obtained;

1994 – using over-sowing, hybrids of F<sub>1</sub> generation were obtained;

1995 – using over-sowing, hybrids of F<sub>2</sub> generation were obtained;

1996 – using over-sowing, hybrids of F<sub>3</sub> generation were obtained, out of them elite plants were selected;

1997 – 12 elite plants progeny were sown in the breeding first year nursery, out of them six lines were selected for further sowing;

1998 – 6 best lines were sown in the breeding second year nursery, a plot size 2 m<sup>2</sup>, in one replication, remains of the best three lines were combined giving them No. L 28650, which was sown in a previous variety testing, a plot size 10 m<sup>2</sup>, in four replications;

1999–2004 – competitive variety trial, a plot size 10 m<sup>2</sup>, in six replications;

2001–2004 – variety testing in crop rotation under organic management, a plot size 20 m<sup>2</sup>, in four replications;

2003–2004 – testing of value for cultivation and use in plant variety testing stations in Valmiera, Saldus and Višķi;

2003–2004 – distinctness, uniformity, stability test (DUS test) by UPOV method in Poland.

2005–2006 – test of value for cultivation and use under organic farming system at the State Priekulī Field Crops Breeding Institute and Study and Research Farm „Vecauce” of the Latvia University of Agriculture.

Oat breeding work was performed on crop rotation fields of the State Stende Cereals Breeding Institute. The soils are well cultivated, sod slightly podzolic loamy sand with pH<sub>KCl</sub> of 5.4–6.6 in arable layer, organic matter 1.6–2.5%, plant available P<sub>2</sub>O<sub>5</sub> 183–355 mg/kg, K<sub>2</sub>O 170–264 mg/kg, previous crop – potatoes.

Fertilization was carried out before sowing applying the mineral fertilizer *Kemira NPK 18:9:9*, calculated in nitrogen active ingredient 60 kg ha<sup>-1</sup>. Weed control was done by harrowing performed twice (prior to oat seed germination and in the tillering stage of plant growth), and spraying with the herbicide *granstar* – 12 g ha<sup>-1</sup>. Sowing was performed in optimal terms and grain was harvested using Sampo – 130. Phenological observations were done on the field. In the winter period, morphological, agronomic and technological properties of the oat grain were analysed in the laboratory of the institute.

## Results and Discussion

Out of the hybrid combination P 4175 elite plants' progeny, seeds of the best three lines: L 28600, L 28601, L 28602 (excess of the seed sown in the breeding 2nd year nursery) were combined and sown in a preliminary variety testing nursery giving to a line a new number L 28650. That year meteorological conditions were favourable for the growth and development of oat plants and line L 28650 was also recorded as among the most productive ones. This oat line produced a good grain yield 7.01 t ha<sup>-1</sup> compare to 6.39 t ha<sup>-1</sup> obtained from the standard variety 'Laima',  $\gamma_{0.05} = 0.34$ . Oat plants of this line were characterized by a strong and stable stem, which was indicative of higher lodging resistance than that of the standard variety 'Laima'. This oat line was characterized by a uniform stand. Visual examination showed that the combined lines of oats had been phenotypically similar. These traits were the chief selection criteria, which allowed this oat line to be included in a competitive variety trial.

Competitive variety trial. Over the period of 1999 to 2003, the oat line L 28650 was investigated in a competitive variety trial. Meteorological conditions during five test years were comparatively different. This is indicated by fluctuations in the productivity levels both of the standard variety and the promising line (Table 1). In years less favourable to the growth and development of oats (1999 and 2002) grain yield obtained from the line L 28650 did not significantly differ from grain yield obtained from the standard variety 'Laima', respectively +0.03 and +0.16 t ha<sup>-1</sup>, ( $\gamma_{0.05}$  respectively 0.73 and 0.29 t ha<sup>-1</sup>). But in years more favourable to oat development (2000, 2001 and 2003) the productivity of this line was significantly higher compare to the standard variety. Overall, in competitive variety trials the average productivity level of the line L 28650 significantly surpassed the productivity level of the standard variety. When analysing yield structural elements, oat breeders found on average 4 grains more in a panicle of the line L 28650 than in the panicle of the standard variety 'Laima'. The 1000 grain weight was equivalent to the standard variety 'Laima', respectively 33.99 and 33.66 g, but the 4-year average number of generative stems per 1 m<sup>2</sup> differed only by 15 stems for both oat varieties. Considering the seeding rate of 550 seeds per 1 m<sup>2</sup> suitable to oats, the results of the analysis showed that the tillering of plants in a sowing is not characteristic for both oat varieties.

Oat line L 28650 was an average by a day earlier and higher in lodging resistance (+2 points). Results of testing oats to loose smut (*Ustilago avenae*) on an artificial infection background showed that line L 28650 was highly resistant to this disease as not a single infected panicle was found in the experimental plots (for the standard variety 'Laima' – on average 5 panicles out of 100 plants). Line L 28650 also showed also resistance to oat leaf crown rust (*Puccinia coronifera*) and powdery mildew (*Erysiphe graminis*).

Table 1. Characteristics of the oat variety 'Stendes Darta' in competitive variety trial (1999-2003)

Indices	Varieties	Years					Mean
		1999	2000	2001	2002	2003	
Grain yield, t ha <sup>-1</sup>	standard 'Laima'	3.54	5.33	5.25	4.28	5.32	5.05
	'Stendes Darta'	3.57	5.95	5.67	4.44	5.90	5.49
+/- t ha <sup>-1</sup> to 'Laima'	'Stendes Darta'	+0.03	+0.62	+0.42	+0.16	+0.58	+0.44
	$\gamma_{0.05} =$	0.73	0.34	0.41	0.29	0.29	0.21
1000 grain weight, g	standard 'Laima'	34.30	38.50	29.10	34.59	32.47	33.66
	'Stendes Darta'	34.90	36.80	29.70	35.13	34.34	33.99
Volume weight, g l <sup>-1</sup>	standard 'Laima'	510.0	506.0	453.0	511.0	495.5	491.4
	'Stendes Darta'	521.0	507.0	468.0	524.0	500.0	499.8
Hull content, %	standard 'Laima'	23.50	24.00	25.90	25.00	25.20	25.03
	'Stendes Darta'	22.00	24.50	25.50	24.20	24.70	24.73
Crude protein content, %, (x 5,7)	standard 'Laima'	10.40	11.63	11.64	10.01	11.15	11.11
	'Stendes Darta'	10.85	10.59	12.43	10.52	10.41	10.99
Crude fat content, %	standard 'Laima'	6.31	5.97	5.45	6.36	5.70	5.87
	'Stendes Darta'	6.63	6.61	5.37	6.74	6.20	6.23
Plant height, cm	standard 'Laima'	90	112	106	95	115	107
	'Stendes Darta'	82	118	107	95	119	104
Spikelets per panicle, pieces	standard 'Laima'	*	32.0	13.4	22.6	21.8	22.5
	'Stendes Darta'	*	36.4	20.0	25.8	23.8	26.5
Number of generative tillers per 1 m <sup>2</sup>	standard 'Laima'	*	520	610	528	504	540
	'Stendes Darta'	*	474	626	516	485	525
Resistance to <i>Ustilago avenae</i> , panicles /100 plants	standard 'Laima'	25	11	8	0	0	5
	'Stendes Darta'	0	0	0	0	0	0
Days from sowing to heading	standard 'Laima'	103	105	110	94	108	104
	'Stendes Darta'	103	104	109	92	107	103

Husk content, volume weight, crude protein and crude fat contents are the most important quality characteristics of the oat grain yield. The oat line L 28650 showed that hull content in the grain yield was lower than in the yield of the standard variety 'Laima', respectively 24.73 and 25.03% on the average in five test years. The highest hull content was recorded in 2001, respectively 25.50 and 25.90%. That year this parameter somewhat exceeded the maximum limit value - 25% of the grain hull content standard set by the holding company „Rīgas dzirnavnieks”.

Results of the oat grain chemical quality evaluation showed, that line L 28650 had a tendency to accumulate more fat in grain than the standard variety 'Laima' did. The crude fat content in grain dry matter was on average by 0.46% higher than that of the variety 'Laima'. (In the following test years it was found that starch content in the grain of the oat 'Stendes Darta' was also by 2 to 4% higher than in the oat 'Laima'.) This gives evidence that the grain of the new oat variety is higher in energy value, which is an important showing when using grain for animal feed.

Starting research in organic farming systems, variety testing of cereals in organic crop rotation was established at the State Stende Plant Breeding Station in 2001 to study the suitability of oat varieties and promising lines released in Latvia to this farming system. Results of testing oat line L 28650 and the standard variety 'Laima' under organic conditions (Table 2) also showed a tendency that yielding capacity of the promising line between years was equal to that of the standard variety and on average in the three test years even significantly surpassed the standard (+ 0.32 t ha<sup>-1</sup>,  $\gamma_{0.05} = 0.24$  t ha<sup>-1</sup>). Line L 28650 was high in lodging resistance. The uniform stand of this oat line contributed to weed suppression, which is a good precondition to recommend the variety for growing under organic conditions. The evaluation of grain quality characteristics show the increase in husk content under conditions of limited nutrient levels in the soil. However, this 3-year average

index for the line was by 0.5% lower compared to the standard variety. Values of other analysed parameters are presented in Table 2.

Table 2. Oat variety testing under organic farming conditions (Stende, 2001-2004)

Indices	Varieties	Years			Mean
		2002	2003	2004	
Grain yield, t ha <sup>-1</sup>	standard 'Laima'	2.66	3.24	4.37	3.42
	'Stendes Darta'	2.76	3.87	4.59	3.74
+/- t ha <sup>-1</sup> to 'Laima'	'Stendes Darta'	+0.1	+0.63	+0.22	+0.32
	$\gamma_{0.05} =$	0.32	0.57	0.29	0.24
Grain result above 1.9 x 2 mm sieve, %	standard 'Laima'	89.9	96.2	95.2	93.7
	'Stendes Darta'	89.1	93.8	89.0	90.6
1000 grain weight, g	standard 'Laima'	34.20	34.05	34.90	34.4
	'Stendes Darta'	33.40	34.80	34.70	34.3
Volume weight, g l <sup>-1</sup>	standard 'Laima'	506.0	506.0	515.0	509.0
	'Stendes Darta'	496.0	502.0	503.0	500.0
Husk content in yield, %	standard 'Laima'	28.9	27.3	25.4	27.2
	'Stendes Darta'	27.4	27.1	25.6	26.7
Crude protein content, %, (x 5,7)	standard 'Laima'	12.3	10.2	11.1	11.2
	'Stendes Darta'	12.0	10.4	10.3	10.9

Results obtained in the competitive variety trial nursery and in other experiments showed that the oat line L 28650 combines several traits due to which this line surpassed the standard variety 'Laima' and which are significant for grain breeders and consumers, such as lodging resistance, high disease resistance, lower hull content and somewhat, but at the same time significantly, a higher productivity level.

State variety trial. The oat line L 28650 with the denomination 'Stendes Darta' (initially also 'Dārta') in 2003 was brought to the Plant Variety testing department of VAAD to test value for cultivation and use under conventional farming conditions in variety testing institutions in Valmiera, Višķi and Saldus. The 2-year trial results obtained in different regions of Latvia (Table 3) indicated that the productivity level of the oat variety 'Stendes Darta' was equal to the standard variety 'Laima'. On average in the two evaluation years in three trial sites the oat variety 'Stendes Darta' produced grain a yield 5.22 t ha<sup>-1</sup> or by 0.13 t ha<sup>-1</sup> more compare to the grain yield produced by the standard variety 'Laima' ( $\gamma_{0.05} = 0.20$  t ha<sup>-1</sup>). The highest grain yield was obtained in 2004 in trials at the Saldus Variety Testing Station in Kurzeme, lowest – in 2003 in trials in Višķi secondary agricultural school in Latgale.

Table 3. Grain yield of the oat variety 'Stendes Darta' in the State Variety Testing for conventional farming system, t ha<sup>-1</sup> (2003-2004)

Testing site	Varieties	Years		
		2003	2004	Mean
Saldus	standard 'Laima'	6.17	6.24	6.21
	'Stendes Darta'	6.38	6.65	6.52
	+/- t ha <sup>-1</sup> to 'Laima'	+0.21	+0.41	+0.31
	$\gamma_{0.05} =$	0.33	0.27	0.21
Višķi	standard 'Laima'	4.31	5.04	4.68
	'Stendes Darta'	4.44	4.88	4.66
	'Stendes Darta'	0.13	-0.16	-0.02
	$\gamma_{0.05} =$	0.75	0.83	0.56
Valmiera	standard 'Laima'	4.75	4.83	4.79
	'Stendes Darta'	4.96	4.74	4.85
	standard 'Laima'	*	*	*
	'Stendes Darta'	0.21	-0.09	0.06
	$\gamma_{0.05} =$	0.22	0.18	0.14

To evaluate its suitability for organic farming, in 2005 and 2006 the oat 'Stendes Darta' was investigated in the State Variety Testing trials in LLU Study and Research Farm „Vecauce” and the State Priekuli Field Crops Breeding Institute on fields certified for organic farming. The results of these trials confirmed evaluation results beforehand stated by Stende oat breeders that the oat variety 'Stendes Darta' also in organic farming system show yield capacity equal to the standard variety 'Laima' (Table 4).

Table 4. Grain yield of the oat variety 'Stendes Darta' in the State Variety Testing for organic farming system, t ha<sup>-1</sup> (2005-2006)

Testing site	Varieties	Years		
		2005	2006	Mean
Vecauce	standard 'Laima'	5.08	4.68	4.88
	'Stendes Darta'	5.65	4.69	5.17
	+/- t ha <sup>-1</sup> to 'Laima'	+ 0.57	+ 0.01	+ 0.29
	$\gamma_{0.05}^2$	0.66	0.35	0.37
Priekuli	standard 'Laima'	2.79	3.05	2.92
	'Stendes Darta'	2.69	3.08	2.89
	+/- t ha <sup>-1</sup> to 'Laima'	- 0.10	+ 0.03	- 0.03
	$\gamma_{0.05}^2$	0.40	0.57	0.35

On average in the two evaluation years in two test sites the grain yield of the oat 'Stendes Darta' was 4.03 t ha<sup>-1</sup>, which by 0.13 t ha<sup>-1</sup> outyielded the standard variety 'Laima' ( $\gamma_{0.05} = 0.25$  t ha<sup>-1</sup>). In observation years, the grain yield of the oat 'Stendes Darta' fluctuated from 2.69 t ha<sup>-1</sup> (in 2005 in Priekuli) to 5.65 t ha<sup>-1</sup> (in 2005 in Vecauce).

The results from the evaluation of the oat grain quality characteristics in different variety testing sites showed, that hull content in the grain yield of the oat 'Stendes Darta' was on average by 0.8–1.1% lower than in the grain yield of the standard variety 'Laima' (Table 5). In case of breeding oats by conventional farming methods, this index corresponded to food industry requirements (<25%). In trials established on fields under organic farming conditions, hull content in the grain yield was 25% higher both for the standard variety and oat variety 'Stendes Darta'. Results of grain chemical analysis from two variety testing sites, Saldus and Valmiera, showed that on average in two testing years the grain of the oat 'Stendes Darta' accumulated by 2.1 to 3% more starch compare to the standard variety. Results of any other parameters analysed for chemical composition were equal to those of the standard variety when research was conducted both by conventional and organic farming methods.

Table 5. Grain quality of the oat variety 'Stendes Darta' in the State Variety Testing for conventional and organic farming systems (on the average of 2003-2004 and 2005-2006)

Indices	Varieties	Testing site for conventional farming system			Testing site for organic farming system	
		Saldus	Višķi	Valmiera	Priekuli	Vecauce
Volume weight, g l <sup>-1</sup>	standard 'Laima'	470.0	454.5	501.5	492.0	505.0
	'Stendes Darta'	445.0	486.0	495.0	493.0	517.0
Husk content in yield, %	standard 'Laima'	25.50	24.80	25.80	28.00	26.30
	'Stendes Darta'	24.70	23.00	24.70	28.00	27.10
Crude protein content, %, (x 5,7)	standard 'Laima'	12.60	12.55	11.35	11.90	14.2
	'Stendes Darta'	12.60	12.70	11.40	11.80	13.80
Crude fat content, %	standard 'Laima'	6.7	5.9	6.75	6.50	5.60
	'Stendes Darta'	6.66	6.1	6.60	6.50	5.90
Starch content, %	standard 'Laima'	46.2	53.5	47.0	39.2	40.3
	'Stendes Darta'	48.3	52.4	50.0	40.8	39.3
Resistance to lodging, 1-9 points	standard 'Laima'					
	'Stendes Darta'	6	4.5	7	9	9
		6	5	8	9	9

Results from lodging resistance evaluation showed, that under conventional farming conditions the oat 'Stendes Darta' was by 0.5 to 1 point higher in resistance. In the organic farming system, lodging was not identified in oat trials.

### Conclusion

The oat variety 'Stendes Darta' (till the year 2002 line L 28650), which was developed at the State Stende Cereals Breeding Institute over the period from 1993 to 2002 and has been successfully investigated in VCU and DUS tests, is recommended for food and feed grain production in conventional and organic farming systems. Results of the variety trial in Stende and other regions of Latvia suggest that the grain productivity level of the oat 'Stendes Darta' is equal or in particular years significantly higher than the productivity level of 'Laima' – the oat variety most widely bred in Latvia. The oat variety 'Stendes Darta' combines several traits due to which it surpasses the standard variety 'Laima' and which are important for grain breeders and consumers, i.e., better lodging resistance, high disease resistance and lower hull content.

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### AUZU ŠĶIRNE 'STENDES DARTA' UN TĀS VĒRTĒŠANAS REZULTĀTI

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Auzu šķirne 'Stendes Darta' (līnija Nr.28650) ir izveidota Valsts Stendes graudaugu selekcijas institūtā. Tā reģistrēta Valsts Augu aizsardzības dienesta (reģ. Nr. A-8 no 27.12. 2004.) un iekļauta Augu šķirņu katalogā no 2005. gada. Šķirne ir rekomendēta pārtikas un lopbarības graudu ieguvei. Šķirne izveidota hibridizācijas un vairākkārtējas izlases rezultātā. Novērojumi no 1999. līdz 2003. gadam rāda, ka jaunā šķirne ir būtiski ražīgāka nekā standartšķirne 'Laima' ( $+0.44 \text{ t ha}^{-1}$ ,  $R_{S0.05} = 0.21 \text{ t ha}^{-1}$ ). Vidēji pārbaudes periodā līnija L 28650 bija par divām dienām agrināka, ar augstāku veldres noturību (+ 2 balles) un augstāku izturību pret putošo melnplauku, auzu lapu vainagrūsu un miltrasu. Šķirnei raksturīgs augsts tauku saturs graudos (vidēji  $+0.46\%$ , salīdzinot ar šķirni 'Laima'). Šķirne 'Stendes Darta' ir parādījusi stabilus ražības rādītājus un labu konkurētspēju ar nezaļēm arī bioloģiskās saimniekošanas apstākļos.

## CROP MANAGEMENT

### IMPACT OF FERTILIZER RATES ON THE YIELD AND QUALITY OF ALFALFA ON A SOD PODZOLIC LOAMI SAND

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#### **Abstract**

A 5-year field study was conducted near Skriveri, Latvia at the Research Institute of Agriculture of the Latvian University of Agriculture. The results of investigations with alfalfa on well-cultivated sod-podzolic light loamy soil are presented. It is shown that the optimum NPK fertilizer dose in consideration of the size of the yield of dry matter and its nutritive value was  $P_{120}K_{160}$ , comes to  $8.10 \text{ t ha}^{-1}$ . Mineral fertilizers favour the rise of the collection of total protein, with higher doses the collection of protein increased by 1.5 times. It is found that in the optimum trial variant ( $N_{20}P_{120}K_{160}$ ) provided the most alfalfa yield with good quality, the specific nitrogen removal amounted to  $266 \text{ kg ha}^{-1}$ ;  $P_2O_5$  -  $55 \text{ kg ha}^{-1}$  and  $K_2O$  -  $271 \text{ kg ha}^{-1}$ .

**Key words:** alfalfa, NPK fertilizer, dry matter, protein

#### **Introduction**

The yearly deficiency of protein in Latvian feeds is about 20-30%; this is especially apparent during the stalled periods. The existing disproportion between general food value of feeds and their protein leads to significant over expenditure of the feeds and to the rise of the costs of animal produce.

Vegetable fodder protein production growth is possible at the expense of expansion of the sown area and raising the level of the crop yield of alfalfa.

The value of alfalfa as a fodder crop is determined by its macrobionics and high protein content. Alfalfa produces high yields in both clean crops and together with grass. One of the key conditions for the high yields of alfalfa is the optimal introduction of mineral fertilizers. (Marvin, Robert, 2002). This crop, which is cultivated for feed, has a well-known positive reaction to the introduction of phosphoric/potassium fertilizers on sod-carbonated soils (Hall, Smiles, Dickerson, 2000). But data on the mineral nutrition of alfalfa, which is cultivated for feed, when combining different NPK fertilizers on sod-podzol soils, so far are not sufficient (Kindler, 1996).

#### **Materials and Methods**

Field tests were carried out by 24 variants' scheme, which corresponds to 1/9 (6x6x6) of the full factorial experiment (Peregudov, 1981). The results of the research are processed for 5 testing years.

We examined the efficiency of six levels of fertilizers (including zero level), three factors N, P, K, where the unit is equal to: N- 20;  $P_2O_5$ - 60;  $K_2O$ - 80  $\text{kg ha}^{-1}$ .

We studied the cultivation of alfalfa for feed with double harvesting. Values of yields are given in tons of completely dry matter from 1 ha.

Phosphoric fertilizers were introduced manually in the early spring in one step as granulated superphosphate, nitrogen (ammonia nitrate) and potash (potassium chloride) fertilizers – separately, in equal parts, - in spring and after the first hay crop.

The sod-podzol sandy-loam lay with morainic friable loamy sand. The effervescence from HCl from the depth of 80-90 cm, the thickness of topsoil – 28-30 cm,  $pH_{KCl}$  – 6.1-6.4. Contents of available phosphorus  $P_2O_5$  – 60-85  $\text{mg kg}^{-1}$  and available potassium  $K_2O$  – 101-124  $\text{mg kg}^{-1}$  of soil (Egner-Riehm DL method.) Contents of organic matter – 15 – 19  $\text{g kg}^{-1}$  (Tyurin method).

Soil was limed on the basis of full norm by hydrolytic acidity (5 ton ha<sup>-1</sup> of CaCO<sub>3</sub> in form of slate ash), consequently pH<sub>KCl</sub> of the topsoil increased to 6.6-6.8. During the experiments, introducing mineral fertilizers, repeated acidulation did not occur. The seeding rate of alfalfa – 20 kg ha<sup>-1</sup>, space between rows – 15 cm. In the second year of alfalfa’s life – early spring harrowing and in the third year of life - disking.

Data on crops were processed by regression method with application of the model:

$$Y=a_0+a_1N+a_2P+a_3K+a_4N^{0.5}+a_5P^{0.5}+a_6K^{0.5}+a_7(NP)^{0.5}+a_8(NK)^{0.5}+a_9(PK)^{0.5},$$

where: Y is yield, a<sub>0</sub> is absolute term, which defines yield without fertilizers (variant N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>), a<sub>1</sub> to a<sub>9</sub> are coefficients, which define the impact of the NPK fertilizers on yield (MATLab, 2005).

The evaluation of the reliability of values under study was conducted on the level of significance of 0.95. The determination of the quality of grass dry matter, of all agrochemical characteristics of soil and plants, and the examination of grass stand’s formation were conducted according to standard methods.

The meteorological conditions of the vegetative season in the particular years of the studies differed from the long-term average, but did not significantly influence the crop yield of alfalfa.

### Results and Discussion

After the Results of the Plant Breeding Department data, the crop yield of alfalfa’s dry matter on neutral soils was 2.8 to 13.5 tons per ha, and changed by year and variety. As is seen from the table 1, yields are not stable (Results..., 2007). By using mineral fertilizers it is possible to get more stable yields, and by using mathematical models it is possible to plan the introduction of fertilizers.. Therefore, it is very important to know not only the impact of separately introduced fertilizers, but also their interaction. Considering that the soil was weak-acidic, alfalfa developed well in our experiment.

Table 1. Crop yield of alfalfa’s dry matter in 2000-2004, tons per ha, (results of Plant Breeding Department)

Variety	Dry matter yield, t ha <sup>-1</sup>				
	2000 g.	2001 g.	2002 g.	2003 g.	2004 g.
Skriveru	3.2	14.3	10.9	8.9	5.7
Magda	3.0	13.3	10.5	8.5	6.5
ABT-205	2.8	12.6	10.4	8.3	5.5
Jitka	2.7	11.9	9.7	7.8	5.8
Nr.60	3.3	13.5	11.1	9.1	6.7
Vernal	3.4	13.4	10.6	8.8	6.4
Ņiva	2.9	12.3	10.7	8.6	6.6
Multigen	2.2	10.5	8.3	7.9	5.0
Birute	3.5	12.7	10.8	9.5	6.2
Karli	3.6	12.2	10.2	10.7	7.5
Average	3.1	12.7	10.3	8.1	6.2

Least significant difference at p≤0.05=0.16 t ha<sup>-1</sup>

In each year of the study, the crop yield of completely dry matter (DM) of alfalfa (Y<sub>1,2,3</sub> accordingly by the years of usage, and Y<sub>4</sub> – for five testing years) in fertilized variants was, on average, 2.1- 2.4 times higher than in the control variant. This dependence was defined by the following production functions:

$$Y_1=6.22-0.44N^{0.5}+0.45(NP)^{0.5}+0.65(NK)^{0.5}; R^2=0.90$$

$$Y_2=7.11+0.19N+0.54(PK)^{0.5}; R^2=0.81$$

$$Y_3=6.93-0.20N+0.56N^{0.5}+0.52K^{0.5}+0.16(NP)^{0.5}; R^2=0.96$$

$$Y_4=6.93+0.56K^{0.5}+0.20(NP)^{0.5}+0.22(PK)^{0.5}; R^2=0.91$$



Judging by the value of absolute term of equations, the fluctuations of the crop yield of DM, depending on the year of usage, excluding the impact of fertilizers, were insignificant. Generally, yield depended on the level of applied doses of fertilizers, which is indicated by the high value of the determination coefficient ( $R^2$ ).

The increasing doses of nitrogen provided for the continuous growth of alfalfa yields in the second year ( $Y_2$ ) of grass stand usage.

The increasing doses of nitrogen provided for the consecutive continuous growth of the alfalfa yield in the second year of usage of the grass stand. The alfalfa of the first year ( $Y_1$ ) of usage was negatively influenced by separately introduced nitrogen fertilizers, and the alfalfa of the third year ( $Y_3$ ) of usage, after reaching maximal yield with  $N_{40}$ , was influenced negatively.

Persistent influence on the yield of DM was shown by phosphoric and potash fertilizers (Table 2). Increases of yields after their combined introduction grew with the increase of doses. Both types of fertilizers were identical. However, the phosphoric fertilizer was effective only when combined with nitrogen and potash fertilizers. Potash fertilizer was effective both separately and combined with NP fertilizers.

Table 2. The crop yield of the absolutely dry matter ( $Y_4$ ) of alfalfa, depending on fertilizers, in average for 5 testing years.

N kg ha <sup>-1</sup>	P <sub>2</sub> O <sub>5</sub> kg ha <sup>-1</sup>	K <sub>2</sub> O kg ha <sup>-1</sup>			
		0	80	160	240
0	0	6.57	7.14	7.37	7.55
	60		7.36	7.69	8.93
	120		7.45	7.82	8.1
	180		7.52	7.91	8.22
20	0				
	60	6.77	8.56	7.88	7.55
	120	6.85	7.73	8.11	8.14
	180	6.92	7.87	8.26	8.38
40	0				
	60	6.86	7.64	7.97	8.22
	120	6.97	7.85	8.22	8.5
	180	7.07	8.01	8.41	8.71
60	0				
	60	6.92	7.7	8.03	8.28
	120	6.06	7.94	8.31	8.59
	180	7.17	8.13	8.52	8.52

From the paired combinations, the PK fertilizers had the strongest impact slightly weaker – NP fertilizers.

The maximum practically justified growth of the yield of DM was about 1.54 t ha<sup>-1</sup>, and was reached by combining 20 kg ha<sup>-1</sup> of N, 120 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 160 kg ha<sup>-1</sup> of K<sub>2</sub>O.

The recoupage by increase of the yield of DM by introducing potassium and phosphoric fertilizers, as their doses increased, decreased from 5.5 to 1.8 kg of DM for 1 kg of fertilizers. In the range of optimal doses of fertilizers ( $N_{20}P_{120}K_{160}$ ), each kilogram of PK fertilizers additionally gave 3.32 kg of DM of alfalfa.

The crop yield of alfalfa lies in an average correlation with the height of plants ( $r=0.32-0.49$ ). The regression equation of the dependence of plants' height ( $Y_5$ ) from fertilizers corresponds to the following formula:  $Y_5=73.65+5.50(NP)^{0.5}$ ;  $R^2=0.57$ . It is seen from the equation, that the change of the plants' height generally depended on the influence of nitrogen and phosphoric fertilizers. The influence of potash fertilizers was insignificant. The level of alfalfa's sprout-formation lies in close correlation with the crop yield of DM ( $r=0.60-0.75$ ). The number of alfalfa's culms ( $Y_6$ ) changed by years of usage in dependence on the doses of potash and nitrogen fertilizers. The regression equation has the following form:  $Y_6=175.91+24.69 N -62.63N^{0.5}+0.59 (NK)^{0.5}$ ;  $R^2=0.65$ . By the third year of usage, high doses of NK fertilizers led to a fall of the number of plants on 1 sq. m. However, the rest of plants were strenuously tillered, thus, the fall of the crop yield of DM did not

occur. Significant correlation was found between the yield of alfalfa's DM and leaf - bearing ( $r=0.63-0.74$ ). The dependence of plants' leaf - bearing ( $Y_7$ ) from the doses of fertilizers corresponded to the following regression equation:  $Y_7=33.3-1.31 N +3.7N^{0.5}+1.2P^{0.5}+3.9K^{0.5}$ ;  $R^2=0.48$ . Fertilizers increased plants' leaf - bearing, which was: with  $N_0P_0K_0$  – 42.3%; with  $N_0P_{120}K_{160}$  – 43.5%, and with  $N_{20}P_{120}K_{160}$  – 44.6%. Alfalfa is valuable as a high-protein crop. Thus, the content of raw protein (RP) in its leaves was 19.75- 30.57%. In culms this value was lower: 8.37-15.77%. The yield of protein ( $Y_8$ ) depended on both the level of the yield of dry matter ( $r=0.83-0.89$ ), and the doses of fertilizers, accordingly to the following regression equation:  $Y_8=1.16+0.07(NK)^{0.5}+0.08(PK)^{0.5}$ ;  $R^2=0.65$ . The yield of protein generally depended on paired combinations of NK and PK fertilizers, and with higher doses increased by 1.5 times (Table 3).

Table 3. Collection of protein, ( $Y_8$ ) depending on fertilizers, on average for 5 testing years.

N kg ha <sup>-1</sup>	P <sub>2</sub> O <sub>5</sub> Kg ha <sup>-1</sup>	K <sub>2</sub> O kg ha <sup>-1</sup>		
		80	160	240
0	0			
	60	1.24	1.28	1.3
	120	1.28	1.32	1.36
	180	1.30	1.36	1.41
20	0	1.23	1.26	1.28
	60	1.31	1.38	1.42
	120	1.35	1.42	1.48
	180	1.37	1.46	1.52
40	0	1.26	1.3	1.33
	60	1.34	1.42	1.48
	120	1.38	1.47	1.53
	180	1.40	1.5	1.58
60	0	1.28	1.33	1.37
	60	1.36	1.45	1.51
	120	1.40	1.5	1.57
	180	1.42	1.53	1.61

Changing the level of the mineral nutrition, the mineral contents of alfalfa's feed also changed. The content of phosphorus in the alfalfa's leaves was 0.34-0.41%, in culms – 0.21-0.29%. The introduction of nitrogen and phosphoric fertilizers increased this value by 1.3-1.4 times. The impact of potash fertilizers on this value was insignificant.

The content of potassium in the yield of alfalfa was roughly equal in all plant's parts, and was 2.62-3.05%. Single introduction of potassium fertilizers increased the content of potassium in the feed.

The content of calcium in the leaves was 2.42-3.02%, much lower in culms (0.78-0.98%). The accumulation of calcium in alfalfa was positively influenced by NK fertilizers. The impact of potassium fertilizers was negative ( $R^2=0.64$ ). The content of magnesium in the yield of alfalfa varied in the range 0.17-0.35%. Phosphoric fertilizers increased this value.

To estimate the equilibrium of mineral elements in feed, the correlation K: is importants (Ca+Mg). This correlation in DM of alfalfa was optimal – 1.28, and even with higher doses of fertilizers lay within acceptable limits. The removal of nutrients ( $Y_9$ - N;  $Y_{10}$ - P<sub>2</sub>O<sub>5</sub>;  $Y_{11}$ - K<sub>2</sub>O) from the soil with the yield of alfalfa, depending on the doses of mineral fertilizers, occurred according to the following equations:  $Y_9=184.91+10.62 (NK)^{0.5}+13.62(PK)^{0.5}$ ,  $R^2=0.85$ ;  $Y_{10}=44.88+3.95 N+4.74 (PK)^{0.5}$ ,  $R^2=0.64$ ;  $Y_{11}=224.66+11.31N-7.19P+24.45 (PK)^{0.5}$ ,  $R^2=0.80$ .

The nature of effect and efficiency of NPK fertilizers on the yield of DM and the removal of the key nutrients from soil are identical. Thus, the interaction of paired combinations of NK and PK fertilizers positively influenced the removal of nitrogen. The removal of phosphorus depended on the yield of DM and also from fertilizers. The amount of phosphorus, removed by alfalfa, was almost four times less than the removal of potassium and nitrogen. The removal of phosphorus increased with higher doses of phosphoric fertilizers. Single introduction of nitrogen fertilizers increased the removal of potassium, and each step of nitrogen ( $N_{20}$ ) caused an increase in the

removal of potassium by 11.31kg. Single introduction of phosphoric fertilizers decreased the removal of potassium from 217.43 kg ha<sup>-1</sup> (P<sub>60</sub>) to 188 kg ha<sup>-1</sup> with (P<sub>180</sub>).

The level of the total removal of nutrients is determined generally by the crop yields of DM of alfalfa, and less depended on the chemical composition.

### Conclusions

Use of NPK fertilizers is the most effective influencing factor on the crop yield of alfalfa, which is cultivated for feed. With optimal doses of fertilizers (N<sub>20</sub>P<sub>120</sub>K<sub>160</sub>), the average yield of dry matter was 8.10 t ha<sup>-1</sup>.

Mineral fertilizers favour the increase of the total protein. The most significant impact is shown by paired combinations of NP and PK fertilizers – with higher doses the increase of protein increased by 1.5 times.

With the application of N<sub>20</sub>P<sub>120</sub>K<sub>160</sub>, alfalfa removes from the soil with the yield of dry matter 226 kg ha<sup>-1</sup> of N; 55 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub> and 271 kg ha<sup>-1</sup> of K<sub>2</sub>O.

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## LUCERNAS RAŽĪBA UN KVALITĀTE VELĒNU PODZOLĒTĀ AUGSNĒ PIE DAŽĀDIEM MINERĀLMĒSLU LĪMENIEM

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Rakstā apkopoti LLU aģentūras Zemkopības zinātniskā institūta lucernas 5-gadīgie pētījumu rezultāti. Pētījumi veikti velēnu podzolētā augsnē ar dažādām NPK mēslojumu devām. Minerālmēslojums būtiski palielināja lucernas zaļās masas ražu. Pie optimālā NPK mēslojuma attiecības (N<sub>20</sub>P<sub>120</sub>K<sub>160</sub>) ieguva 8.10 t ha<sup>-1</sup> sausas.

Minerālmēslojums sekmēja arī kopējā proteīna ieguvī, pie paaugstinātas devas N<sub>20</sub>P<sub>120</sub>K<sub>160</sub>, proteīna ieguve palielinājās 1.5 reizes. Mēslojot lucernu ar N<sub>20</sub>P<sub>120</sub>K<sub>160</sub>, iegūtā lucernas sausas raža no augsnes iznes N-226 kg ha<sup>-1</sup>, P<sub>2</sub>O<sub>5</sub> – 55 kg ha<sup>-1</sup> un K<sub>2</sub>O – 271 kg ha<sup>-1</sup>.

## THE EFFECTS OF COVER CROPS AND STRAW ON SOIL MINERAL NITROGEN DYNAMICS AND LOSSES FROM ARABLE LAND

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### Abstract

Seeking to estimate the effects of biological measures – various cover crops in combination with different straw incorporation methods on the reduction of soil mineral nitrogen and nitrogen leaching complex research was conducted at the Lithuanian Institute of Agriculture's Joniskelis Experimental Station on clay loam *Gleyic Cambisol* during the period 2003–2005. Undersown legume crops during the post-harvest period gave the largest reduction in mineral nitrogen in the

soil: red clover (*Trifolium pratense* L.) – 15.1 %, white clover (*Trifolium repens* L.) and Italian ryegrass (*Lolium multiflorum* Lamk.) mixture – 16.4 %, compared with the treatment without cover crops. However, having incorporated by a stubble breaker not only stubble but also straw and having applied nitrogen fertiliser (N 45) for its mineralization, the content of mineral nitrogen increased by 15.2 %, compared with the treatment where the plots were stubble–broken without straw. Having incorporated straw with the addition of mineral nitrogen fertiliser, 9.2 % lower  $N_{\min}$  content in the soil was found in the treatment where cover crop white mustard (*Sinapis alba* L.) was sown as a post–crop. In heavy–textured *Cambisol* in spring, higher contents of  $N_{\min}$  in the soil and filtration water were found in the treatments where the biomass of legume crops was incorporated in the autumn. Having incorporated it together with straw, the  $N_{\min}$  content in the soil significantly declined (9.8 %).

**Key words:** clay loam soil, cover crops, mineral nitrogen, nitrogen leaching

### Introduction

Intensive agriculture where the nutritional needs of plants are met by mineral fertilisers, poses a threat to ecological balance (Buciene, 2003). Most research findings suggest that 50–60 % of nitrogen present in surface water is of agricultural origin. Numerous studies have been done and recommendations have been provided on the most suitable fertiliser forms and rates, application timing and methods. However, research on the effects of the technologies used on nutrient, especially nitrogen, immobilisation in the soil after the harvesting of the main crops, when the soil during the post–harvest period stays without any plant cover for a long time, is rather scanty. It was found that intensive crop fertilisation results in increased  $N_{\min}$  content in the autumn (Arlauskiene and Maikstieniene, 2005). Mineral nitrogen build–up and dynamics in the soil depend on soil texture and humus content, conditions of the growing period, crop and soil management technologies, especially fertilisers used (mineral and organic), their rates and application method (Antil *et al.*, 2005). Seeking to reduce environmental pollution, adequate selection of preventive measures is very important when including the nutrients not utilised by plants into the biological turnover cycle. With this end in view, technologies with cover crops that accumulate and localize in the soil the nutrients left in the biomass at the end of summer – during the autumn (winter) period, and during the most intensive leaching complex that prevent the nutrients from being leached, are widely used in Western Europe. Under the effect of biological transformation the incorporated biomass in the following year becomes the nutrient source for crops grown (Reents and Moller, 2000; Thorup–Kristensen, 2006). However, after the incorporation of the nitrogen–rich biomass of cover crops (especially those with a narrow carbon to nitrogen ratio), intensive mineralization during the early spring period is possible, when the content of  $N_{\min}$  increases and there is a risk of its leaching into ground water (Farthofer *et al.*, 2004; Loges *et al.*, 2006). An effective way to prevent nutrient migration into deeper layers during the autumn – is early spring nitrogen inclusion into the soil’s organic compounds. It is the incorporation of the nitrogen–rich biomass of the cover crops into the soil together with carbon –rich cereal straw (Dryslava and Prochazkova, 2002). Experiments carried out in Denmark show that the proper use of straw not only reduces mineral nitrogen excess in the soil but also restores soil humus and increases the stable humic matter content (Luxhoi *et al.*, 2007).

The study was designed to estimate the effects of various cover crops in combination with different straw incorporation methods on the reduction of soil mineral nitrogen and nitrogen leaching complex in clay loams.

### Materials and Methods

Two analogous trials were carried out at the Lithuanian Institute of Agriculture’s Joniskelis Experimental Station during the periods: 2003–2004 (I experiment) and 2004–2005 (II experiment). The soil of the northern part of Central Lithuania’s lowland is *Endocalcari–Endohypogleyic Cambisol*, according to texture – clay loam on silty clay with deeper lying sandy loam (p2 / m2 / p1). The soil agrochemical properties in the 0–20 cm layer were as follows:  $pH_{KCl}$ –

6.4, humus content of 21–22 g kg<sup>-1</sup>, plant available P and K – 0.052 and 0.220 g kg<sup>-1</sup> of soil respectively.

Experimental design:

Factor A. Winter wheat (*Triticum aestivum* L.) straw management methods: I. Straw – removed from the field; II. Straw – chopped and incorporated.

Factor B. Stubble breaking and cover crops: 1. Without cover crop, stubble not broken; 2. Without cover crop, stubble broken; 3. Undersown cover crop – red clover (*Trifolium pratense* L.); 4. Undersown cover crop – mixture of white clover (*Trifolium repens* L.) and Italian ryegrass (*Lolium multiflorum* Lamk.); 5. Undersown – cover crop – white mustard (*Sinapis alba* L.) broadcast–sown into winter wheat at wax maturity stage; 6. Aftercrop cover crop – white mustard direct–sown into stubble; 7. Aftercrop cover crop – white mustard direct–sown into stubble–broken soil.

The experiment was conducted in the following rotation sequence: winter wheat + cover crop; spring barley. The effect of cover crops and straw was monitored by growing spring barley (*Hordeum vulgare* L.). Winter wheat ‘Ada’ and spring barley ‘Ula’ were grown following conventional technology and fertilised by N 60, P 26.4 and K 49.8. The sowing time of cover crops was for undersown red clover and a mixture of white clover and Italian ryegrass – beginning of April, for broadcast – undersown white mustard – end of July, for aftercrop white mustard – beginning of August (the very day after the cereal harvest). According to the experimental design the winter wheat stubble was broken by a combined breaker composed of goose feet coulters cutting 10–12 cm soil layer, disks incorporating plant residues and straw and bar rollers levelling the soil surface. Various sowing methods of cover crops were studied: undersowing – by drilling perennial grasses into winter wheat in spring; broadcast undersowing – by spreading the seeds of white mustard at winter wheat wax maturity; aftercrops – by direct drilling into stubble and into the surface loosened by a combined stubble breaker after harvesting. In order to adequately place the seeds of cover crops into the clay loam soil, the wedge – type coulters of the *Amazona D7* sowing machine were fitted with special additions to make a deeper furrow and to achieve better placement of seed. The seed rate when sowing white mustard by the sowing machine *Amazona D7* was 18 kg ha<sup>-1</sup>, when sowing broadcast the rate was increased by 30 %. The seed rate of undersown red clover was 15 kg ha<sup>-1</sup>, of white clover mixed with Italian ryegrass 8 + 7 kg ha<sup>-1</sup>. Mineral nitrogen fertilisers (N 45) were applied after winter wheat harvesting: in background – straw removed from the field (I) to white mustard and in background – straw chopped and incorporated (II) for straw mineralisation – in all treatments excluding undersown red clover and mixture of white clover and Italian ryegrass). Soil samples for mineral nitrogen (N<sub>min.</sub>) determination were taken before the incorporation of cover crops and straw into the soil and early in spring before barley sowing from the soil profile layer 0–40 cm. Mineral nitrogen (NO<sub>3</sub> + NH<sub>4</sub>) was measured by the distillation and colorimetry method (in 1 N KCl extraction). To measure nutrient leaching, wells-piezometers were set up (depth 0–80 cm) after winter wheat harvesting in each background in two replications to collect the filtration water. In water samples the N–NO<sub>3</sub> content were determined by cadmium reduction methods.

The rate of rainfall during the August–September period in 2003 accounted for 63.7 % of the long–term mean, however sufficient rainfall after crop emergence determined good crop establishment and growth. In winter and early spring (2004) the rate of precipitation was lower compared with the long–term mean, as a result, the probability of nutrient leaching was low. During the September–November period of 2004 after the incorporation of straw and plant biomass, the rate of precipitation was 43 % higher than the long–term mean. The winter of 2004–2005 was milder (especially in January, when the air temperature was 4.3 °C higher) than usual. However, the winter was long and the weather became warmer only in April before crop sowing. The experimental data were processed by ANOVA and STAT ENG.

## Results and Discussion

In the agrocenoses on the soils satiated with soil–depleting cereals cover crops during post–harvest period additionally accumulate organic matter and biogenic elements, better utilise the growing season and protect the soil from degradation. The largest amount of aboveground mass 2.55 t ha<sup>-1</sup>

of dry matter was produced by undersown red clover with a longer growing season. The largest amount of white mustard aboveground mass was formed in the plots of the treatments where the seed was sown into stubble–broken soil or directly sown into the stubble 2.43 and 2.53 t ha<sup>-1</sup> of dry matter, respectively.

Experiments carried out early in October before the incorporation of cover crops into the soil show that the content of mineral nitrogen in the upper 0–40 cm soil profile was on average through all experimental treatments 0.0067 g kg<sup>-1</sup>. Nitrate and ammonia nitrogen accounted for 45.9 and 54.1 % of its content. On the background of straw, having spread mineral nitrogen fertiliser for its more rapid mineralization, N<sub>min.</sub> tended to increase (on average 1.5 %) (Table 1). The higher mineral nitrogen content on the background of straw was determined by ammonia nitrogen. Spread on the soil but not incorporated into the soil straw partly protected the soil from large temperature variations and conserved productive moisture reserves thus creating favourable a climate for the emergence and development of cover crops.

Table 1. Effect of cover crops sowing and straw using methods on the soil (layer 0–40 cm) mineral nitrogen content (g kg<sup>-1</sup> soil) in autumn before the incorporation of the biomass of the cover crop (mean date from 2003–2004)

Cover crops and their sown methods (B)	Straw using methods (A)					
	straw removed from the field		straw chopped and incorporated		means for factor B	
	N <sub>min.i</sub> g kg <sup>-1</sup>	relative number, %	N <sub>min.i</sub> g kg <sup>-1</sup>	relative number, %	N <sub>min.i</sub> g kg <sup>-1</sup>	relative number, %
Without cover crop, stubble not broken	0.0070	100	0.0076	108.6	0.0073	100
Without cover crop, stubble broken	0.0066	94.3	0.0076	108.6	0.0071	97.3
Undersown cover crop – red clover	0.0060	85.7	0.0065	92.9	0.0062	84.9
Undersown cover crop – mixture of white clover and Italian ryegrass	0.0063	90.0	0.0058	82.9	0.0061	83.6
Undersown cover crop – white mustard broadcast–sown into winter wheat at wax maturity stage	0.0065	92.9	0.0066	94.3	0.0066	90.4
Aftercrop cover crop – white mustard direct–sown into stubble	0.0070	100.0	0.0072	102.9	0.0071	97.3
Aftercrop cover crop – white mustard direct–sown into stubble–broken soil	0.0073	104.3	0.0069	98.6	0.0071	97.3
Means for factor A	0.0067	100	0.0068	101.5	0.0068	
LSD <sub>05</sub> (A) = 0.00030; LSD <sub>05</sub> (B) = 0.00057; LSD <sub>05</sub> (AB) = 0.00080						

Having incorporated cereal roots and stubble shallowly at 10–12 cm by the stubble breaker, the contents of the mineral nitrogen declined, which created a reduction in the mineral nitrogen content of 5.7 %, compared with that in the treatment with unbroken stubble. However, having incorporated by a stubble breaker not only stubble but also straw and having applied nitrogen fertiliser (N 45) for its mineralization, the content of mineral nitrogen increased by 15.2 %, compared with the treatment where the plots were stubble–broken without straw.

Seeking to reduce nitrogen accumulation in the soil, which increases the risk of leaching, white mustard was sown as an aftercrop. On the background without straw having broken stubble and having applied mineral nitrogen fertiliser for the start growth of mustard, the content of mineral nitrogen was the highest, its content increased by 4.3 and 10.6 %, respectively, compared with the check and stubble–broken treatments. In an analogous treatment only having incorporated straw into the topsoil by a stubble breaker nitrogen fixation increased and there was less mineral nitrogen in the soil. The effect of white mustard as an aftercrop on mineral nitrogen content in the soil depended on the sowing method of white mustard. White mustard undersown at cereal early wax maturity (and having incorporated the start dose of mineral nitrogen fertiliser), was at the intensive nutrient utilisation stage (Thorup–Kristensen, 2006), therefore better utilised both soil and mineral

fertiliser nitrogen and reduced  $N_{\min}$  in the soil by 7.1 %, compared with the check treatment. However, mineral nitrogen tended to increase having sown mustard into stubble after harvesting, especially in the soil where straw had been spread.

On both backgrounds undersown legumes significantly increased the revivification of mineral nitrogen in the soil profile. Literature sources indicate that in autumn cover crops can reduce mineral nitrogen content in the soil by 20–25 kg ha<sup>-1</sup> (Farthofer *et al.*, 2004). Undersown grasses grown as cover crops have a well-developed root system, as a result, after cereal harvesting they better utilise the nitrogen remaining in the soil compared with white mustard as an aftercrop, moreover they do not need mineral nitrogen fertilisers. After the incorporation of the cover crops biomass there was less mineral nitrogen on the background without straw where red clover was grown, with straw – white clover and ryegrass mixture, which made up 14.3 and 17.1 % less, compared with the check treatment.

The mineral nitrogen content in the soil profile in spring had some effect on the nitrogen concentration in the soil filtration water (Table 2). On both straw utilisation backgrounds nitrate nitrogen content in the soil filtration water was significantly increased by the biomass of legume crops incorporated in the autumn. As literature sources indicate, nitrate nitrogen depends on the nitrogen content accumulated in the cover crops biomass, and according to the nitrogen content in the soil solution the plants can be ranked in the following order: legumes > legume and non-legume mixture > non-legumes (Rinnofner *et al.* 2005).

Table 2. Effect of cover crops and straw using methods on the nitrate nitrogen (N–NO<sub>3</sub>) leaching from the soil in spring period (mean data from 2004–2005)

Cover crops (B)	Straw using methods (A)					
	straw removed from the field		straw chopped and incorporated		means for factor B	
	mg l <sup>-1</sup>	relative number, %	mg l <sup>-1</sup>	relative number, %	mg l <sup>-1</sup>	relative number, %
Without cover crop	6.11	100	7.80	127.7	6.96	100
Undersown cover crop – red clover	15.50	253.7	12.0	196.4	13.75	197.6
Aftercrop cover crop – white mustard	13.1	214.4	8.16	133.6	10.63	152.7
Means for factor A	11.57	100	9.32	80.6	10.44	

LSD<sub>05</sub> (A) = 3.141; LSD<sub>05</sub> (B) = 4.440; LSD<sub>05</sub> (AB) = 6.282

The use of straw for fertilisation reduced (on average 9.8 %) nitrate nitrogen content in filtration soil solution. Having incorporated straw, after legume crops, the content of nitrate nitrogen in filtration water declined by 11.3 %, white mustard by 18.9 %, compared with analogous treatments without straw. This nitrate nitrogen concentration in soil filtration water is not high. The data of long-term experiments conducted in Lithuania suggest that the average nitrate nitrogen concentration in lysimetric water of different soils ranged from 49.2 to 83.7 mg l<sup>-1</sup> (Tyla, 1995).

In spring after the incorporation of the cover crops biomass and straw the content of nitrogen in heavy-textured soil at the 0–40 cm layer was found to be almost the same as in autumn – 0.0068 g kg<sup>-1</sup> soil or 34.0 kg ha<sup>-1</sup> (in average) (Table 3). The data of the trials conducted in Austria indicate that after the cover crops incorporation, in spring the content of mineral nitrogen markedly increases (in the 0–30 cm layer – 60 kg ha<sup>-1</sup>, in the 0–120 cm layer – 120 kg ha<sup>-1</sup>) (Farthofer *et al.*, 2004). At the beginning of the plant growing season mineral nitrogen varied in a slightly different way than in autumn. The greatest increase in  $N_{\min}$  content in the soil occurred after the incorporation of red clover and white clover and the ryegrass mixture. This results from the fact that during the period of autumn, winter and early spring during the breakdown of incorporated biomass of legumes, a higher content of nitrogen is released compared with that after incorporation of non-legume cover crops. After red clover and white clover with ryegrass mixture,  $N_{\min}$  increased by 18.3 and 17.5 %, straw incorporation reduced its content by 3.1 and 5.2 %, compared with analogous data in autumn. Having incorporated non-legume crop – white mustard the content

of mineral nitrogen declined on both backgrounds (except for the treatment where mustard was sown into broken stubble), which is indicated by other authors (Reents *et al.*, 2000).

Table 3. Effect of cover crop sowing and straw using methods on the soil (0–40 cm) mineral nitrogen content ( $\text{g kg}^{-1}$  soil) in spring (mean data from 2004–2005)

Cover crops and their sowing methods (B)	Straw using methods (A)					
	straw removed from the field		straw chopped and incorporated		means for factor B	
	$N_{\text{min.}}$ $\text{g kg}^{-1}$	relative number, %	$N_{\text{min.}}$ $\text{g kg}^{-1}$	relative number, %	$N_{\text{min.}}$ $\text{g kg}^{-1}$	relative number, %
Without cover crop, stubble not broken	0.0075	100.0	0.0069	92.0	0.0072	100.0
Without cover crop, stubble broken	0.0068	90.7	0.0068	90.7	0.0068	94.4
Undersown cover crop – red clover	0.0071	94.7	0.0067	89.3	0.0069	95.8
Undersown cover crop – mixture of white clover and Italian ryegrass	0.0074	98.7	0.0061	81.3	0.0068	94.4
Undersown cover crop – white mustard broadcast–sown into winter wheat at wax maturity stage	0.0062	82.7	0.0065	86.7	0.0063	87.5
Aftercrop cover crop – white mustard direct–sown into stubble	0.0067	89.3	0.0061	81.3	0.0064	88.9
Aftercrop cover crop – white mustard direct–sown into stubble–broken soil	0.0077	102.7	0.0067	89.3	0.0072	100.0
Means for factor A	0.0071	100	0.0065	91.5	0.0068	
LSD <sub>05</sub> (A) = 0.00023; LSD <sub>05</sub> (B) = 0.00042; LSD <sub>05</sub> (AB) = 0.00060						

The greatest reduction in mineral nitrogen content occurred having incorporated straw into broken and unbroken stubble (10.5 and 9.2 %, respectively), compared with the respective data obtained in the autumn. A reduction of 15.3 % in mineral nitrogen was also recorded having direct–sown mustard into stubble with straw, compared with respective data in the autumn.

In spring ammonia nitrogen accounted for the largest share (59.6 %) than nitrate nitrogen (40.4 %) in total mineral nitrogen. After straw incorporation a significant reduction in mineral nitrogen contents occurred compared with the treatments where the straw was removed. Here the content of the mineral nitrogen was 8.0 % lower, compared with the check treatment. Autumn stubble breaking with and without straw tended to reduce mineral nitrogen content in the soil 9.3 %, compared with the check treatment, or by 1.4 %, compared with the treatment where straw was spread in the autumn. The highest content of mineral nitrogen was found on the background without straw, like in the autumn having incorporated white mustard sown into broken stubble. The non–legume cover crop, white mustard, significantly reduced the mineral nitrogen content in the soil, whereas legumes tended to increase mineral nitrogen.

## Conclusions

Undersown legume crops during the post–harvest period gave the largest reduction in mineral nitrogen in the soil: red clover (*Trifolium pratense* L.) – 15.1 %, white clover (*Trifolium repens* L.) and Italian ryegrass (*Lolium multiflorum* Lamk.) mixture – 16.4 %, compared with the treatment without the cover crop. Having incorporated cereal straw and having applied nitrogen fertiliser (N 45) for its mineralization, the content of mineral nitrogen increased by 15.2 %, compared with the treatment where the plots were stubble–broken without straw. While incorporating straw with the addition of mineral nitrogen fertiliser (N 45), 9.2 % lower  $N_{\text{min.}}$  content in the soil was found in the treatment where cover crop white mustard was sown as the aftercrop. In spring, higher contents of  $N_{\text{min.}}$  in the soil and filtration water were found in the treatments where the biomass of legume crops was incorporated in the autumn. Having incorporated it together with straw, the nitrate nitrogen content in the soil filtration water declined (9.8 %).



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## UZTVĒRĒJAUGU UN SALMU EFEKTIVITĀTE AUGSNES MINERĀLĀ SLĀPEKĻA DINAMIKĀ ARAMZEMĒ

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Lai noskaidrotu dažādu uztvērējaugu un to kombināciju ar salmiem bioloģiskās iespējas samazināt augsnes minerālā slāpekļa izskalošanos minerālaugsnēs, Lietuvas Zemkopības institūta Joniškēļu izmēģinājumu stacijā smilšmāla augsnē *Gleyic Cambisol* periodā no 2003.-2005. gadam tika ierīkoti kompleksi izmēģinājumi. Tauriņziežu pasēja pēcnovākšanas periodā nodrošināja lielāko minerālā slāpekļa samazinājumu: salīdzinājumā ar kontroli, sarkanais āboliņš (*Trifolium pratense* L.) par 15.1 %, baltā āboliņa (*Trifolium repens* L.) un daudzziēdu airenes (*Lolium multiflorum* Lamk.) maisījums- par 16.4 %. Taču, atliekās iekļaujot ne tikai rugājus, bet arī salmus un to mineralizācijai lietojot slāpekļa mēslojumu (N 45), minerālā slāpekļa saturs pieauga par 15.2 %, salīdzinot ar rugāju variantu bez salmiem. Iekļaujot salmus ar minerālā slāpekļa mēslojuma piedevu, par 9.2 % zemāks  $N_{min}$ . saturs augsnē tika konstatēts apstrādē, kur kā uztvērējaugs tika sēta baltā sinepe (*Sinapis alba* L.) Smagās *Cambisol* augsnēs pavasarī augstāks  $N_{min}$ . saturs augsnē un filtrācijas ūdenī tika konstatēts variants, kur rudenī tika iekļauta tauriņziežu biomasa. Iekļaujot to kopā ar salmiem,  $N_{min}$ . saturs augsnē būtiski samazinājās (9.8 %).

## THE EFFECT OF MINERAL AND ORGANIC FERTILIZERS ON POTATO TUBER YIELD AND QUALITY

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### Abstract

Experiments were conducted at the Lithuanian Institute of Agriculture's Perloja Experimental Station during the period 1994-2005. They were designed to estimate the effects of organic and mineral fertilizer combinations on potato tuber yield and quality. The soil of the experimental site is sandy loam *Hapli Albic Luvisol*. In the long-term experiment the tests were done in a four – course crop rotation. Potatoes were grown without and with farmyard manure (40 t ha<sup>-1</sup>) combined with various mineral fertilization (N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>; N<sub>90</sub>P<sub>90</sub>; P<sub>90</sub>K<sub>120</sub>; N<sub>90</sub>K<sub>120</sub>; N<sub>90</sub> P<sub>90</sub>K<sub>120</sub>).

The long-term experimental data suggest that farmyard manure (FYM) increased potato tuber yield by 35 - 82 %, depending on fertilizer combinations. Mineral fertilizer efficacy on the background without FYM was by 28 % higher. The highest tuber yield increases (3.8–6.0 t ha<sup>-1</sup>) were obtained having used fertilizer combinations with nitrogen. These combinations increased tuber yield on both backgrounds by 32-93 %. Potato crops applied with only mineral fertilizers contained higher starch and dry matter contents in tubers compared with those applied with FYM and mineral fertilizers. Fertilizer combinations with potassium tended to reduce starch and the dry matter content in tubers.

**Key words:** potatoes, starch, dry matter, farmyard manure, mineral fertilizers

### Introduction

Organic and mineral fertilization improves light-textured soils' physical properties and water and warmth regime. Systematic fertilization not only increases crop yield but also alters its quality, and results in the higher buildup of nutrients in the yield (Bagdoniene *et al.*, 1998). Crop yield and mineral fertilizer efficacy depend on the contents of available phosphorus, potassium and mineral nitrogen in the soil (Simanaviciene *et al.*, 1996, Shield *et al.*, 1997). It has been found that nutrients present in mineral fertilizers are more effective than the equivalent amount of these nutrients present in farmyard manure (Bagdoniene *et al.*, 1998), therefore mineral fertilizer efficacy for potatoes was noticeably higher than that of organic fertilizer (Antanaitis *et al.*, 2000). Depending on the mineral fertilizer forms, rates and nutrient ratios, the contents of dry matter, starch, protein and other substances may either increase or decrease. Excessive nitrogen fertilization reduces starch, dry matter and sugar contents in tubers and potatoes go bad more rapidly during storage (Simanaviciene *et al.*, 1996). This results from the fact that nitrogen promotes growth of potato vines, and when lasting drought occurs, the vines that have grown the largest, are most dramatically affected and the growing season is extended, therefore by the time of harvesting, tubers may not have been able to mature completely and reach maximal dry matter content, the starch is accumulated less rapidly, and part of it is used for respiration (Amberger *et al.*, 1997). Dry matter content is affected by various factors, among which the most significant are the following ones: tuber maturity, growth character, plant nutrient and water uptake (Haris, 1992). They were designed to estimate the effects of organic and mineral fertilizer combinations on potato tuber yield and quality.

### Materials and Methods

The long term tests were done in a four-course crop rotation (1. winter rye, 2. potatoes, 3. spring barley, 4. lupine and oats mixture for green manure). Potatoes were grown with and without farmyard manure (FYM), according to the following mineral fertilization scheme: 1. N<sub>0</sub> P<sub>0</sub>K<sub>0</sub> 2. N<sub>90</sub>P<sub>90</sub> 3.P<sub>90</sub>K<sub>120</sub> 4. N<sub>90</sub>K<sub>120</sub> 5.N<sub>90</sub> P<sub>90</sub>K<sub>120</sub>.

The soil of the experimental site is sandy loam *Hapli Albic Luvisol*. Soil agrochemical characteristics: pH – 4.1-5.7, P<sub>2</sub>O<sub>5</sub> - 187-320, K<sub>2</sub>O– 193-251 mg kg<sup>-1</sup>, humus – 1.10-1.34 %.

Potatoes and other crop rotation plants were cultivated following the soil and crop management practices recommended for light soils. Depending on the weather conditions, potatoes were planted at the end of April – first half of May. The tests involved potato cultivars ‘Vokē’ and ‘Goda’ planted at a seed rate of 3.5 - 4 t ha<sup>-1</sup> and replicated four times.

Mineral fertilizers – ammonium nitrate, granulated superphosphate and potassium chloride were spread in spring prior to potato planting and were incorporated into the soil by a harrow. Solid FYM 40 t ha<sup>-1</sup> was applied in the autumn before ploughing. Chemical plant protection agents were used according to the need against the Colorado beetle and potato late blight. Before harvesting, 5 potato plants per plot were removed for tuber sampling (in total 40 samples). The tubers were tested for starch content (g kg<sup>-1</sup>) by specific weight Demin method and for dry matter content. Experimental data were processed by ANOVA and correlation-regression analysis methods.

## Results and Discussion

The present paper presents the results from the last three rotations (IX (1994-1997), X (1998-2001) and XI (2002-2005)). The data averaged over 12 years suggest that potato tuber yield, starch and dry matter contents varied in relation to organic and mineral fertilization and the weather conditions.

Moisture is an important factor when seeking high crop yields in light-textured soils. Comparison of the three rotations indicated that the highest yields were obtained in the XI rotation, when the precipitation amount that fell during the growing season amounted to 392 mm, while the lowest yields were obtained in the IX rotation with a mean precipitation amount of as low as 279 mm. The relationship between the yield and precipitation amount is expressed by the equation: without FYM -  $y = 0.0044x + 3.6455$ ,  $r^2 = 0.9578$ ; with FYM -  $y = 0.0073x + 4.9076$ ,  $r^2 = 0.9997$ . Mineral fertilizer efficacy for potatoes on the background without FYM was by 28 % higher (Table 1).

Under the effect of these fertilizers potatoes yielded best having used fertilizer mixtures with nitrogen. Higher starch and dry matter contents were identified in potato tubers grown without FYM. Phosphorus did not have any appreciable effect on tuber quality. Mineral fertilizers, especially in combinations with potassium fertilizer, tended to reduce both starch and dry matter content.

Table 1. The effect of mineral and organic fertilizers on potato tuber yield, starch and dry matter contents (Perloja, data averaged over 1994-2005)

Mineral fertilizers	Organic fertilizers							
	Without manure				With manure			
	Rotation							
	IX	X	XI	average	IX	X	XI	average
	Yield t ha <sup>-1</sup>							
0	5.33	6.76	7.13	6.41	10.30	11.92	12.67	11.63
N <sub>90</sub> P <sub>90</sub>	9.10	10.88	11.34	10.44	14.85	15.40	17.40	15.88
P <sub>90</sub> K <sub>120</sub>	7.73	8.07	8.44	8.08	12.05	13.57	14.75	13.46
N <sub>90</sub> K <sub>120</sub>	7.55	10.44	12.68	10.22	12.48	15.43	18.26	15.39
N <sub>90</sub> P <sub>90</sub> K <sub>120</sub>	12.30	12.43	12.48	12.40	15.35	16.32	18.22	16.63
LSD <sub>05</sub>	0.83	0.63	0.91	2.24	0.94	1.06	1.23	1.44
	starch content g kg <sup>-1</sup>							
0	179.5	165.8	165.9	170.4	171.8	147.7	156.1	158.5
N <sub>90</sub> P <sub>90</sub>	184.5	157.7	161.5	167.9	176.8	144.9	154.1	158.6
P <sub>90</sub> K <sub>120</sub>	158.0	146.3	146.9	150.4	156.0	137.6	144.3	146.0
N <sub>90</sub> K <sub>120</sub>	151.8	149.2	144.3	148.4	152.3	130.5	141.0	141.2
N <sub>90</sub> P <sub>90</sub> K <sub>120</sub>	163.0	144.1	150.3	152.5	155.3	133.3	145.5	144.7
LSD <sub>05</sub>	0.85	0.86	0.51	0.88	1.07	0.73	0.45	0.58
	dry matter g kg <sup>-1</sup>							
0	242.9	240.8	241.7	241.8	224.6	226.4	218.8	223.3
N <sub>90</sub> P <sub>90</sub>	247.7	243.7	239.1	243.5	224.8	232.1	219.2	225.4
P <sub>90</sub> K <sub>120</sub>	209.9	225.9	220.3	218.7	208.3	201.5	210.8	206.9
N <sub>90</sub> K <sub>120</sub>	207.5	227.0	218.6	217.7	206.1	217.9	218.4	214.1
N <sub>90</sub> P <sub>90</sub> K <sub>120</sub>	215.3	225.5	222.3	221.0	210.5	205.1	211.7	209.1
LSD <sub>05</sub>	1.00	1.80	1.53	1.08	0.79	2.35	3.28	1.11

It was found that application with FYM and mineral fertilizers gave a tuber yield increase of 61 %, compared with the yield obtained using only mineral fertilizers (Repšiene *et al.*, 2006). Other authors indicate that incorporation of 50 and 60 t ha<sup>-1</sup> FYM can give a tuber yield increase of 20 and 23 %, respectively (Ciganov *et al.*, 2001, Simanaviciene *et al.*, 2001).

Our experimental evidence shows that depending on the mineral fertilizer combination, FYM increased potato tuber yield by on average 35 - 82 %, compared with potatoes applied with only mineral fertilizers (Fig. 1). The effects of FYM on potato tuber yield is described by the following regression equation  $y = 0.8634x + 6.3874$ ,  $r^2 = 0.9654$ . FYM application improves potato growth, development and nutrition conditions, extends nutrient uptake time, which in turn results in a higher tuber yield (Birietiene *et al.*, 1994).

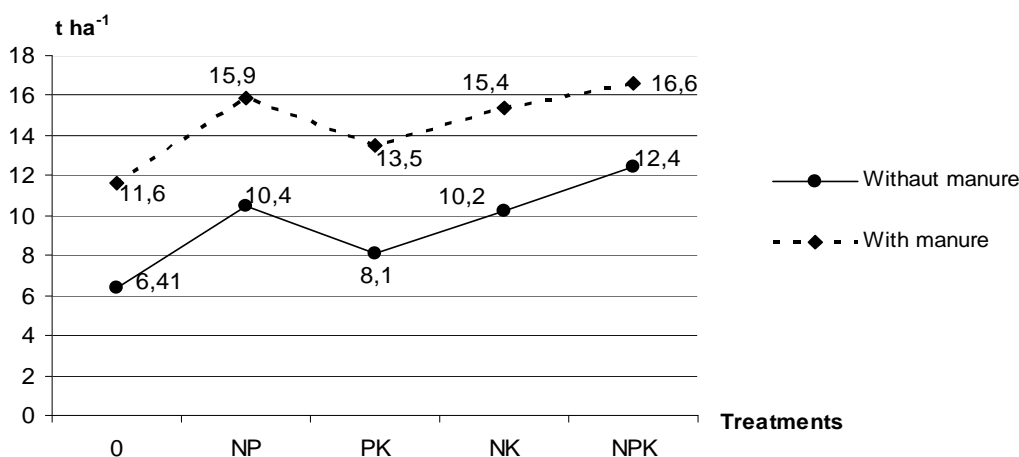


Figure 1. The effect of organic and mineral fertilizers on potato tuber yield, t ha<sup>-1</sup>.

To produce 20 t ha<sup>-1</sup> of tuber and 10 t ha<sup>-1</sup> vine yield, potatoes utilize about 100 kg of nitrogen, 40 kg of P<sub>2</sub>O<sub>5</sub> and 180 kg of K<sub>2</sub>O from the soil (Simanaviciene *et al.*, 2001). It was determined that in the soils high in phosphorus, phosphorus fertilization did not result in any appreciable tuber yield increase (Mažvila, 1998). Research done in Germany has shown that, taking into account the status of potassium in the soil, potassium fertilizers used may not result in any yield increase, and potassium chloride may reduce starch content in tubers (Pienz, 1999). In our research the most vital element for potatoes was nitrogen. The highest yield increases (3.8-6.0 t ha<sup>-1</sup>) were obtained using fertilizer combinations with nitrogen (Figure 1). The combination of only PK increased the yield only insignificantly. The highest dry matter and starch contents were accumulated in the tubers of crops fertilized with mineral, not organic fertilizers (Stimumar *et al.*, 1990).

However, other researchers have reported that higher starch contents in tubers accumulate when the crop is fertilized with only FYM or with FYM and low mineral nitrogen and potassium fertilizer rates (Lazauskas *et al.*, 2001).

Our findings suggest that tuber starch content and dry matter directly depended on FYM fertilization. These relationships can be expressed by the following equations: starch -  $y = 0.7737x + 2.7637$ ,  $r^2 = 0.9688$ , dry matter -  $y = 0.5957x + 7.9624$ ,  $r^2 = 0.8643$ .

Potatoes applied with only mineral fertilizers had by 4.5 - 11.9 g kg<sup>-1</sup> higher starch content, by 3.6 - 18.5 g kg<sup>-1</sup> higher dry matter content compared with the fertilization treatment applied with 40 t ha<sup>-1</sup> FYM and mineral fertilizers. However, mineral fertilizers, compared with unfertilized treatment, reduced starch content by 1.5 -12.9 and dry matter content by 0.7 - 10.0 percent (Figure2).

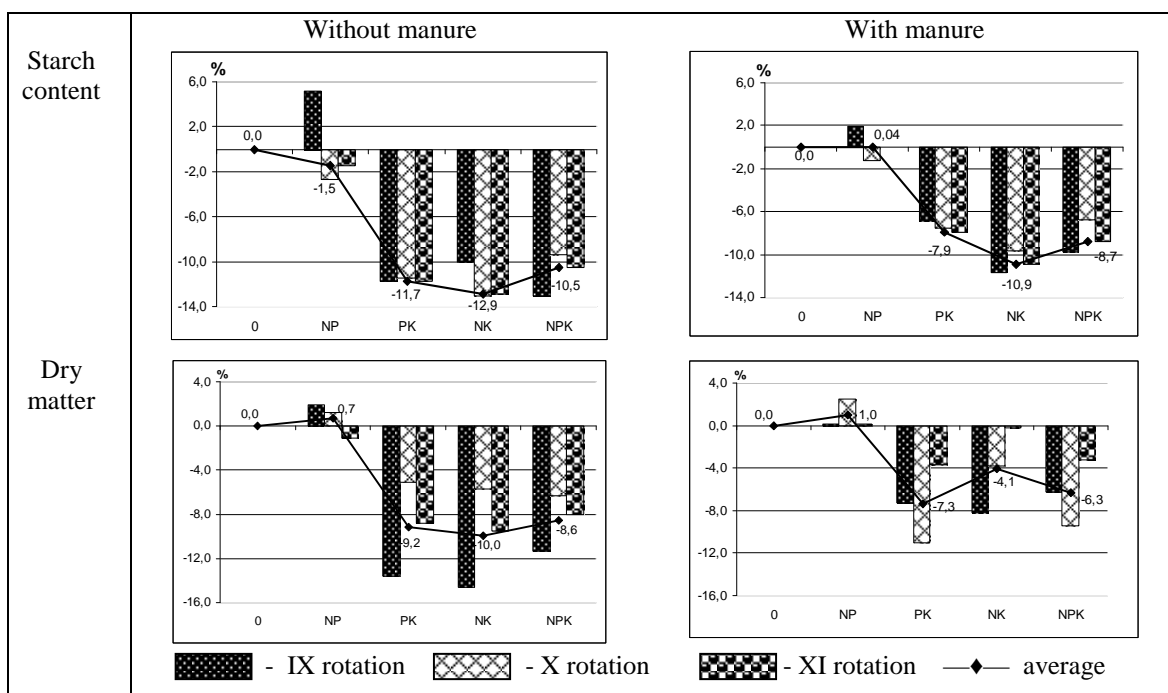


Figure 2. Starch and dry matter variation (percent) in tubers in relation to mineral fertilizer combinations.

To achieve high tuber quality it is recommended to fertilize less with nitrogen and to increase phosphorus and potassium fertilizer rates (Vos, 1999). With the application of moderate potassium fertilizer rates starch content in tubers increases, compared with that in tubers that had received low potassium fertilization. However, with more abundant potassium application the contents of starch and dry matter tend to decline (Pienz, 1999).

Our research evidence shows that potassium fertilizers reduced starch content in tubers by on average 1.3-2.2, and dry matter by 0.9-2.4 percentage units, compared with completely unfertilized or fertilized with only NP tubers (Figure 2). The variations of starch and dry matter contents in potato tubers grown on the background of FYM were lesser than those of potatoes grown without FYM.

## Conclusions

Depending on fertilizer combinations, farmyard manure gave a potato tuber yield increase of 38 - 82 %.

Of mineral fertilizers, the most effective were found to be the phosphorus and potassium combinations with nitrogen fertilizer, under the effect of which the yields increased by 32-93 %.

Potatoes fertilized with only mineral fertilizers had higher starch content and the highest dry matter contents, compared with those fertilized with FYM and mineral fertilizers.

Fertilizer combinations with potassium tended to decline starch and dry matter contents in tubers.

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## MINERĀLĀ UN ORGANISKĀ MĒSLOJUMA IETEKME UZ KARTUPEĻU BUMBUĻU RAŽU UN KVALITĀTI

**Baniuniene A., Zekaite V.**

Izmēģinājumi tika veikti Lietuvas Zemkopības institūta Perloja izmēģinājumu stacijā laika periodā no 1994.-2005. gadam. Tie tika plānoti, lai novērtētu organiskā un minerālmēslojuma kombināciju ietekmes uz kartupeļu bumbuļu ražu un kvalitāti. Ilglaicīgais izmēģinājums tika veikts smilšmāla augsnē *Hapli Albic Luvisol* četrū-lauku augu sekā. Kartupeļi tika audzēti bez un ar kūtsmēsliem (40 t ha<sup>-1</sup>) kombinācijā ar dažādu minerālmēslojuma devu (N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>; N<sub>90</sub>P<sub>90</sub>; P<sub>90</sub>K<sub>120</sub>; N<sub>90</sub>K<sub>120</sub>; N<sub>90</sub>P<sub>90</sub>K<sub>120</sub>).

Dati liecina, ka atkarībā no mēslojuma kombinācijas kūtsmēsli (FYM) palielina kartupeļu bumbuļu ražu par 35 - 82 %. Minerālmēsli iedarbība fonā bez FYM bija līdz 28 % augstāka. Visaugstākie bumbuļu ražas pieaugumi (3.8–6.0 t ha<sup>-1</sup>) tika iegūti, izmantojot mēslojuma kombinācijas ar slāpekli. Šīs kombinācijas palielināja bumbuļu ražu abos fonos par 32-93 %. Minerālmēsli fonā bumbuļiem bija augstāks cietes un sausnas saturs. Mēslojuma kombinācijas ar kāliju sekmēja cietes un sausnas saturu samazināšanos bumbuļos.

## OPTIMIZATION OF ENVIRONMENTALLY FRIENDLY CULTIVATION TECHNOLOGY OF OILSEED RAPE UNDER LATVIA AGROECOLOGICAL CONDITIONS.

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### Abstract

Field trials were carried out on the soddy-podzolic sandy clay soil of the Research institute of agriculture in Skrīveri. The effect of increasing the rates of nitrogen and potassium fertilizers (N<sub>60</sub>K<sub>60</sub>, N<sub>80</sub>K<sub>80</sub>, N<sub>100</sub>K<sub>100</sub>, N<sub>120</sub>K<sub>120</sub>, N<sub>140</sub>K<sub>140</sub>) kg ha<sup>-1</sup> on the spring oilseed rape and quality was tested against the phosphorus background P<sub>70</sub>. During the trial were tested half rates (0.5 t ha<sup>-1</sup>) of the container mixtures of growth regulators and fungicides: Moddus + Folicur, Moddus + Juventus; Cycocel + Folicur, Cycocel + Juventus, by spraying them throughout plant florescence-bud formation. The results of three-year field trials show that the application of nitrogen and potassium

fertilizers ensured a positive influence on the seed yields and the quality of seeds of spring oilseed rape. The highest seed yield was obtained when introducing  $N_{120}K_{120} - 3.25 \text{ t ha}^{-1}$ . The use of NK fertilizers promoted the accumulation of protein by 22-24 %, but decreased the content of oil 47-44 % in the seeds. Totally, oil yield made up 977-1341  $\text{kg ha}^{-1}$  depending on the rates of mineral fertilizers. The highest oil yield – 1341  $\text{kg ha}^{-1}$  was obtained when  $N_{120}K_{120}$  was applied. The application of the studied mixtures of preparations ensured the yield increase of seeds 0.62-0.91  $\text{t ha}^{-1}$  and oil yield to 282-441  $\text{kg ha}^{-1}$ . The best preparation mixture was the Cycocel + Folicur, what provided the oil yield 1389  $\text{kg ha}^{-1}$ .

**Key words:** spring rape, fertilizers, growth regulators, fungicides, yield

### Introduction

Beside seed productiveness, during the cultivating of spring rape, of great importance is the biochemical content of the seed rape that characterize the food and nutrient values of the culture. The research made by many scientists proves that the content of the seed rape, to a great extent, depends on weather conditions, the variety, and the agrotechnical cultivation methods (Яковчик, 2006, ЖОЛИК, 2006). Nutrient deficiency quickly causes interruption of growth, yield and seed quality inhibition of spring oilseed rape. Phosphorus is necessary for the formation of the strong assemblage of rootlets, increase of seed yields, and acceleration of maturation. Oilseed rape plants are supplied with phosphorus mainly at the expense of reserves in soil (70-80 %) as oilseed rape assimilates well the background phosphorus from the soil (Schroder, 1992).

There is no consensus of opinion in the scientific literature regarding the influence of potassium on the spring oilseed rape seed yields and seed quality. According to V.P. Savenkov (2000), application of potassium fertilizers has not lead to substantial increase in the seed yields, and had just a slight influence on the protein content and oil content of oilseed rape seeds. The tests carried out in Germany show that full-value nutrition increases both the seed yield, by 0.02-0.03  $\text{t ha}^{-1}$ , and the oil content in the seeds.

It is considered that the basic element that affects the yield and the seed quality is the nitrogen fertilizer. Provided that a correctly set dose is applied, nitrogen increases the seed yields while overdosage can promote lodging of plants, outbreak of fungi diseases and the reduction of oil content in seeds (Шпаар и др., 1999). In order to achieve sufficient formation of the seed yields components, a split of the introduced doses of nitrogen fertilizer is recommended. The first dose of fertilizer 60-80  $\text{kg ha}^{-1}$  was introduced before sowing, or immediately after it while the second - 40-60  $\text{kg ha}^{-1}$  – at the stage of formation of stems (Beer *et al.*, 1990; Cramer, 1990; Feger, Orlovius, 1995; Finck, 1991; Fruchtenicht *et al.*, 1993; Sturm *et al.*, 1994).

The influence of the growth regulators on the yield of seeds was studied, in essence, on the winter rape, for the purpose of the shortening of the stem of plants, the stimulation of the formation of lateral flights and auxiliary buds, reduction of the danger of lodging, guaranteeing the high content of oil and uniform ripening of pods on the main and lateral flights (Makowski, Gienapp, 1998; Vořak a kolektiv, 2000; Шпаар и др., 1999).

There is an assumed hypothesis that the use of nitrogen-potassium fertilizers and container mixtures of growth regulators and fungicides affect the yield and the quality of spring rapeseeds.

### Materials and methods

Field trials in 2001– 2004 were conducted in soddy - podzolic sandy clay pH- 6.2, organic matter content 33  $\text{g kg}^{-1}$  (method of Turin),  $P_2O_5$  content (high) 195  $\text{mg kg}^{-1}$ ,  $K_2O$  content (medium) 147  $\text{mg kg}^{-1}$  (DL method). The clay parameters fit for oilseed rape cultivation. Their predecessor was autumn fallow. Conventional farming techniques were used. Prior to sowing the spring oilseed rape `Olga`, there were applied mineral fertilizers  $P_2O_5$  70  $\text{kg ha}^{-1}$  in the form of superphosphate,  $K_2O$  in the form of potassium chloride, and nitrogen in the form of ammonium nitrate. The following NK rates were studied: 0, 60, 80 (60+20), 100 (60+40), 120 (60+60) and 140 (60+80)  $\text{kg ha}^{-1}$ .  $N_{60}K_{60}$  was introduced as a basic fertilizer, and at the stage of stem formation 20-80  $\text{kg ha}^{-1}$  according to variants.

Field trials were tested using the following preparations with the aim to increase the impact performed by the applied growth regulators/fungicide mixture on the seeds yield and quality and efficiency concerning disease elimination: fungicide Folicur (tebucanazole 125  $\text{g l}^{-1}$ , triadimephone

100 g l<sup>-1</sup>), Juventus (metconazole– 60 g kg l<sup>-1</sup>), and plant growth regulators. Moddus 250 e.k (trinexapac-ethyl 250 g l<sup>-1</sup>) and Cycocel 750 (chlormequate chloride 750 g l<sup>-1</sup>). During the trials preparations were tested applying half doses (0.5 l ha<sup>-1</sup>), creating the preparation mixtures: Modus + Folicur, Modus + Juventus; Cycocel + Folicur; Cycocel + Juventus, by spraying them throughout plant florescence-bud formation (GS 50). As a rule, preparation effectiveness within mixtures increased. Data analysis was done by ANOVA (Arhipova, Bāliņa, 2003). Interactions between factors were calculated using the dispersion analysis.

## Results and Discussions

There is a point of view that that mineral fertilizers had exerted a positive influence on growth and development of spring oilseed rape plants and this, in the end, redounded considerably upon the volume and quality of harvest of seeds (Пилюк, Белявский, 1999; Буряков, 1990). In our research, the seed yields of spring oilseed rape 'Olga' fluctuated from 2.24 – 3.25 t ha<sup>-1</sup>. The use of nitrogen and potassium fertilizers at the rate of 60 kg ha<sup>-1</sup> to 120 kg ha<sup>-1</sup> substantially increased the oilseed yield. The further increase of nitrogen and potassium rates did not lead to rise of the spring oilseed rape seed yields (Figure 1). The highest seed yield was obtained when introducing N<sub>120</sub>K<sub>120</sub> – 3.25 t ha<sup>-1</sup> rape oilseeds. The increase of harvest when introducing nitrogen 60-140 kg ha<sup>-1</sup> and potassium 60-140 kg ha<sup>-1</sup> fertilizers was 0.64-1.01 t ha<sup>-1</sup> (LSD<sub>0,05</sub>= 0.31 t ha<sup>-1</sup>).

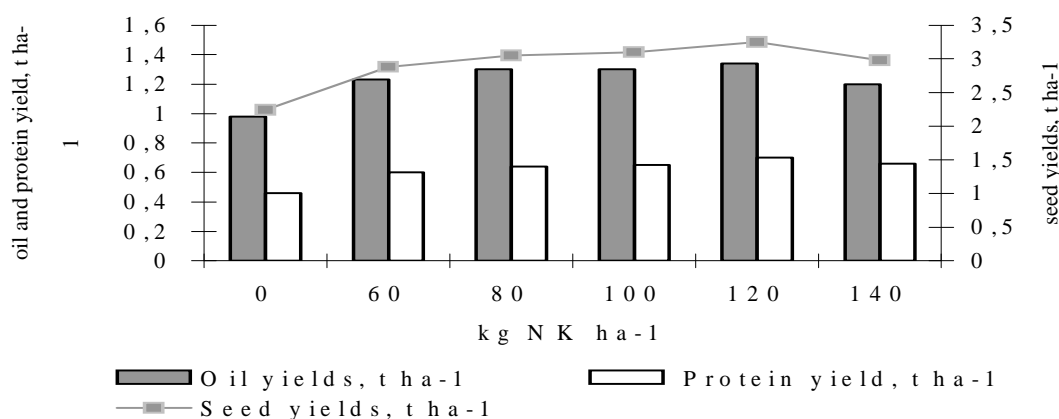


Figure 1. Productivity of spring rape oilseeds in dependence of NK mineral fertilizers

Mineral fertilizers caused a considerable increase in protein content of the spring oilseed rape seed yields and that corresponds to the research results of V.P. Savenkov (Савенков, 2007). As a result of the introduction of nitrogen and potassium fertilizers, crude protein content increased by 0.47-2.10% (Figure 2). The change in crude protein content in spring oilseed rape seeds under the influence of different rates of NK fertilizers can be represented with straight-line regression equation  $y = 0.0139x + 21.81$ ;  $R^2 = 0.8841$  (Figure 3). The value of the coefficient of correlation ( $r = 0.91$ ) points to a close linear relationship existing between the searched factors. With the increase of NK fertilizer rate, the oil content gets lower for 0.86 – 3.54 %, and there has been noted a close correlative feedback ( $r = -0.97$ ). The change of oil content is reflected by regression equity  $y = -0.0243x + 47.753$ ;  $R^2 = 0.9325$  (Figure 4). The quality indicators are in a close reciprocal reverse correlative dependence ( $r = -0.98$ ).



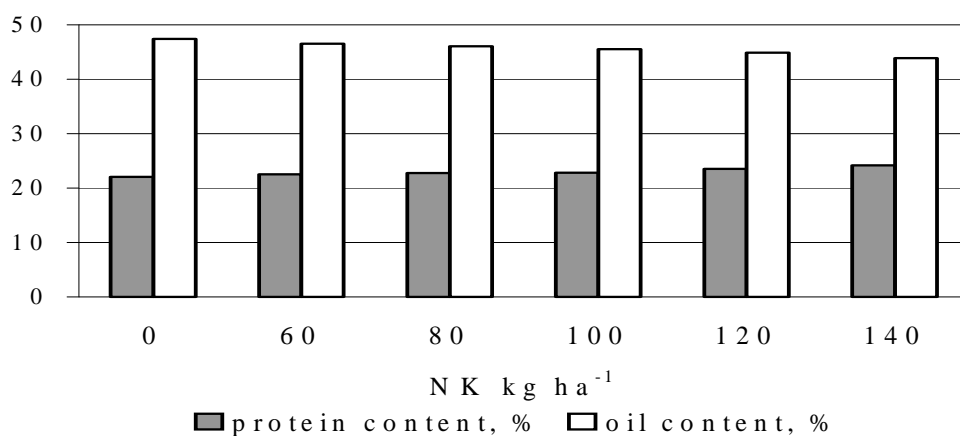


Figure 2. Oil and protein content in spring rape seeds in dependence of NK mineral fertilizers

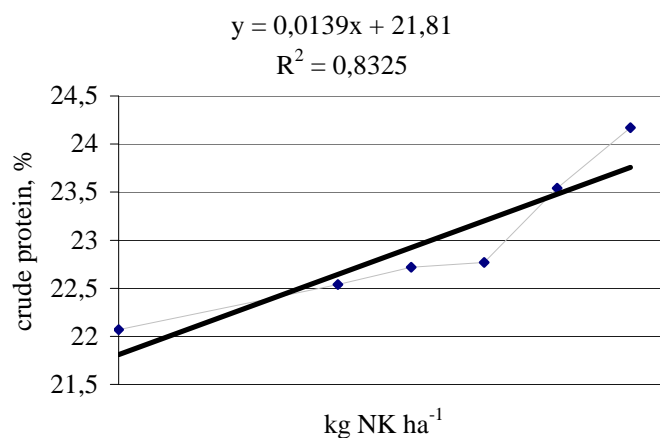


Figure 3. Linear regression interrelation between the content of protein in the seeds of spring rape and the rates of the NK fertilizers

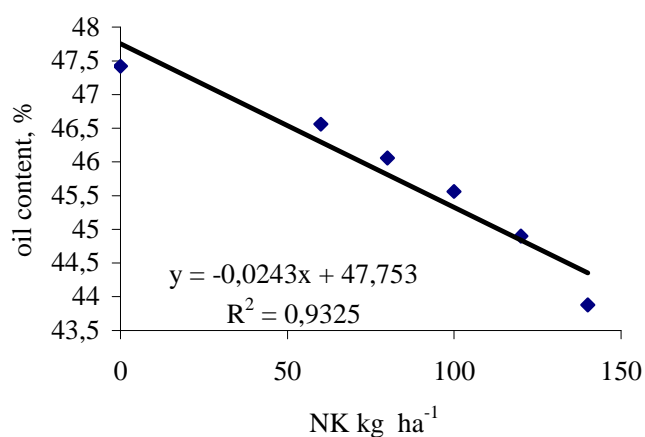


Figure 4. Linear regression interrelation between the content of oil in the seeds of spring rape and the rates of the NK fertilizers

In spite of the reduction in the oil content of the seeds, the total yield of oil made up 977-1341 kg ha<sup>-1</sup> depending on rates of mineral fertilizers. The highest yield of oil - 1341 kg ha<sup>-1</sup> was obtained when introducing 120 kg ha<sup>-1</sup> of nitrogen and 120 kg ha<sup>-1</sup> of potassium fertilizers.

The results of field experiences confirm that the application of growth regulators in the mixture with the fungicides had a positive impact on the yields of the seeds and seed quality of spring rape. (Table 1). On average, throughout all the three years, thanks to the application of growth regulators and fungicide mixtures the summer rape seed yields essentially increased ( $F=20.16 > F_{crit} = 3.68$ ;  $P = 5.61E-0.5 < 0.05$ ). The increase of yield was 0.62-0.91 t ha<sup>-1</sup> ( $LSD_{0,05} 0.35$  t ha<sup>-1</sup>). The applying of preparations, a full rate, or in mixture with smaller rates, did not cause any important differences. The highest growth within the seed yield 0.91 t ha<sup>-1</sup> was ensured when applying the container mixture of Cycocel + Folicur, during the developing of rape (GS 50). Applying the fungicides in a mixture with growth regulators, in the beginning stage of rape budding, a steadier blossoming and ripening of pods on the main and lateral shoots was ensured increasing the accumulation of oil content in the seeds by 0.78 – 2.14 %. The most effective method was the applying of the mixture Cycocel + Juventus, and Cycocel + Folicur when the oil yield made up 1310-1389 kg ha<sup>-1</sup>. The research results confirmed that applying the mixture of preparations only once, in smaller doses, with the aim to increase the productiveness and quality of rapeseeds simultaneously reduced the chemical load upon one unit of the area, and reduced pollution, therefore the research was justified.

Table 1. The influence of different mixtures of growth regulators and fungicides on the spring rape `Olga` yield and seed quality

Variants	Seed yield, t ha <sup>-1</sup>	Protein		Oil	
		content, %	yield, kg ha <sup>-1</sup>	content, %	yield, kg ha <sup>-1</sup>
Control	2.30	22.75	489	45.08	948
Moddus AS 30 un Folicur AS 70	2.92	22.96	627	45.86	1230
Moddus + Folicur AS 50	3.04	21.16	629	46.40	1288
Moddus + Juventus AS 50	2.96	22.36	619	46.54	1260
Cykocel + Folicur AS 50	3.21	22.54	670	47.22	1389
Cykocel + Juventus AS 50	3.04	22.43	635	47.20	1310
$LSD_{0,05}$	0.35	0.94	74.99	1.02	160.8

### Conclusions

The results of the four-year field trials show that the introduction of nitrogen and potassium fertilizers at the rate from N<sub>60</sub>K<sub>60</sub> to N<sub>140</sub>K<sub>140</sub> kg ha<sup>-1</sup> considerably increased the yields of spring oilseed rape. A significant increase of seed yields 1.01 t ha<sup>-1</sup> was obtained when introducing N<sub>120</sub> K<sub>120</sub> kg ha<sup>-1</sup> and oil yield 364 kg ha<sup>-1</sup>.

The introduction of mineral fertilizers exerts a considerable influence on the qualitative content of the oil seeds of spring oilseed rape. The increase in the rates of nitrogen and potassium fertilizers leads to increases in protein content and a decrease in the oil content of the seeds.

Applying fungicides and growth regulators in mixtures, in minified doses, provides evenness of florescence and legume ripening, and essential increase of rape oilseed yield, improving its quality as well.

The application of the studied mixtures of preparations ensured the yield increase of seeds 0.62-0.91 t ha<sup>-1</sup> and oil yield to 282-441 kg ha<sup>-1</sup>.

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## VIDEI DRAUDZĪGU AUDZĒŠANAS TEHNOLOĢIJU OPTIMIZĀCIJA EĻĻAS RAPSIM LATVIJAS AGROEKOĻOĢISKĀJOS APSTĀKĻOS

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LLU Zemkopības zinātniskajā institūtā Skrīveros velēnu vāji podzolētā mālsmilts augsnē vasaras rapša sējumos veikti izmēģinājumi, kuros uz fosfora P<sub>70</sub> fona pētīta slāpekļa un kālija mēslojuma devu: (N<sub>60</sub>K<sub>60</sub>, N<sub>80</sub>K<sub>80</sub>, N<sub>100</sub>K<sub>100</sub>, N<sub>120</sub>K<sub>120</sub>, N<sub>140</sub>K<sub>140</sub>) kg ha<sup>-1</sup> ietekme uz sēkļu ražu un tās kvalitāti. Izmēģinājumos pārbaudīti sekojoši preparātu maisījumi ar samazinātām devām (0.5 l ha<sup>-1</sup>): Moddus + Folikūrs, Moddus + Juventus, Cikocels + Folikūrs, Cikocels + Juventus. Maisījumi izsmidzināti augu ziedpumpuru veidošanās fāzē. Trīs gadu lauka izmēģinājumos iegūtie rezultāti rāda, ka NK mēslojums pozitīvi ietekmēja vasaras rapša augšanu un attīstību, sēkļu ražu un tās kvalitāti. Vislielāko sēkļu ražas pieaugumu – 3.25 t ha<sup>-1</sup> nodrošināja N<sub>120</sub>K<sub>120</sub> deva. NK mēslojuma pielietošana veicināja kopproteīna uzkrāšanos (22-24%), bet koptauku samazināšanos (47-44%) sēklās. Augstāko eļļas iznākumu – 1341 kg ha<sup>-1</sup> nodrošināja mēslojuma deva N<sub>120</sub>K<sub>120</sub>. Vidēji trijos gados augšanas regulatoru un fungicīdu maisījumu pielietošanas ietekme uz vasaras rapša sēkļu ražu bija būtiska. Salīdzinājumā ar kontroli, ražas pieaugums sastādīja 0.62-0.91 t ha<sup>-1</sup>. Labākais preparātu maisījums bija Cikocels + Folikūrs, kas nodrošināja 1389 kg ha<sup>-1</sup> eļļas iznākumu.

## EFFICIENCY ESTIMATION OF SOIL DEEP-TILLAGE FOR WINTER WHEAT BY USING PRECISION TECHNOLOGIES OF CROP CULTIVATION

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### **Abstract**

For the completion of the work the following aim is set: the establishing of soil deep-tillage efficiency in winter wheat industrial areas in conditions of rugged field macro relief using data of the farm management local GIS and soil research. The work was performed at the Latvia University of Agriculture Research and Study farm "Vecauce" from 2005 to 2007. During the research in stationary with GPS established points lightly soil penetration resistance and soil moisture spots were measured to the depth of 50 cm and soil samples were taken to determine its

grading composition in the topsoil. Harvest was detected with combine CLASS LEXION 420 GPS facilities. It was established that deep loosening of soil should be carried out when the penetrometric resistance of soil exceeded 600 kPa cm<sup>-2</sup>.

**Key words:** GPS, GIS, soil resistance, soil moisture

**Introduction**

During recent years several elements of precision agriculture are appearing in the Latvian farms, firstly, the equipment of grain harvesters for the formation of the yield charts, next, the application of the yield chart is connected with the investigation into soil properties (Lapins et al. 2007). This means that the farms should form their own local geographic information system (GIS). Research materials of the soil properties form the basis of the introduction of differentiated, GIS-based resources-saving soil tillage. Already in previous years the research of the possibilities to optimize the measures of soil tillage by using the GPS was conducted in Latvia (Lapins et al. 2006). The aim of the research was to investigate correlations among yield, soil resistance, soil humidity in different meteorological circumstances, as well as to evaluate the efficiency of deep soil tillage for winter wheat in autumn 2005. That would allow for the adjustment of the criteria recommended for implementing site specific soil tillage in the farms.

**Materials and Methods**

Field trials were carried out at the Research and Study farm “Vecauce” of the Latvia University of Agriculture during the years 2005 to 2007. Winter wheat was investigated in 47 stationary points set up by GPS; the crop rotation was winter wheat after winter wheat. Meteorological conditions were different between the trial years: the average air temperatures were above the long term mean in both trial years, especially in the second part of the year 2006 (Figure 1). The average temperature of July 2006 was 3.5 °C higher than that observed over the long term. Along with insufficient precipitation this caused rapid ripening and the early harvesting of winter wheat. The total of precipitations was low in both trial years, but during April – August in 2007 it was lower if compared to the same period in 2006 despite a high amount of precipitation in August 2006 (Figure 2).

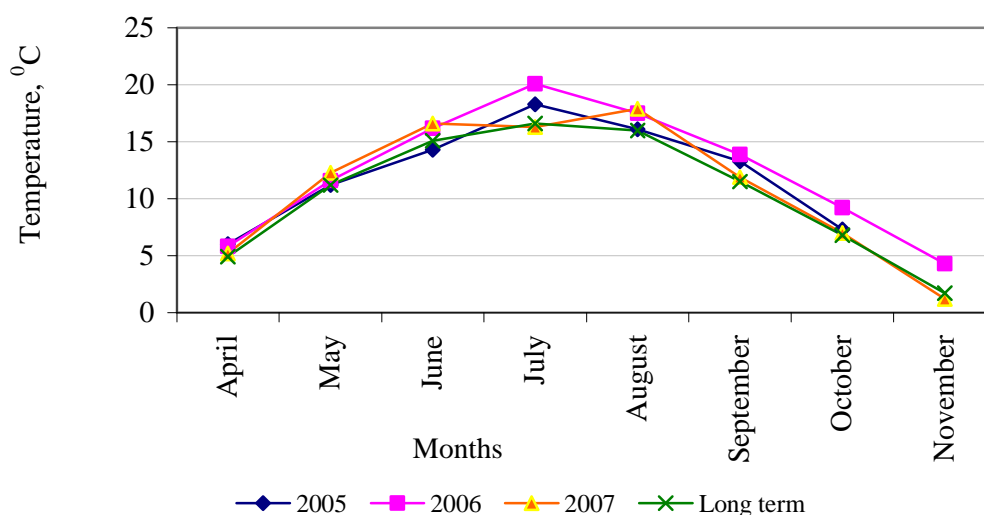


Figure 1. The average day and night air temperatures in the years 2005 – 2007, °C (In specific year from Metpole in Vecauce, long-term average – from Dobeles HMS)

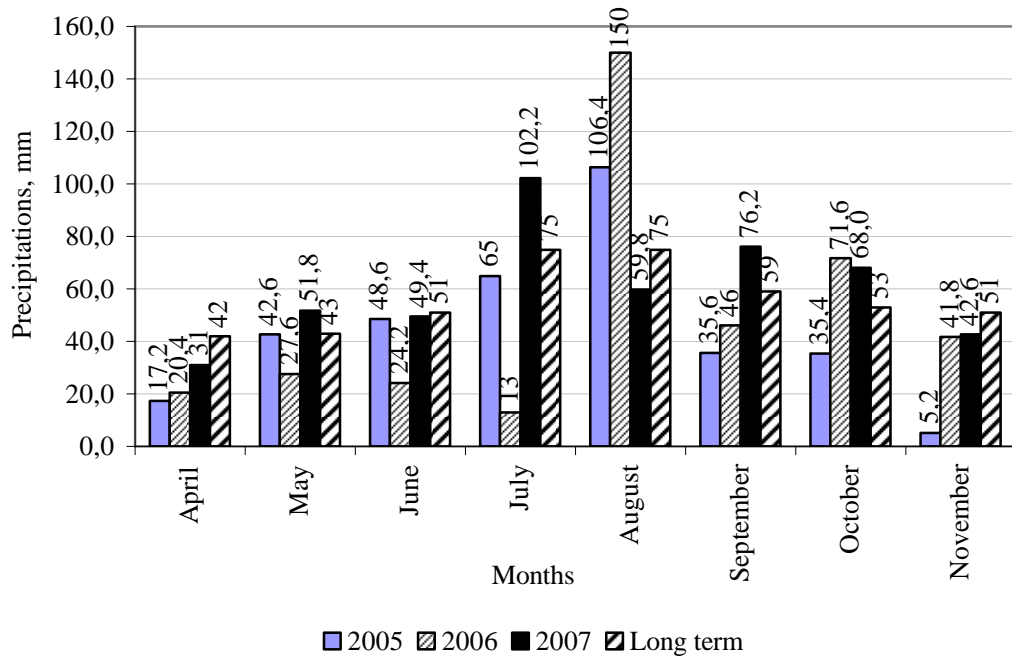


Figure 2. The average amount of precipitation in the years 2005 – 2007, mm  
(In specific year from Metpole in Vecauce, long-term average – from Dobeles HMS).

Soil characteristics in “Kurpnieki” field: sod podzolic loam soil, humus content 14 - 91 g kg<sup>-1</sup>, soil reaction pH<sub>KCL</sub> 6.0 - 7.4, phosphorus content 102 - 394 mg kg<sup>-1</sup> and potassium content 102 - 333 mg kg<sup>-1</sup>. Relief – wavy low-land, area with marked mezzo-relief (Figure 3). Field has a closed drainage system.

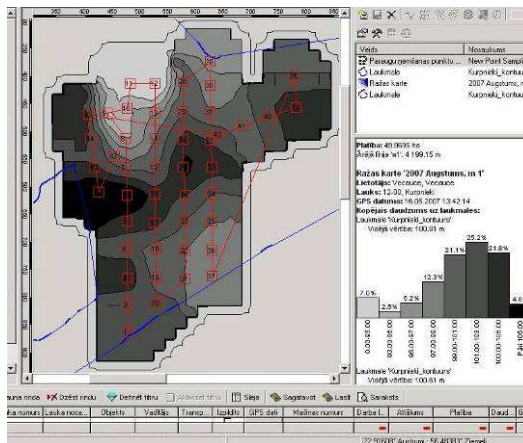


Figure 3. Cartogram of surface altitude in “Kurpnieki” field, m.

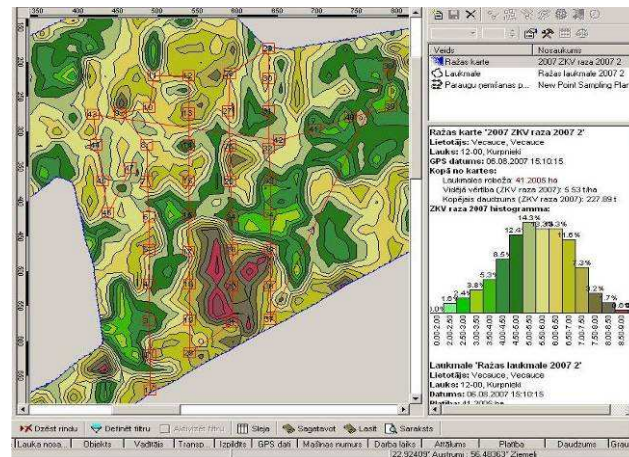


Figure 4. Winter wheat grain yield cartogram of “Kurpnieki” field in year 2007.

Deep soil tillage (until 50 cm) in autumn 2005 was done before the sowing of winter wheat. The same agrotechnology on the entire field was used for growing of winter wheat ‘Tarso’ and the principle of the single difference was applied at all 47 stationary observation points. For the determination of the point coordinates the GPS receiver Germin iQ 3600 was used. The yield was determined by means of the yield maps developed from the Claas Lexion 420 GPS, and the AGROCOM software was used (Figure 4). The altitude of stationary observation points was determined by using Magelan eXplorist 600. Soil moisture in the arable layer and under it was determined by Eijkelkamp Agrisearch equipment.

**Results and Discussions**

Winter wheat yields indicated a significant negative effect of on deep soil tillage (Figure 5).

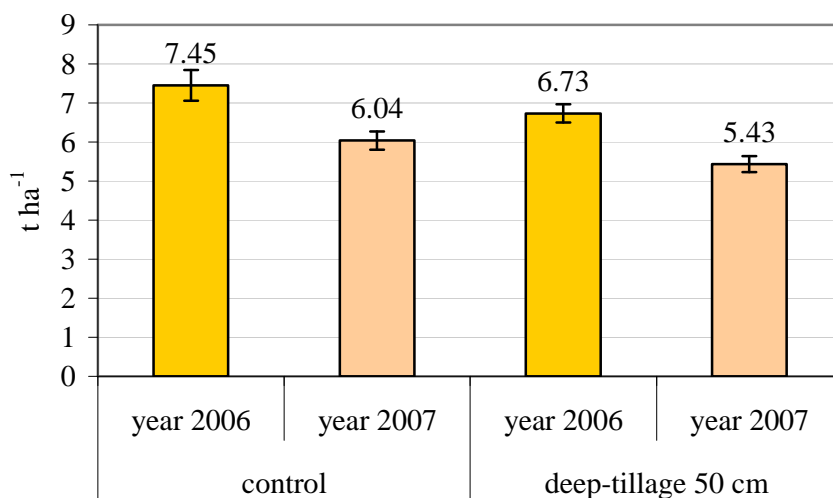


Figure 5. Changes of winter wheat yields after deep soil tillage, t ha<sup>-1</sup>.

A marked negative effect of deep soil tillage was observed in 2006 when there was extremely low precipitation. This was confirmed by the analysis of the results of the effect of soil humidity differences' on the formation of the winter wheat yield (Figure 6). In both trial years the soil humidity was below the optimum which is 25% of the soil capacity.

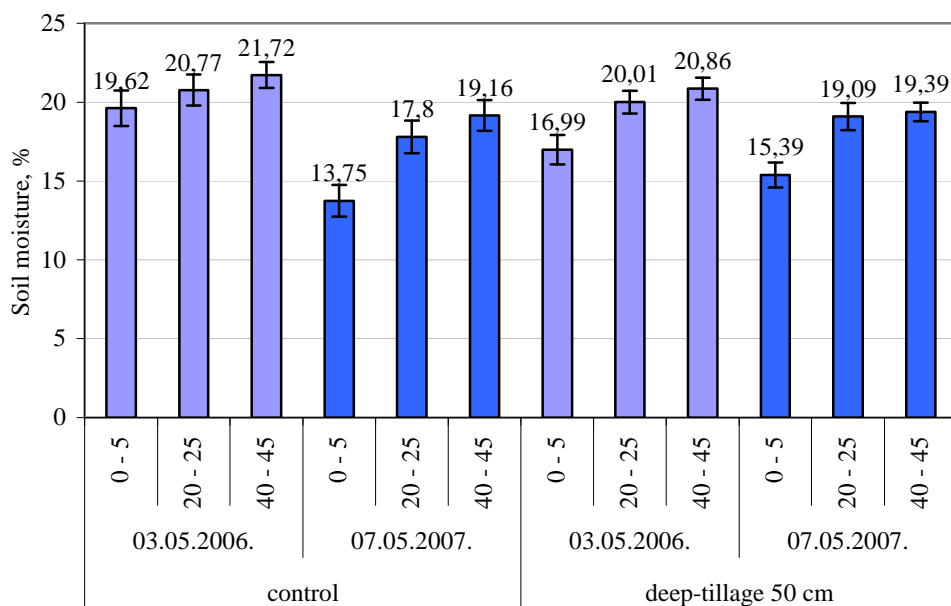


Figure 6. Changes in soil humidity after deep soil tillage.

The evaluation of the indices of soil penetration resistance in the sowing of winter wheat from autumn 2005 until spring 2006 (Figure 7) shows that the soil penetration resistance was significantly higher in the autumn in all soil layers where it was detected. It was significantly lower though in the spring after wintering - only 318 kPa cm<sup>-2</sup> in a depth of 20 – 30 cm.

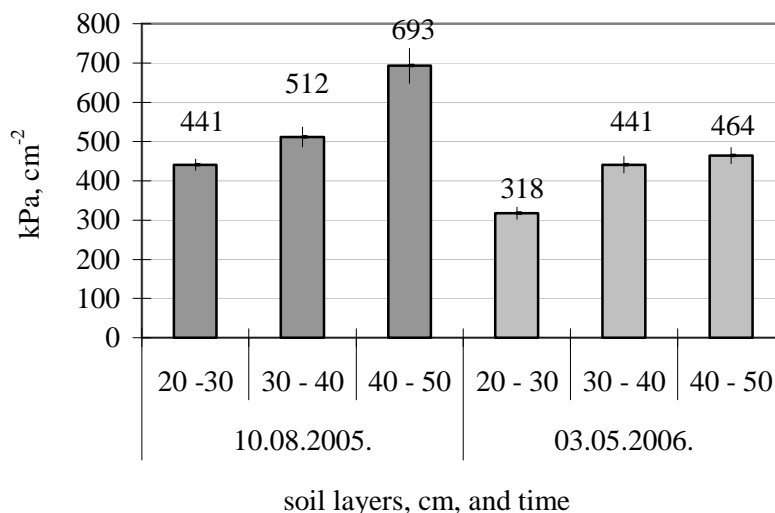


Figure 7. Characterization of the changes of soil penetration resistance ( $\text{kPa cm}^{-2}$ ).

It was established that the negative effect of higher sub-soil resistance (above  $600 \text{ kPa cm}^{-2}$ ) on winter wheat yield appeared in the spring.

A evaluation of changes in soil penetration resistance after deep soil tillage in the years 2006 and 2007 confirmed that soil resistance (Figure 8) and humidity are both co-linear factors.

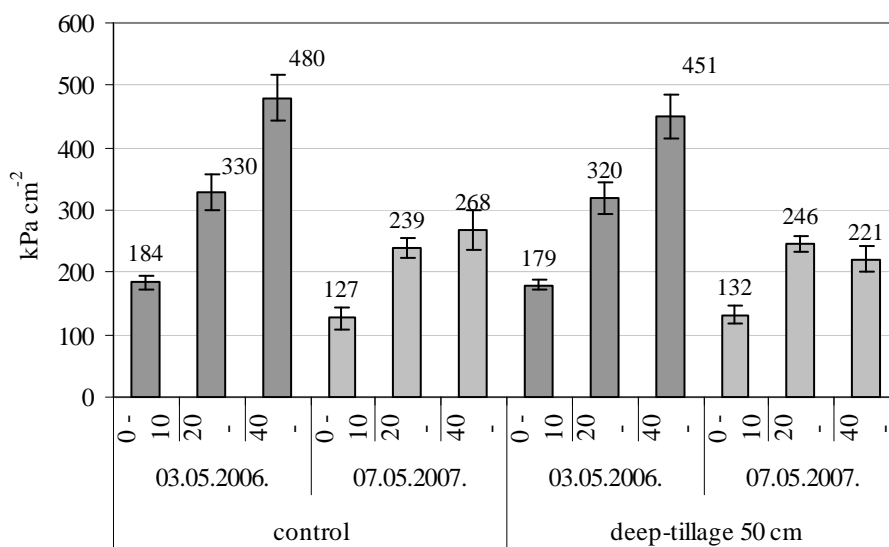


Figure 8. Characterization of the changes of soil penetration resistance ( $\text{kPa cm}^{-2}$ ) after deep soil tillage

Evaluation of winter wheat yields' differences in stationary observation points with different altitudes (meters above the sea level) showed that the yields were higher mainly in the lower areas of the slope (Figure 9).

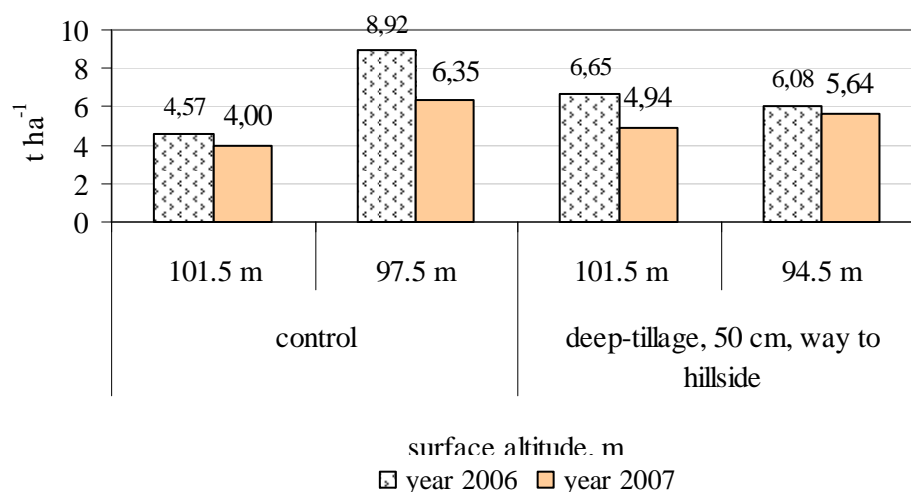


Figure 9. The effect of changes in relief on winter wheat yield

During 2006 (characterized by comparatively low precipitation) this correlation was mostly expressed during the vegetation period of winter wheat in the control treatment. In the points where deep soil tillage was used in 2005 the yield had a tendency to increase. The cause of the yield differences was the increase of soil humidity in the slope in both control and deep tillage variants, with deep tillage in the direction of the slope (Figure 10).

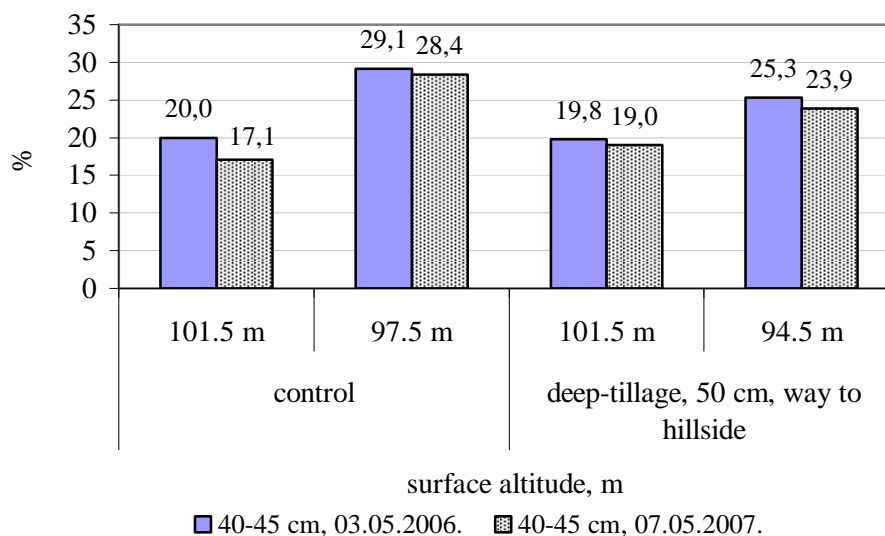


Figure 10. The effect of changes in relief on soil humidity

Soil humidity differences between control and deep tillage variants can be explained by the water flow through the marl of tine of the soil deep tillage equipment. In the lower points of the slope soil humidity (25.3%) was higher than on top of the hill (19.8%) in the same marl (Figure 10). After deep soil tillage across the slope in the comparison points (with altitudes 104 and 101 m above sea level) the soil humidity in spring 2007 were 16.6 and 16.4%, but in the spring of 2007 14.3 and 19.6%.

### Conclusions

Deep soil tillage is necessary if the soil penetration resistance exceeds  $600\ kPa\ cm^{-2}$  in depths of 40-50 cm. However, there exists a possibility of a decrease in the winter wheat yield in years with not enough precipitation because of uneven mezzo-relief.



The cause of the decrease in the winter wheat yield after deep soil tillage to the depth of 50 cm was the decrease in the soil humidity in the higher areas of mezzo-relief.

Soil humidity (in % of soil capacity) and soil penetration resistance are mutually co-linear factorial traits.

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## AUGSNES DZIĻIRDINĀŠANAS EFEKTIVITĀTES VĒRTĒJUMS ZIEMAS KVIEŠIEM IZMANTOJOT PRECĪZĀS LAUKKOPIBAS TEHNOLOĢIJAS

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Darba mērķis - noteikt augsnes dziļirdināšanas efektivitāti ziemas kviešiem ražošanas platībās neizlīdzināta lauka makroreljefa apstākļos, izmantojot saimniecības lokālās ĢIS un augšņu izpētes datus. Izmēģinājumi iekārtoti SIA LLU MPS „Vecauce”. No 2005.- 2007. gadam stacionāros ar GPS noteiktos punktos, veikti augsnes penetrometriskās pretestības un mitruma mērījumi augsnes slāņos līdz 0.50 m dziļumam, noteikts augsnes granulometriskais sastāvs aramkārtā. Raža noteikta izmantojot kombaina CLASS LEXION 420 GPS iespējas. Konstatēts, ka augsnes dziļirdināšana izpildāma, ja augsnes penetrometriskā pretestība pārsniedz 600 kPa cm<sup>-2</sup>.

## THE EFFECT OF NITROGEN NUTRITION ON THE PRODUCTIVITY OF WINTER TRITICALE IN THE SOILS OF CENTRAL LITHUANIA

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## Abstract

During the period 2000–2004 field trials with winter triticale were conducted at the LIA in Dotnuva on a light loam *Endocalcari - Epihypogleyic Cambisol*. The goal of the field trials was to determine the optimal conditions for winter triticale nitrogen nutrition and to estimate nitrogen fertilizer efficacy taking into account mineral nitrogen content in the soil.

Our experimental evidence suggests that nitrogen fertilizers were net effective every year, the regularities of grain yield variation resulting from fertilizer application also differed. A grain yield increase of 19.5–24.0 % was obtained through nitrogen fertilizer application. A rate of N<sub>90</sub> was found to be optimal for triticale in our trials. Fertilizer efficacy is presented in kilos – averaged data suggest that when winter triticale had received N<sub>60</sub>, N<sub>90</sub> and N<sub>120</sub>, 1 kg of fertilizer nitrogen produced 19.9±7.46 kg, 16.5±6.00 kg and 12.7±5.02 kg of grain, respectively. Additional fertilization of triticale was effective only in the normally wet years.

Having fertilized triticale with N<sub>90</sub> and N<sub>120</sub>, in the years conducive to the spread of diseases (2000–2001), a significant yield increase was obtained through fungicide application.

**Key words:** winter triticale, yield, efficacy of nitrogen fertilizers.

## Introduction

Exhibiting a high yield potential, winter triticale is a promising crop. The area currently sown with triticale in Lithuania is steadily increasing and this increase has been determined by the availability

of high-yielding, winterhardy, thick-stemmed, rather satisfactorily drought and disease resistant varieties. In terms of yield, winter triticale compares well with many winter wheat varieties. The grain chemical composition of triticale determines its rather wide application possibilities: the grain is used in the food industry, flour in confectionery, for beer, spirits, and starch production (Seguchi *et al.*, 2000). In grain protein, the ratio of amino acids content is suitable for livestock feeding (Kuzeev *et al.*, 1997; Alaru *et al.*, 2003, Mikulionienė *et al.*, 2002). Triticale is an excellent raw material for the expanding bioethanol industry to be used for environment-friendly fuel production. Winter triticale is well suited for growing on various-textured soils and its cultivation is rational not only from the viewpoint of productivity but also from the viewpoint of the optimal maintenance of soil physical and chemical properties (Petraitis *et al.*, 2002; Malecka *et al.*, 2004).

There has been very little research done so far under Lithuania's conditions on triticale fertilization, which is one of the links in cultivation technology, with a view to maximally exploiting varietal genetic productivity potential. There were also no tests done designed to estimate the role of soil mineral nitrogen in winter triticale nutrition and to ascertain the yield, yield increase and fertilizer efficacy as influenced by mineral nitrogen ( $N_{\min}$ ). The objective of the study was to identify optimal nitrogen nutrition conditions for winter triticale grown on Central Lithuania's soils and to estimate nitrogen fertilizer efficacy in relation to the mineral nitrogen content in the soil.

### Materials and Methods

*Experimental site.* Field experiments were conducted during the period 1999-2004 at the Lithuanian Institute of Agriculture in Dotnuva on a light loam *Endocalcari - Epihypogleyic Cambisol* by a conventional field experiment method.

According to the values of agrochemical parameters, the soil  $pH_{KCl}$  was 6.0-7.0 (measured potentiometrically), plant available phosphorus and potassium contents - 129-206  $mg\ kg^{-1}$  ( $P_2O_5$ ) and 140-201  $mg\ kg^{-1}$   $K_2O$ , respectively (A-L methods), humus content 1.8-2.1 % (Tyurin) and total nitrogen content 0.12-0.14 % (Kjeldahl).

The soil at the 0-40 cm depth was relatively low in plant available mineral nitrogen ( $N-NO_3+N-NH_4$ , measured:  $N-NO_3$  – ionometrically,  $N-NH_4$  spectrophotometrically) ranging from  $38.0\pm 0.73$  to  $55.2\pm 0.93$ . On average, in spring at the beginning of the triticale growing season,  $N_{\min}$  content at the 0-40 cm soil layer from which plants utilize nutrients most intensively at the beginning of the growing season varied within the 38.0-55.2  $kg\ ha^{-1}$  range, at low or moderate variation ( $V = 87-17.3\%$ ). Having added up  $N_{\min}$  present at 0-40 and 40-60 cm soil layers, it was noted that in different years it varied within the 55-70  $kg\ ha^{-1}$  range, ( $V = 7.3-10.9\%$ ). The distribution of  $N_{\min}$  content in the soil profile was as follows: at the 0-40 cm depth on average 68-78 %, at the 40-60 cm depth 22-32 % of the total  $N_{\min}$  content in the 0-60 cm depth.

The distribution of nitrate nitrogen ( $N-NO_3$ ) in the soil profile was similar to that of  $N_{\min}$ : at the 0-40 cm soil layer - 67-82 % of readily plant-available  $N-NO_3$  content, the other 18-33 % was distributed within the deeper 40-60 cm layer. Ammonia nitrogen ( $N-NH_4$ ) in separate experimental years accounted for 33 - 46 % of the mineral nitrogen content present at the 0-40 cm depth.

*Experimental design:* 1. Not fertilized ( $N_0\ P_0\ K_0$ )/ 2.  $P_{60}K_{60}$  (background F)/ 3.  $N_{60}$  in spring (BBCH 25-29)/ 4. F+ $N_{30}$  in autumn + $N_{60}$  in spring (BBCH 25-29)/ 5. F+  $N_{60}$  in spring (BBCH 25-29)/ 6. F+  $N_{90}$  in spring (BBCH 25-29)/ 7. F+  $N_{60}$  in spring (BBCH 25-29) +  $N_{30}$  at the beginning of booting (BBCH 30-32)/ 8. F+  $N_{120}$  in spring (BBCH 25-29)/ 9. F+  $N_{90}$  in spring (BBCH 25-29) +  $N_{30}$  at the beginning of booting (BBCH 30-32)/ 10. F+  $N_{90}$  in spring (BBCH 25-29) +Tilt (BBCH 47-59)/ 11. F+  $N_{120}$  in spring (BBCH 25-29) +Tilt (BBCH 47-59). BBCH - scale describes the phenological development of cereals.

Treatments 2 and 4-11 received the same phosphorus and potassium fertilization level -  $P_{60}K_{60}$ .

According to the experimental design, triticale grown in the plots of two treatments (10 and 11) was sprayed with the fungicide Tilt ( $0.5\ l\ ha^{-1}$ ) at BBCH 47-59. Winter triticale was preceded by perennial grasses of the second year of use. At the end of tillering in the spring the triticale crop was sprayed with a mixture of herbicides and growth regulators, insecticides were used according to the need.

The winter triticale cv. *Tewo* was sown for 2000 and 2001-year harvest. After it had been removed from the National List of Plant varieties, the cv. *Tornado* was sown for the 2002-2004 harvest.

Winter triticale was thrashed at complete maturity and grain yield data were adjusted to 15 % moisture.

*Meteorological conditions*, recorded at the Dotnuva Weather Station differed between the experimental years. Of the five experimental years, two were extremely dry and warmer than usual, three years were normally wet. The year 2000 was distinguished by a warm and dry spring – in April the mean monthly temperature was by +5.7 °C higher than the long-term mean, and the amount of precipitation was as low as 19 % of the mean long-term mean – 7.6 mm. During the period of intensive growth and development – in May and June humidity was close to normal, and in July the amount of rainfall was twice as high (185 %) as the long-term mean. The weather conditions during the 2000-2001 winter period were similar to long-term means. In 2001 the amount of rainfall that fell during the entire growing season corresponded to long-term mean, however its distribution was very uneven – 45 % of the total amount of rainfall that fell during the growing season occurred in July. Moreover, the mean air temperature of July was by 3.5 °C higher than the long-term mean. During the first months of the year 2002 the amount of rainfall was from 22 to 53, and exceeded the long-term mean by 91 %. Such heavy precipitation might have resulted in lower mineral nitrogen content in the soil in the spring of 2002. During the growing season of 2002 the weather was warm with several heat waves and exceeded the mean air temperature of individual months by 2.3 °C – 3.2 °C. In April the plants were exposed to stress resulting from dramatic temperature variations (from -5.5°C to +19.2°C) and shortage of moisture, since only about half (56 %) of the mean long-term rate fell. In June and July in some days the air temperature reached a striking maximum to 34-35 °C. The amount of rainfall during the summer growing season made up 57 % of that period's mean long-term rate. In the autumn of 2002 having sown winter triticale, the amount of rainfall that fell in October was record-breaking – 2.5 times as high as the long-term mean. The winter period was characterised by a slightly lower amount of precipitation than usual. The year 2003 was somewhat drier than the norm, the amount of precipitation that fell during the growing season was 80 % of the long-term mean. Of the summer growing season, July was noted for hot weather, with the daily mean temperature by 3°C higher than the long-term mean. In 2004 almost all the growing season was dry – the amount of precipitation that fell in April, May and June was accordingly 29, 53 and 70 % of the long-term mean. The sum total of precipitation that fell during the growing season was 72 % of the long-term rate.

Statistical grain yield data processing was done using analysis of variance. Correlations between grain yield, yield increase in different expressions and nitrogen fertilizer rates and mineral nitrogen were determined and regression equations were calculated following the directions in special literature (Littl *et al.*, 1981, Tarakanovas *et al.*, 2003). Symbols used in the paper: \* and \*\* statistically significant at 95 % and 99 % probability level; LSD<sub>05</sub> – the least significant difference at 95 % probability level; V % - variation coefficient.

### Results and Discussion

Nitrogen fertilization is one of the major and most efficient means to increase yield, control yield formation processes and improve yield quality. The findings on nutrition of triticale, which is a relatively undemanding crop in terms of cultivation conditions, are scarce in literature. Different nitrogen rates are often indicated for winter triticale. On the background of P<sub>100</sub>K<sub>100</sub> an optimal nitrogen rate is indicated to be 80 kg ha<sup>-1</sup> (Paponov *et al.*, 1999), more recent research suggests that the highest winter triticale yield was achieved through a nitrogen rate not lower than 120 kg ha<sup>-1</sup> (Malecka *et al.*, 2004), other researchers have reported optimal nitrogen rates to be from 60 to 120 kg ha<sup>-1</sup> (Bulavina, 1993), 160 kg ha<sup>-1</sup> or even 180 kg ha<sup>-1</sup> (Cimrin *et al.*, 2004; Mut *et al.*, 2005). Nitrogen fertilizer efficacy during 2001-2004 was sufficiently high and grain yield increases through its application were statistically significant (Table 1)

Table 1. The effect of fertilization and fungicides on grain yield t ha<sup>-1</sup>

<i>Treatment</i>	<i>Year</i>					<i>Mean.</i>	
	2000	2001	2002	2003	2004	t ha <sup>-1</sup>	<i>relative values</i>
Without fertilizers	8.34	3.86	7.30	4.01	5.78	5.86	100
P <sub>60</sub> K <sub>60</sub> (background F)	8.59	4.46	7.32	3.94	4.73	5.81	99.1
N <sub>60</sub> in spring	8.58	5.76	8.13	4.93	7.46	6.97	119.0
F+N <sub>30</sub> in autumn +N <sub>60</sub> in spring	8.53	5.85	7.64	5.84	7.53	7.08	1208
F+ N <sub>60</sub> in spring	8.66	6.15	7.76	5.14	7.31	7.00	119.5
F+ N <sub>90</sub> in spring	8.56	6.08	8.01	5.92	7.87	7.29	124.4
F+ N <sub>60</sub> in spring + N <sub>30</sub> at beginning of booting (BBCH 30-32)	8.20	5.95	8.04	5.79	8.20	7.24	123.5
F+ N <sub>120</sub> in spring	7.89	6.08	7.73	6.27	7.99	7.19	122.7
F+ N <sub>90</sub> in spring + N <sub>30</sub> at beginning of booting (BBCH 30-32)	7.81	6.28	7.99	5.78	8.46	7.26	124.0
F+ N <sub>90</sub> in spring +Tilt (BBCH 47-59)	9.34	6.99	8.91	5.70	7.95	7.78	132.7
F+ N <sub>120</sub> in spring +Tilt (BBCH 47-59)	9.21	7.12	7.72	6.13	8.58	7.75	132.3
LSD <sub>05</sub>	0.676	0.447	0.839	0.669	0.962	0.727	

Only the year 2000 stood out when nitrogen fertilizers did not increase the yield but grain yield even in the check treatment amounted to 8.34 t ha<sup>-1</sup>. It is likely that this was determined by the pre-crop – perennial grasses of the second year of use that contained a high content of clover, and the atmospheric nitrogen fixed by clover and accumulated in the soil might decline nitrogen fertilizer effect on triticale yield. Low nitrogen fertilizer efficacy could be also responsible for crop lodging in 2000. The data averaged over the five experimental years suggest that triticale grown without fertilizers produced a grain yield of 5.86 t ha<sup>-1</sup>, and a yield increase of 19.5-24.0 % resulting from nitrogen fertilization was obtained, compared with the check treatment.

Yield increases on the background of PK, that resulted from single spring-applied nitrogen rates 60, 90 and 120 kg ha<sup>-1</sup> were different during the experimental years and varied substantially – the variation in different fertilization levels was as high as 81-88 %. In 2001 and in 2002, which was especially warm it increased with a nitrogen rate up to 90 kg ha<sup>-1</sup>. In 2003 and 2004 nitrogen fertilizers were the most effective – with increasing single rates to 120 kg ha<sup>-1</sup>, the yield increased. Averaged data indicate that nitrogen rates of 60, 90 and 120 kg ha<sup>-1</sup> gave grain yield increases of 1.20±0.447 t ha<sup>-1</sup>, 1.49±0.540 t ha<sup>-1</sup> and 1.52±0.602 t ha<sup>-1</sup>, respectively.

Having estimated nitrogen fertilizer efficacy, expressed as kg grain per 1 kg of fertilizer nitrogen, it was found that with nitrogen rates of 60, 90 and 120 kg ha<sup>-1</sup> applied to triticale, 1 kg of fertilizer nitrogen gave on average 19.9±7.46 kg, 16.5±6.00 kg and 12.7±5.02 kg grain, respectively.

Based on the experimental data, the correlation of triticale grain yield with mineral and nitrate nitrogen contents present at different soil layers (0-40 cm and 0-60 cm) was mathematically estimated. It was found that the contents of both nitrate and mineral nitrogen in the soil 0-40 cm and 0-60 cm layers correlated similarly with triticale grain yield data. The data of separate experimental years show that in ¾ of the cases tested (75 %) the correlation was weak and at 95 % the probability level statistically insignificant. The data averaged over the 5 experimental years indicate that yield correlation with N-NO<sub>3</sub> and mineral nitrogen amounts at the 0-60 cm soil layer were moderate and statistically significant. A slightly weaker, but close to moderate correlation was found between the grain yield and contents of plant available nitrogen forms at the 0-40 cm soil layer (Table 2).

Table 2. Correlation coefficients between triticale grain yield ( $y$ , t ha<sup>-1</sup>) and mineral and nitrate nitrogen contents ( $x$ ) present at different soil layers

Year	Nitrate nitrogen (N-NO <sub>3</sub> )		Mineral nitrogen (N min)	
	0-40 cm	0-60 cm	0-40 cm	0-60 cm
2000	0.31	0.50	0.31	0.37
2001	0.39	0.37	0.32	0.33
2002	0.20	-0.016	0.37	0.04
2003	-0.66	-0.59	-0.61	-0.56
2004	-0.72*	-0.61*	-0.40	-0.33
Avg. over 5 yrs.	-0.49**	-0.58**	-0.41**	-0.53**

The correlations between winter triticale yield and nitrogen fertilizer rates are presented in Table 3. The strength of correlation varied from weak and statistically insignificant at the 95 % probability level ( $\eta = 0.35$ , in 2002) to strong and significant at the highest 99 % probability level ( $\eta = 0.92$ , in 2001). In separate experimental years nitrogen fertilizers were responsible for 13 to 84 % yield data variation. However, the data averaged over the 5 experimental years show that only 11 % yield variation was related to the nitrogen fertilizer rate.

While calculating the dependence of nitrogen fertilizer efficacy on nitrogen content in the soil and the nitrogen fertilizer rate, we took nitrate and mineral nitrogen content present at the 0-60 cm depth, as the most appropriate indicator that defines nitrogen abundance in the soil, since at the beginning of the growing season, before the main spring fertilization, a large part of mineral nitrogen present in the soil (about 30 %) was found at the 40-60 cm depth. In the year 2000 there was no grain yield correlation with these parameters. The data from 2001-2004 period indicate that similar yield correlation in terms of strength and significance, was determined when adding up both nitrate and mineral nitrogen content with fertilizer nitrogen content (Table 3). The correlation was moderate or strong, in 60 % of the cases tested – statistically significant at the 99 % probability level. During the experimental period, the sum of N-NO<sub>3</sub> and N<sub>min</sub> and nitrogen applied with fertilizers present at the 0-60 cm soil layer determined from 24 to 88 % and from 24 to 93 % of the yield, respectively. The data averaged over the 5 experimental years show that the correlation between the yield and total soil mineral nitrogen forms and fertilizer nitrogen content was weak but statistically significant ( $r=0.31^*$ ).

 Table 3. Correlation coefficients between the yield ( $y$ , t ha<sup>-1</sup>) and nitrogen fertilizer rate ( $x_1$ , kg ha<sup>-1</sup>) and total contents of nitrogen present in the soil and applied with fertilizer ( $x_2$  and  $x_3$ , kg ha<sup>-1</sup>)

Year	Correlation coefficients		
	H	R	r
	$x_1$ – nitrogen fertilizer rate	$x_2$ – sum of N-NO <sub>3</sub> and fertilizer nitrogen at the 0-60 cm soil layer	$x_3$ – sum of N min and fertilizer nitrogen at the 0-60 cm soil layer
2000	0.50*	0.05	0.04
2001	0.92**	0.88**	0.87**
2002	0.35	0.49	0.49
2003	0.73**	0.96**	0.97**
2004	0.75**	0.94**	0.93**
Avg. over 5 yrs.	0.32**	0.31*	0.31*

In plant nutrition diagnostics tests nitrogen fertilizer efficacy is defined by yield increase or calculated figure – percentage yield. It is calculated by dividing the yield of each experimental plot by the highest yield obtained in the experiment. The correlation of winter triticale separate year's and percentage yield with soil nitrogen – nitrate and mineral present at the 0-40 and 0-60 cm depth was determined (Table 4). In most cases the correlation between the mentioned indicators was best described by a parabola of the second degree, however, the correlation was significant not in all the cases studied. The contents of both N-NO<sub>3</sub> and N<sub>min</sub> at the 0-40 cm soil depth correlated very similarly with percentage yield, the correlation ranged from moderate to strong. At the 0-60 cm depth, the percentage yield correlated stronger with soil N<sub>min</sub> than with N-NO<sub>3</sub>, but it was

statistically significant only in 40 % of cases. The data from 5 experimental years suggest that average percentage yield correlations with soil nitrogen were most often represented by a linear equation, indicating an inverse moderately strong correlation ( $r = -0.50-0.51$ ) which was significant only in half of the cases.

Table 4. The relationship between percentage yield (y) and N<sub>min</sub> and N-NO<sub>3</sub> at the 0-40 and 0-60 cm soil layers

Year	Denomination of trait x	Equation	r/η	dxy	F <sub>Fisher</sub>
2000	N-NO <sub>3</sub> 0-40 cm	$y = -25.28 + 1.4134x - 0.0191x^2$	0.63	0.40	3.3
2001		$y = -3.07 + 0.3016x - 0.0056x^2$	0.70*	0.50	6.4
2002		$y = -3.86 + 0.4495x - 0.0103x^2$	0.64	0.41	4.9
2003		$y = -1.68 + 0.2165x - 0.0044x^2$	0.84*	0.71	7.4
2004		$y = 2.18 - 0.0513x$	0.71*	0.50	9.4
Avg. Over 5 yrs.		$y = -1.78 - 0.0523x + 0.0007x^2$	0.54	0.29	2.8
2000	N-NO <sub>3</sub> 0-60 cm	$y = -2.69 + 0.1888x - 0.0024x^2$	0.61	0.37	1.5
2001		$y = -3.40 + 0.1728x - 0.0017x^2$	0.38	0.14	0.1
2002		$y = -4.57 + 0.3499x - 0.0054x^2$	0.65*	0.42	5.8
2003		$y = -3.43 + 0.2273x - 0.0029x^2$	0.79*	0.62	5.6
2004		$y = 2.32 - 0.0415x$	0.61*	0.38	5.4
Avg. Over 5 yrs.		$y = 1.28 - 0.0099x$	0.50**	0.23	15.9
2000	N min 0-40 cm	$y = -2.74 + 0.01640x - 0.0018x^2$	0.70*	0.49	5.5
2001		$y = -42.00 + 1.5463x - 0.0139x^2$	0.55	0.30	2.3
2002		$y = -7.41 + 0.4367x - 0.0056x^2$	0.51	0.26	1v3
2003		$y = -13.57 + 0.6851x - 0.0081x^2$	0.90**	0.81	17.7
2004		$y = 10.35 - 0.3734x + 0.036x^2$	0.47	0.22	0.6
Avg. Over 5 yrs.		$y = 1.34 - 0.0096x$	0.50**	0.25	17.7
2000	N min 0-60 cm	$y = -5.39 + 0.2004x - 0.0016x^2$	0.69	0.47	5.0
2001		$y = -50.49 + 1.4507x - 0.0102x^2$	0.57	0.33	3.2
2002		$y = -16.09 + 0.6058x - 0.0053x^2$	0.72*	0.52	8.7
2003		$y = -14.61 + 0.4950x - 0.0039x^2$	0.84*	0.71	10.9
2004		$y = 9.96 - 0.2679x + 0.0020x^2$	0.39	0.15	0.4
Avg. over 5 yrs.		$y = 1.54 - 0.0101x$	0.51	0.26	18.6

Extra fertilization of winter triticale with N<sub>30</sub> rate in the middle of booting stage, when at the beginning of the growing season 60 and 90 kg ha<sup>-1</sup> rates of nitrogen had been applied, gave a low and insignificant yield increase in most cases. In 2003 and 2004 extra fertilization for N<sub>60</sub> – applied triticale was slightly more effective – the grain yield was by 0.65 and 0.89 t ha<sup>-1</sup> higher compared with the treatments fertilized once at N<sub>60</sub> and the yield increase was significant at a slightly lower than 95 % probability level. An additionally applied N<sub>30</sub> rate for triticale fertilized with N<sub>90</sub> in spring was ineffective, since in the experimental years with considerable moisture deficit the grain tended to dry and maturity was accelerated, therefore additionally applied nitrogen remained unutilised.

The importance of the fungicide on triticale productivity increased in wet years when conditions for disease occurrence were favourable. In 2000 and 2001 when during the growing season the amount of precipitation was close to the long-term mean (114.2 % and 98 %, respectively) and warm weather prevailed, conditions conducive for the spread of disease were created and the efficacy of the fungicides was high. Triticale fertilized with N<sub>90</sub> and N<sub>120</sub> and sprayed with Tilt produced a statistically significant yield increase – 0.78-0.91 and 1.32-1.04 t ha<sup>-1</sup>, respectively, compared with triticale that received the same fertilization but was not applied with fungicides. At a higher nitrogen fertilization level the fungicide gave a higher yield increase.

## Conclusions

Nitrogen fertilizers were effective for winter triticale and significantly increased grain yield by on average 19.5 – 24.0 %. Averaged data suggest that on PK background, N<sub>60</sub>, N<sub>90</sub> and N<sub>120</sub> gave a grain yield increase of 1.20±0.447 t ha<sup>-1</sup>, 1.49±0.540 t ha<sup>-1</sup> and 1.52±0.602 t ha<sup>-1</sup>. Additional winter triticale fertilization was effective in normally wet years; dry weather in separate experimental

years was unfavourable for the uptake of additionally applied fertilizer nitrogen, therefore the yield increase obtained was insignificant.

Having estimated the dependence of winter triticale grain yield on nitrogen fertilizer rates, in most cases – five out of six were determined to be statistically significant, moderately strong or strong correlation ( $\eta=0.50^*-0.92^{**}$ ), nitrogen fertilizers determined 13-84 % grain yield variation.

The relationship between the winter triticale yield and the total nitrogen fertilizer and nitrate (N-NO<sub>3</sub>) and mineral nitrogen (N<sub>min</sub>) content at the 0-60 cm soil depth was identified. In terms of strength and significance, the correlations differed little when comparing nitrate and mineral nitrogen and in 60 % of the cases studied were statistically significant at a 99 % probability level.

Having estimated fertilizer efficacy in percentage yield, a slightly higher correlation was determined with mineral nitrogen content at the 0-60 cm soil layer than with nitrate nitrogen content found at the same depth. With increasing mineral nitrogen content in the soil, the efficacy of nitrogen fertilizers tended to decline.

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## SLĀPEKĻA MĒSLOJUMA IETEKME UZ ZIEMAS TRITIKĀLES RAŽĪBU LIETUVAS VIDIENES AUGSNĒS

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Laika periodā no 2000.-2004. gadam Dotnuvā Lietuvas Zemkopības institūtā viegla smilšmāla augsnēs (*Endocalcari – Epihypogleyic Cambisol*) ar mērķi noteikt optimālos apstākļus mēslošanai ar slāpekli un novērtēt slāpekļa mēslojuma iedarbību, ņemot vērā minerālā slāpekļa saturu augsnē, tika veikti lauka izmēģinājumi ar ziemas tritikāli.

Rezultāti liecina, ka mēslošana ar slāpekli ne katru gadu ir efektīva, graudu raža atšķirīgi variē ar mēslojuma devas ietekmē. Lietojot slāpekļa mēslojumu, tika iegūts graudu ražas pieaugums 19.5–24.0 %. Izmēģinājumos kā optimālā slāpekļa norma tritikālei tika noteikta N<sub>90</sub>. Dati liecina, ka ar katru mēslojuma kilogramu ziemas tritikālei pie devas N<sub>60</sub>, N<sub>90</sub> un N<sub>120</sub>, ražas pieaugums ir attiecīgi 19.9±7.46 kg, 16.5±6.00 kg un 12.7±5.02 kg graudu. Tritikāles papildus mēslošana bija efektīva tikai normāli mitros gados.

Pie mēslojuma devas N<sub>90</sub> un N<sub>120</sub> slimību izplatībai labvēlīgos gados (2000–2001), nozīmīgs ražas pieaugums tika iegūts, izmantojot fungicīdus.

## NITROGEN MANAGEMENT EFFECTS ON SPRING WHEAT YIELD AND PROTEIN

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### Abstract

Wheat fields in Latvia are spatially variable as to soil fertility and crop productivity. Therefore, there is interest in applying variable rates of fertilizers across the landscape. The general objective of this research is to determine the relationships among yield, protein and N rates using the regression analysis of yield data in order to optimize the rate of nitrogen fertilizer strategies in spring wheat.

A three year long (1999- 2001) field study was conducted near Skriveri, Latvia, at the Research Institute of the Agriculture of Latvia University of Agriculture with spring wheat (*Triticum aestivum* L.) 'Munk' on the optimisation of nitrogen fertilizers. The field trials were conducted on two kinds of Luvisol soil: loam and loamy sand. The influence of the preceding crops- grass, grain and potatoes on efficiency of different nitrogen fertilizer levels (0; 50; 100; 150; 200; 250; kg ha<sup>-1</sup>) was investigated.

Nitrogen fertilizer has considerably affected the crop yield. The increase of the norm of nitrogen fertilizer from 50 kg ha<sup>-1</sup> up to 100 kg ha<sup>-1</sup> and 150 kg ha<sup>-1</sup> was significant, but a further increase was not significant. The essential difference was observed among all the predecessors as well. The nitrogen fertilizers increased protein content in a linear way and that was dependent on the preceding crops and soil texture.

**Key words:** spring wheat, nitrogen fertilizers, protein.

### Introduction

The rising prices of agriculture production's raw materials and the increase of environmental overload promote more effective agriculture production. More and more importance is given to the fertilization of cultivated plants that must be appropriate to the cultivated soil character and the potential productiveness of plants. In particular, this should refer to field crop fertilization with nitrogen fertilizer since this definitely raises the level of grain productiveness, and nitrogen is also the basic building block of protein, and as a consequence, levels N in the soil have a large influence on grain protein concentration (Flower D. B., 2003). Therefore efficient wheat production systems emphasize the important role that N fertilizer management has in optimizing grain yield and the maintenance of grain quality standards. Nitrogen provision must correspond to the requirements of the plants, taking into account the use of nitrogen from the soil as well. The aim of the work is to explain the influence of three kinds of preceding crops upon the optimal nitrogen rates in two different textures of soil, with different granulometric content, upon grain yield and crude protein content within spring wheat grain.

### Material and Methods

A three-year study was conducted during 1999–2001. The spring wheat (*Triticum aestivum* L.) variety 'Munk' was grown in two different textures of Luvisol soil: loam: pH<sub>KCl</sub> - 6.3, organic matter – 23 mg kg<sup>-1</sup> (Tyurin's method), available phosphorus-100 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>, available potassium - 135 mg K<sub>2</sub>O kg<sup>-1</sup> (DL-method) and loamy sand: pH<sub>KCl</sub> - 5.7, organic matter – 22 mg kg<sup>-1</sup> (Tyurin's method), available phosphorus -142 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>, available potassium - 92 mg K<sub>2</sub>O kg<sup>-1</sup> (DL-method). The influence of the preceding crops - grass, grain and potatoes on the efficiency of six different levels of nitrogen fertilizer rates (0; 50; 100; 150; 200; 250; kg ha<sup>-1</sup>) applied 60 % before drilling, and 40 % at spring wheat stage EC 29 as ammonium nitrate, was investigated. Spring wheat was drilled close to the optimum date (the end of April) at a rate 600 seeds per m<sup>2</sup>. One herbicidal (Granstar 15 g ha<sup>-1</sup>), one fungicidal (Tango 0.8 l ha<sup>-1</sup>), and insecticidal (Bi-58 1 l ha<sup>-1</sup>) was applied to control weeds, pathogens, and insects in spring wheat. The data mathematical processing was performed using the MS Excel linear, polynomial regression and anova analysis.



The economically optimal wheat yield was calculated by Danish Agricultural Advisory Service program.

### Results and Discussion

In the course of three year long field-tests, the wheat grain yield fluctuated, on average, from 2.23 to 4.25 t ha<sup>-1</sup>, and the crude protein content, on average, from 10.57 to 15.90 %. Nitrogen rate 50 kg ha<sup>-1</sup> and its increase up to 100 and 150 kg ha<sup>-1</sup> improved the grain yield remarkably ( $\gamma_{0.05} = 0.382$  t ha<sup>-1</sup>). Nevertheless, further increase of nitrogen rates did not create a remarkable increase of grain yield. Within all variants the formation of crude protein in grain was promoted by the increase of nitrogen fertilizer. To characterize the changes within spring wheat grain yield, for the most part, the second grade regression curve was applied and, in several cases, the third grade polynomial equity was needed.

Changes within spring wheat grain crops grown in sandy loam after potatoes are described by the third grade polynomial regression curve  $y = 3E-07x^3 - 0.0001x^2 + 0.0201x + 3.3984$  (Picture 1). This change model of spring wheat grain crop represents 99.3 % of the cases. The determination ratio –  $R^2 = 0.9929$ . F-test's p-value 0.010622 is below 0.05; therefore the regression equity is a statistically important explanation of the changes within the grain crops.

The economically optimal crop constituted 4.22 t ha<sup>-1</sup>, and was obtained applying 73 kg ha<sup>-1</sup> N rate. Crude protein within spring wheat grain increased according to the linear regression  $y_p = 0.0148x_p + 10.359$  (Figure 1) which, in statistically crucial terms, explained the changes within crude protein values since value F-test's p-value 0.000273 was below 0.05 corresponding to 97.3 % data of field-tests ( $R^2 = 0.9731$ ). Taking into account the crude protein content within spring wheat grain, the optimal nitrogen fertilizer when spring wheat was grown in sandy loam after potatoes, should be increased from 73 to 77 kg ha<sup>-1</sup> since only this or a bigger nitrogen fertilizer rate would ensure grain with a crude protein content above 11.5 % that is supposed to be the critical borderline designed for the growing of qualitative food cereals (Commission Regulation EC 824/2000, Ruža A. 1998).

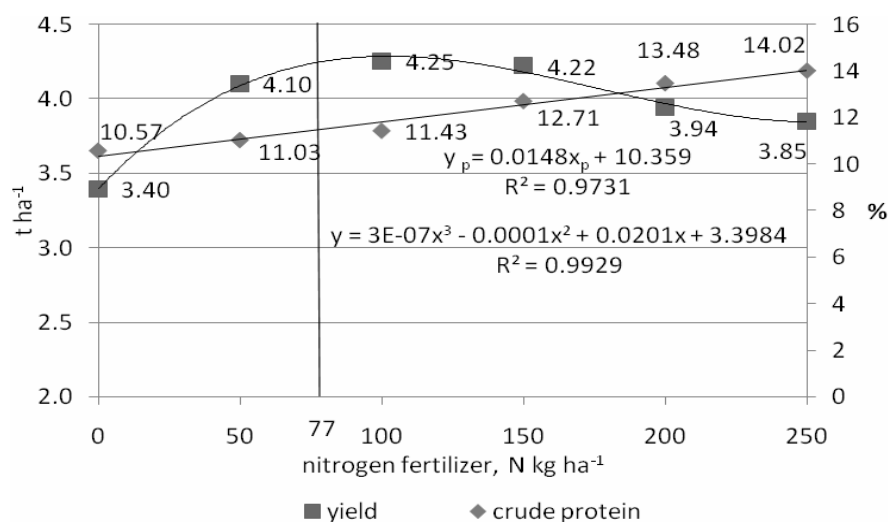


Figure 1. Yield and crude protein changes within loamy sand after potatoes

Changes within spring wheat grain yield grown in loamy soil after potatoes are represented by the polynomial regression equity  $y = -5E-05x^2 + 0.0129x + 2.9488$  (Figure 2), and they can explain 89.3 % of the changes within spring wheat grain yield ( $R^2 = 0.893$ ). The standard error of the expected value is 0.150093. F-test's p-value 0.0346 is below 0.05; therefore the regression equity is a statistically essential explanation of the changes within spring wheat grain yield. The crude protein content increased in correspondence with the linear connexion  $y_p = 0.0145x_p + 11.282$  that is a statistically essential explanation of the crude protein changes since F-test's p-value 0.001787 is below 0.05 within 93.2 % descriptions of field-test data ( $R^2 = 0.9318$ ).

To the spring wheat grown in loamy soil after potatoes, in order to obtain economically optimal yield: 3.85 t ha<sup>-1</sup>, there should be applied a 112 kg ha<sup>-1</sup> N fertilizer rate. Already the 15 kg ha<sup>-1</sup>

nitrogen fertilizer rate provided grain with 11.5 % crude protein content proving that a high level of crude protein content could be obtained with minor nitrogen fertilizer rates if spring wheat was grown in loamy soil after potatoes.

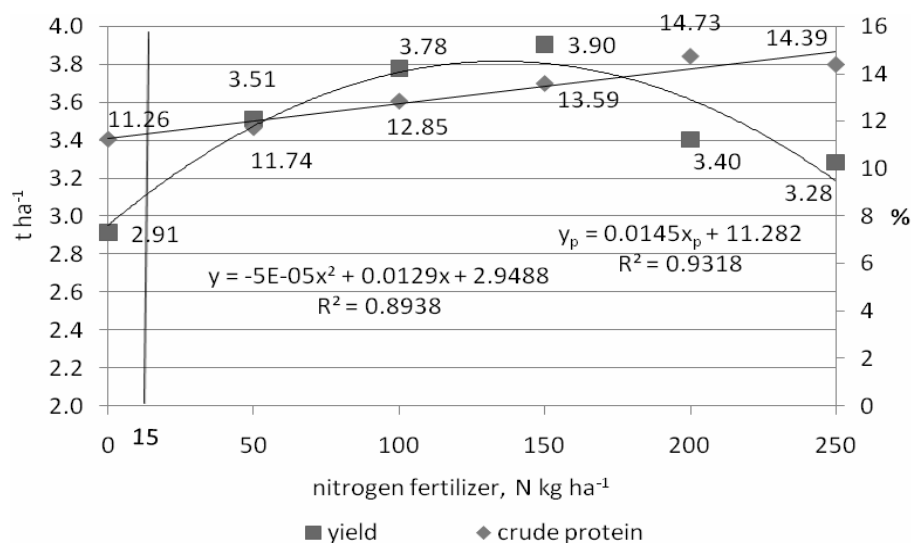


Figure 2. Yield and crude protein changes within loam after potatoes

Changes within the yield of spring wheat grown in sandy loam after cereals are demonstrated by the second stage polynomial regression equity  $y = -5E-05x^2 + 0.0159x + 2.7308$  (Figure 3). This equity represents 96.2 % of changes within cereal yield caused by the increase of nitrogen fertilizer rate,  $R^2 = 0.962$ . The standard mistake of the expected value is 0.126929. And this points to the successfully chosen, data describing the mathematical model. F-test's p-value 0.0072 is below 0.05; therefore the regression equity is statistically essential for the explanation of the changes within cereal yield. Statistically essential crude protein value changes are explained (Figure 3) by the linear regression equity  $y_p = 0.0119x_p + 11.135$  since F-test's p-value 0.001914 is below 0.05 describing 92.9 % of the field-test data ( $R^2 = 0.9294$ ).

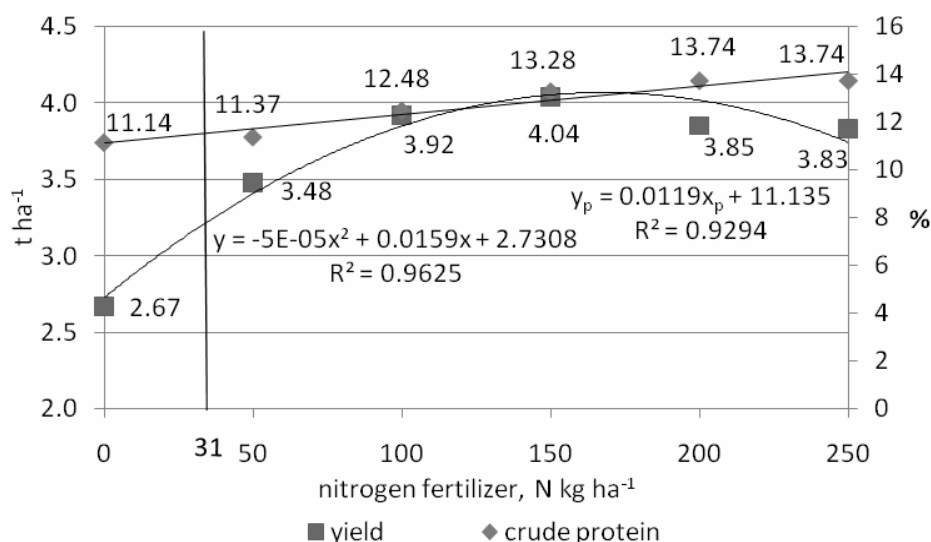


Figure 3. Yield and crude protein changes within loamy sand after cereals

The economically profitable nitrogen fertilizer rate for sandy loam after cereals has been fixed at 120 kg ha<sup>-1</sup>, in order to obtain 4.00 t ha<sup>-1</sup> of spring wheat grain. The critical crude protein content within wheat grain could be gained already with a 31 kg ha<sup>-1</sup> nitrogen fertilizer rate, and the

determination of the economical optimum of nitrogen fertilizer would not be further affected (Figure 3).

The changes within spring wheat grain yield grown in loamy soil after cereals, are represented by the second stage regression equity  $y = -3E-05x^2 + 0.0113x + 2.3204$  (Figure 4) that explains 90.1 % of the cases,  $R^2 = 0.901$ . F-test's p-value 0.031 is below 0.05; therefore the regression equity is a statistically essential explanation of the changes within the cereal yield. The crude protein increase within spring wheat grain should be described with the linear regression equity  $y_p = 0.0129x_p + 12.662$  that is a statistically essential explanation of the crude protein changes since F-test's p-value 0.01242 is below 0.05 describing 82.4 % of the test data ( $R^2 = 0.8237$ ).

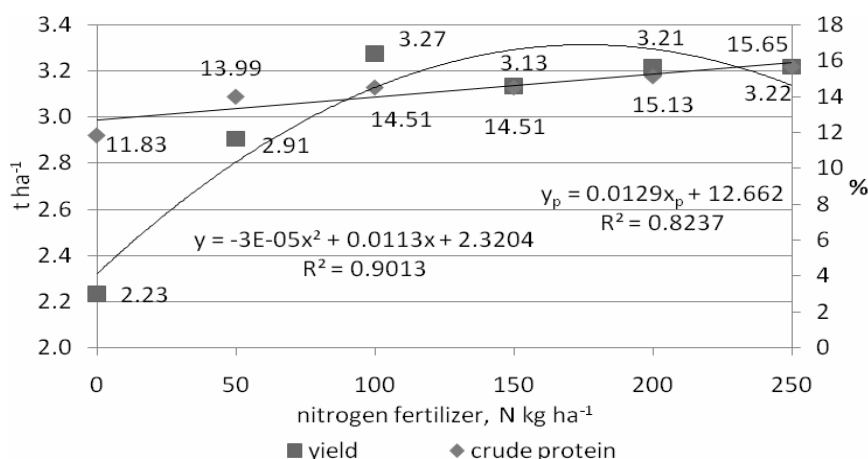


Figure 4. Yield and crude protein changes within loam after cereals

For spring wheat yield grown in loamy soil, to attain the economically optimal level: 3.23 t ha<sup>-1</sup>, there should be applied a 94 kg ha<sup>-1</sup> N fertilizer rate, and, in addition, the critical crude protein content border 11.5 % was attained already applying the variant without nitrogen fertilizer, and that did not affect the optimal yield estimates.

In the third stage the polynomial regression equity  $y = 2E-07x^3 - 0.0001x^2 + 0.0132x + 2.6319$  characterizes the changes within spring wheat grain yield grown in sandy loam after grass (Picture 5). To fix the optimum of nitrogen fertilizer, the third stage polynomial regression equity was applied that essentially explained the changes within the grain yield when influenced by a increased nitrogen fertilizer rate (F-test's p-value 0.00118 below 0.05). Spring wheat grown in experimental conditions, in order to obtain the optimal yield, would need only a 61 kg ha<sup>-1</sup> N fertilizer rate. Such regularity corresponds to 99.9 % of cases,  $R^2 = 0.999$  (Figure 5). The crude protein content within spring wheat grain is reflected by the linear equity  $y_p = 0.013x_p + 11.701$  that was statistically essential since the F-test's p-value 0.002621 was below 0.05 when describing 91.8 % of the field-test data ( $R^2 = 0.9176$ ).

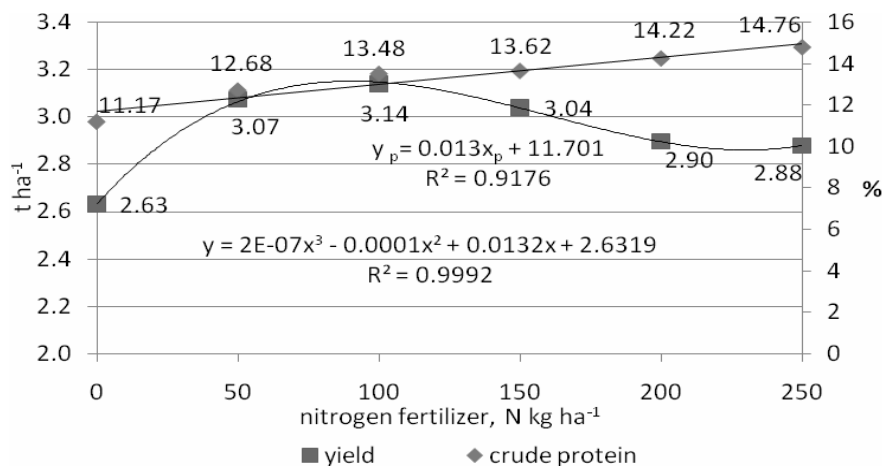


Figure 5. Yield and crude protein changes within loamy sand after grass

With of  $61 \text{ kg ha}^{-1}$  N rate could be obtained supposedly  $3.11 \text{ t ha}^{-1}$  of grain crop. The critical crude protein content within the grain supposedly would be attained already with minor nitrogen fertilizer rates, not affecting the optimal norm of nitrogen fertilizer.

For spring wheat grown in loamy soil after grass, in order to demonstrate the changes within the grain yield, the most appropriate was the second stage polynomial regression equity  $y = -2\text{E-}05x^2 + 0.0069x + 2.749$  (Figure 6).

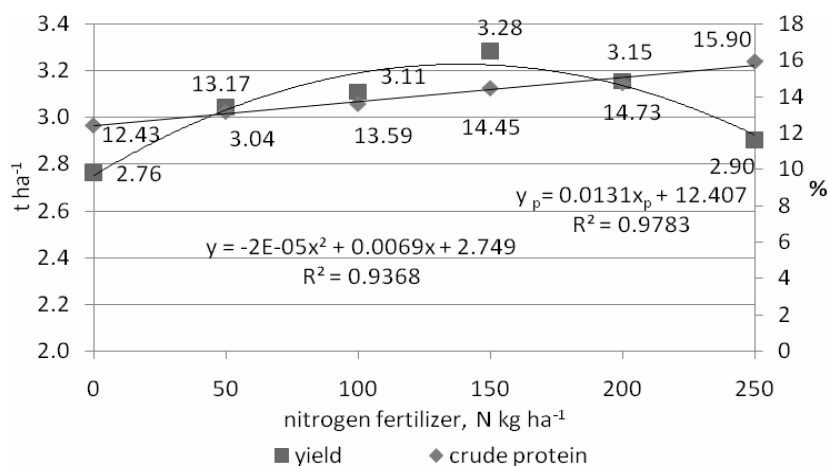


Figure 6. Yield and crude protein changes within loam after grass

The polynomial regression model can explain 93.7 % of changes within spring wheat grain yield affected by the increase of the nitrogen rate. The determination ratio is  $R^2 = 0.937$ . Changes within the grain yield of spring wheat grown in loamy soil after grass is represented at the second stage polynomial regression equity (Figure 6), the standard mistake of the expected value being 0.0604. F-test's p-value 0.0158 is below 0.05, therefore the regression equity is a statistically essential explanation of the changes within spring wheat grain yield.

The crude protein content within spring wheat grain increased in a linear way:  $y_p = 0.0131x_p + 12.407$ , that is a statistically essential explanation of the crude protein value since F-test's p-value 0.000178 is below 0.05 describing 97.8 % field-test data (determination ratio  $R^2 = 0.9783$ ). Based on the regression equity, the spring wheat grown in experimental conditions, in order to obtain economically optimal yield –  $3.03 \text{ t ha}^{-1}$  should need only  $45 \text{ kg ha}^{-1}$  N fertilizer rate, and the crude protein level would definitely be above the critical border since even without nitrogen fertilizer the obtained spring wheat grain yield contained 12.43 % of crude protein.

Changes within crude protein after the same preceding crop in soils with a different texture when increasing the nitrogen fertilizer rate were rather similar. The crude protein content within grain was more affected by various preceding crops whereas the granulometric content of soil affected the crude protein only within the variant without nitrogen fertilizer. Fixing the economically optimal nitrogen fertilizer rate for spring wheat and grain crop, the crude protein content, in most cases, was not a limiting factor if it is supposed that an 11.5 % high crude protein content within the grain is sufficient for qualitative grain standards. Only within sandy loam after potatoes, fixing the economically optimal nitrogen fertilizer norm had to be slightly increased in order to attain the crude protein content's 11.5 % borderline within the grain, and the reason could be the decline of soil nitrogen mineralizing potential due intertilled crops grown in the previous year, especially within lighter soils.

## Conclusions

The essential increase of spring wheat grain yield was insured by a  $50 - 150 \text{ kg h}^{-1}$  high nitrogen fertilizer rate. Further increase of nitrogen fertilizer did not create an essential increase of wheat grain yield.

The results of the polynomial regression demonstrate that the optimal nitrogen fertilizer rates applied in soils with different granulometric contents after various preceding plants were from 45 to 120 kg ha<sup>-1</sup>.

With economically optimal nitrogen fertilizer rates, all the variants, except the variant after potatoes in sandy loam, provided a crude protein content within the grain above 11.5 %.

The crude protein content within different types of grain increased, in direct ratio, with the increase of nitrogen rate – up to 200, 250 kg ha<sup>-1</sup>.

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## SLĀPEKĻA MĒSLOJUMA IETEKME UZ VASARAS KVIEŠU GRAUDU RAŽU UN KOPPROTEĪNA SATURU

**Jermušs A., Vigovskis J.**

Latvijas teritorijā vasaras kviešu lauki gan augsnes auglības gan kultūraugu produktivitātes ziņā ir dažādi. Tādējādi pastāv ieinteresētība lietot dažādas minerālmēsļu normas. Šī pētījuma mērķis ir noskaidrot likumsakarības starp graudu ražu, kopproteīna saturu un dažādu slāpekļa normu izmantojot graudu ražas datu regresijas analīzi slāpekļa mēslojuma normas optimizēšanai vasaras kviešiem.

Slāpekļa mēslojuma efektivitātes noteikšanai Skrīveros, Latvijas Lauksaimniecības Universitātes aģentūras Zemkopības Zinātniskā Institūta dažāda granulometriskā sastāva (mālsmilts un smilšmāla) Luvisol tipa augsnēs no 1999. līdz 2001. gadam tika ierīkoti lauka izmēģinājumi ar vasaras kviešiem ‘Munk’ pēc atšķirīgiem priekšaugiem – zālāja, graudaugu un kartupeļiem ar dažādām slāpekļa minerālmēsļu normām (0; 50; 100; 150; 200; 250; kg ha<sup>-1</sup>).

Slāpekļa mēslojums būtiski ietekmēja graudu ražu. Slāpekļa mēslojuma normas palielināšana no 50 līdz 100 un 150 kg ha<sup>-1</sup> bija būtiska, bet turpmākai slāpekļa normas palielināšanai nebija būtiska. Visi pētītie priekšaugu varianti arī būtiski ietekmēja graudu ražu. Pieaugošs slāpekļa mēslojums lineāri palielināja kopproteīna saturu vasaras kviešu graudos, kas bija atkarīgs no priekšauga un augsnes granulometriskā sastāva.

## THE AGRONOMIC AND QUALITY CHARACTERISTICS OF SPRING CEREALS GROWN AT DIFFERENT INPUT LEVELS OF FERTILIZERS AND CHEMICALS

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### Abstract

The spring cereals are the most important grains in Estonian farming. The input of fertilizers and chemicals used by farmers for spring cereal production is quite different. Successful farmers prefer high input. They utilize high levels of fertilizers, herbicides, fungicides, insecticides and growth regulators (Ministry of Agriculture, 2008).

The aim of this paper was to investigate the influence of the different levels of fertilizers and chemicals to the agronomic and quality characteristics of spring wheat, barley and oats.

The trial was established with two varieties of each spring cereal at the Jõgeva Plant Breeding Institute during 2006-2007. Two input levels of fertilizers and chemicals (high and low input) were

used. Four fertilizer doses, treatment by herbicides and insecticides were carried out in low input level (LI). The same fertilizer rates, treatments by fungicides, insecticides, leaf fertilizers and growth regulator were included in high input level (HI). Unfavourable weather conditions (drought) for cereals were observed. The results of this trial showed that there were significant differences between the agronomic and quality parameters of spring cereals at different input levels. Significant yield decrease and protein content increase was estimated in HI conditions. The plant height of wheat and oat varieties decreased with use of HI. There was no significant influence of the inputs to the length of the growing period in drought conditions.

**Key words:** spring cereals, fertilizers, chemicals, agronomic and quality characteristics

### Introduction

The spring cereals are the most important grains in Estonian farming. In the production of spring cereals farmers are advised to utilize high input of fertilizers and chemicals by sales-companies. Large amounts of chemicals and fertilizers play an important role in environmental pollution (Muurinen *et al.*, 2006). Moreover, the high rates of fertilizer, particularly high N rate, prolong the growing period, reduce lodging resistance and susceptibility to diseases and increase the protein content of spring cereals (Forsberg and Reeves, 1995; Araus, 2002). Therefore, it is important to apply the proper amount of chemicals and fertilizers for minimizing the pollution risk.

The aim of this study was to investigate the effect of different input levels of fertilizers and chemicals to grain yield, quality, length of growing period, plant height and the number of productive tillers of spring cereals.

### Materials and Methods

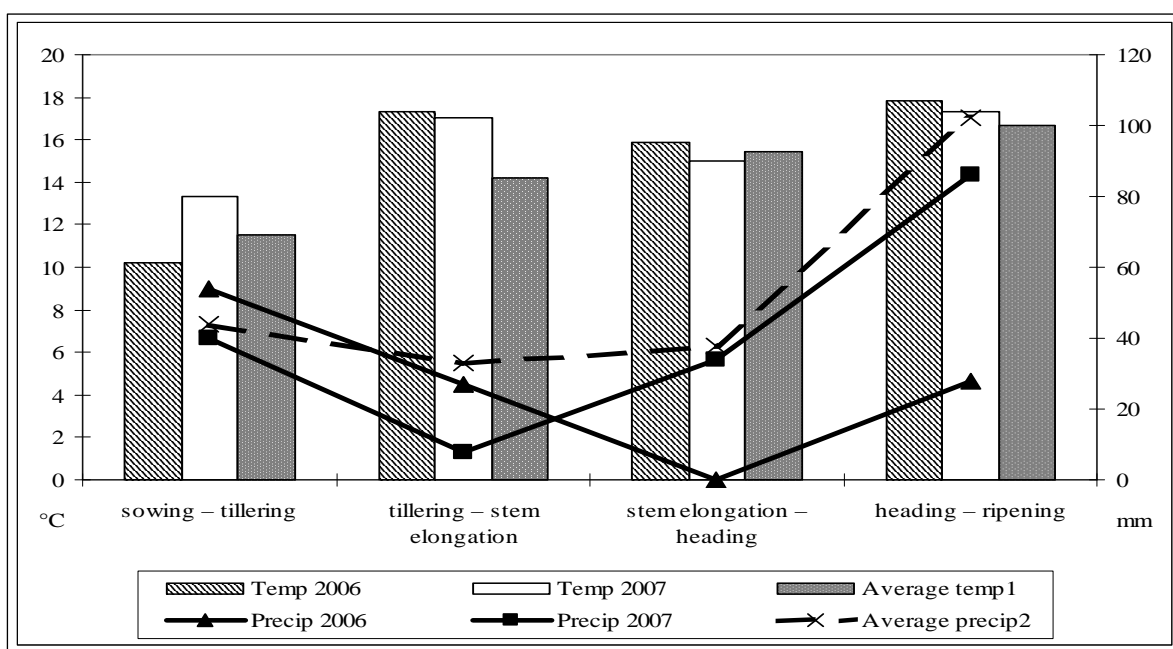
The trial was carried out with two wheat (Vinjett, Monsun), barley (Anni, Class) and oat (Villu, Flämingsprofi) varieties at the Jõgeva Plant Breeding Institute on Calcaric (Eutric) Cambisol (FAO classification) soil (clay loam,  $pH_{KCl}$  5.5) in 2006–2007. The trial was established on 9 m<sup>2</sup> plots in three replications. The plots were sown at density of 600 (wheat, oat) and 500 (barley) germinating seeds per m<sup>2</sup>. For both inputs 4 levels of fertilization (N0 K0 P0 kg ha<sup>-1</sup>; N60 P13 K23 kg ha<sup>-1</sup>; N100 P22 K39 kg ha<sup>-1</sup>; N140 P31 K54 kg ha<sup>-1</sup>) were applied. N0 P0 K0 kg ha<sup>-1</sup> was the control level. The complex fertilizer (containing 18 g kg<sup>-1</sup> N, 4 g kg<sup>-1</sup> P and 7 g kg<sup>-1</sup> K) at the rates of 0, 333, 556, 778 kg ha<sup>-1</sup> before sowing was used. Two chemical treatments – low (LI) and high input (HI) were utilized.

Low input. Herbicide Lintur 0.12 kg ha<sup>-1</sup> + MCPA 0.5 l ha<sup>-1</sup> in stage BBCH 13-14 in 2006 and 2007. Insecticide Danadim 40 EC 1.0 l ha<sup>-1</sup> in stage BBCH 33-34 in 2006 and Proteus OD 0.6 l ha<sup>-1</sup> in stage BBCH 13-14 in 2007.

High input. Low input plus:

- 1.growth regulator Kemira CCC 1.0 l ha<sup>-1</sup> for wheat and for barley in stage BBCH 21-29 and for oat in stage BBCH 31-32;
- 2.leaf fertilizer in stage BBCH 21-25 by Folicare (12 g kg<sup>-1</sup> N, 20 g kg<sup>-1</sup> P, 7 g kg<sup>-1</sup> K) 8 kg ha<sup>-1</sup>;
- 3.leaf fertilizer in stage BBCH 51-56 by Folicare (18 g kg<sup>-1</sup> N, 8 g kg<sup>-1</sup> P, 15 g kg<sup>-1</sup> K) 8 kg ha<sup>-1</sup>;
- 4.leaf fertilizer in stage BBCH 73-75 by Folicare (10 g kg<sup>-1</sup> N, 2 g kg<sup>-1</sup> P, 33 g kg<sup>-1</sup> K) 8 kg ha<sup>-1</sup>;
- 5A.fungicide in 2006: Tilt 250 EC 0.5 l ha<sup>-1</sup> for all cereals in stage BBCH 29-30;
- 5B.fungicides in 2007: Folicur EW 250 1.0 l ha<sup>-1</sup> in stage BBCH 13-14 for barley and in stage BBCH 50-51 for oat. Two fungicide applications for wheat: Falcon 0.8 l ha<sup>-1</sup> in stage BBCH 30-31 and Folicur EW 250 0.6 l ha<sup>-1</sup> in stage BBCH 45-46.

The weather conditions were unfavourable for spring cereals during the testing years. High air temperature and deficiency of precipitation for spring cereals were observed. The most heavy drought conditions were estimated during the period from stem elongation to heading in 2006 and from tillering to stem elongation in 2007 (Figure 1).



<sup>1</sup> – average of long-term temperature (1922–2005), <sup>2</sup> – average of long-term precipitation (1922–2005)

Figure 1. The average air temperature and precipitation in different growing stages during 2006–2007

The yields of spring cereals were weighed and converted to  $860 \text{ g kg}^{-1}$  dry matter content. Grain protein content was measured by total nitrogen using the Kjeldahl method ( $\text{N} \times 6.25$ ). Data was analyzed by factorial analysis of variance using the Agrobases statistics software (Agrobases<sup>TM</sup>, 1999).

### Results and Discussion

The results show that there were significant differences between the agronomic and quality parameters of spring cereals at different input levels.

Factors influencing yield and quality. Grain yield was the influenced most by the fertilizer rate (coefficient of determination 55%) (Table 1). The influence of other factors turned out to be much less. The results of the trial indicated that the number of productive tillers depended primarily on two factors – species (61%) and fertilizer rate (23%). The variation of 1000 kernel weight was influenced equally by the year and species (40 and 44% accordingly). The variation of volume weight almost entirely depended on the species (96%). The husk of the kernels reduced the weight volume weight and this was the reason why wheat volume weight was higher than barley and oat (Halverson and Zeleny 1988; Brouwer and Flood, 1995).

Oats showed the lowest level of this trait. Protein content in the kernels was mostly determined by the fertilizer rate (76%). Wheat had the highest protein content. The length of the growing period was influenced by the species (65%) and by the year (28%). Barley had the shortest growing period (average 91 days) followed by oats (94 days) and wheat (100 days). Variation of plant height was determined mainly by input (29%), fertilizer rate (28%) and species (21%). Oat plants were the longest and barley remained the shortest.

Table 1. Relative importance of factors (coefficients of determination) and their probability<sup>1</sup>

Characteristics of spring cereals	Coefficients of determination %							
	Year	Species	Input	Fertilizer rate	Fertilizer rate x input	Species x year	Species x input	Species x fertilizer rate
Yield	–	9***	10***	55***	10***	9***	2**	–
Productive tillers	–	61***	1*	23***	–	7***	–	4**
1000 kernel weight	40***	44***	–	–	–	5*	–	–
Volume weight	1***	96***	1***	–	–	1***	–	–
Protein content	–	6***	8***	76***	–	–	–	–
Growing period	28***	65***	–	–	–	2*	–	–
Plant height	–	21***	29***	28***	3**	5***	8***	–

<sup>1</sup>probability \*\*\*  $P \leq 0.001$ ; \*\*  $0.001 < P \leq 0.01$ ; \*  $0.01 < P \leq 0.05$

Comparison of inputs. HI had a significant negative effect on the yield of the wheat varieties (Vinjett, Monsun) (Table 2). The differences were  $-631 \text{ kg ha}^{-1}$  and  $-415 \text{ kg ha}^{-1}$ , accordingly. The same effect on barley (Anni difference  $-596 \text{ kg ha}^{-1}$ , Class  $-448 \text{ kg ha}^{-1}$ ) (Table 3) and especially on oat (Villu difference  $-1,088 \text{ kg ha}^{-1}$ , Flämingsprofi  $-1,164 \text{ kg ha}^{-1}$ ) varieties was observed (Table 4).

Table 2. The average characteristics of wheat varieties at two inputs

Characteristics of wheat	Vinjett			Monsun			LSD <sub>0.05</sub>
	LI <sup>1</sup>	HI <sup>2</sup>	Difference	LI <sup>1</sup>	HI <sup>2</sup>	Difference	
Yield, $\text{kg ha}^{-1}$	3,966	3,335	-631*	3,739	3,324	-415*	270
Productive tillers, $\text{pcs m}^{-2}$	373	358	-15	364	344	-20	22
1000 kernel weight, g	35.7	33.8	-1.9*	41.7	42.8	1.1*	0.9
Volume weight, $\text{g l}^{-1}$	747	695	-52*	727	715	-12*	7
Protein content, $\text{g kg}^{-1}$	134	139	5*	128	136	8*	4
Growing period, days	99	99	0	101	101	0	0.9
Plant height, cm	76.0	48.9	-27.1*	67.5	54.6	-12.9*	2.3

\* probability at 0.05; <sup>1</sup> – low input, <sup>2</sup> – high input

HI had no significant effect on the number of the productive tillers of wheat varieties Vinjett and Monsun. A negative influence was found on the barley variety Anni (difference  $-25 \text{ pieces m}^{-2}$ ) and the oat variety Villu (difference  $-32 \text{ pieces m}^{-2}$ ). Impact of HI on varieties Class (barley) and Flämingsprofi (oat) was not observed.

Table 3. The average characteristics of barley varieties at two inputs

Characteristics of barley	Anni			Class			LSD <sub>0.05</sub>
	LI <sup>1</sup>	HI <sup>2</sup>	Difference	LI <sup>1</sup>	HI <sup>2</sup>	Difference	
Yield $\text{kg ha}^{-1}$	4,085	3,489	-596*	3,520	3,072	-448*	242
Productive tillers $\text{pcs m}^{-2}$	588	563	-25*	534	516	-18	23
1000 kernel weight g	44.4	45.6	1.2*	45.7	46.0	0.3	1.2
Volume weight $\text{g l}^{-1}$	695	689	-6	689	686	-3	8
Protein content $\text{g kg}^{-1}$	119	134	15*	126	131	5*	4
Growing period days	90	90	0	92	92	0	0.3
Plant height cm	57.9	53.8	-4.1	56.9	54.4	-2.5	6.3

\* probability at 0.05; <sup>1</sup> – low input, <sup>2</sup> – high input

There was no significant effect of HI to 1000 kernel weight of oats varieties Villu and Flämingsprofi, but affected the wheat varieties (Vinjett, Monsun) and the barley variety Anni. HI decreased grain weight of the variety Vinjett (difference with LI  $-1.9 \text{ g}$ ). On the other hand, grain weight of the varieties Monsun and Anni increased (differences  $1.1 \text{ g}$  and  $1.2 \text{ g}$ , accordingly).



A decline in the volume weight of the wheat varieties (Vinjett -52 g l<sup>-1</sup>, Monsun -12 g l<sup>-1</sup>) and the oat varieties (Villu -23 g l<sup>-1</sup>, Flämingsprofi -20 g l<sup>-1</sup>) occurred in HI. There was no significant decrease of volume weight of the barley varieties.

Using HI the increase of the protein content of all the varieties was observed. The differences between the two inputs were for the wheat varieties 5 g kg<sup>-1</sup> (Vinjett), 8 g kg<sup>-1</sup> (Monsun), for barley 15 g kg<sup>-1</sup> (Anni), 5 g kg<sup>-1</sup> (Class) and for oat 12 g kg<sup>-1</sup> (Villu), 11 g kg<sup>-1</sup> (Flämingsprofi).

The length of the growing period of spring cereals was not prolonged because of the use of different inputs.

Table 4. The average characteristics of oat varieties at two inputs

Characteristics of oat	Villu			Flämingsprofi			LSD <sub>0.05</sub>
	LI <sup>1</sup>	HI <sup>2</sup>	Difference	LI <sup>1</sup>	HI <sup>2</sup>	Difference	
Yield, kg ha <sup>-1</sup>	4,722	3,634	-1,088*	4,931	3,767	-1,164*	265
Productive tillers, pcs m <sup>-2</sup>	500	468	-32*	552	538	-14	23
1000 kernel weight, g	36.2	35.7	-0.5	38.6	39.1	0.5	1.0
Volume weight, g l <sup>-1</sup>	516	493	-23*	470	450	-20*	11
Protein content, g kg <sup>-1</sup>	121	133	12*	116	127	11*	3
Growing period, days	94	94	0	94	94	0	0.3
Plant height, cm	78.7	61.0	-17.7*	77.0	61.3	-15.7*	3.3

\* probability at 0.05; <sup>1</sup> – low input, <sup>2</sup> – high input

HI caused a significant decline of the plant height of wheat and oat. The shortening of plant height was considerable for the wheat variety Vinjett (difference -27.1 cm) and the oat variety Villu (difference -17.7 cm). The plant height of the barley varieties had no significant decrease.

Plant development in the both trial years was influenced by drought conditions during the growth stages from tiller emergence to stem elongation (in 2007) and from heading to ripening (in 2006). Therefore, during both testing years the weather conditions affected negatively the yield and the quality of the cereals. This was the reason why the fertilizer rates influenced yield more than the year as a factor. Drought caused a stress-situation in plants and spraying with chemicals caused additional stress. Stress can reduce the duration of the photosynthetic area and that limits the yield of biomass (Araus, 2002). The contribution of assimilates to final grain yield depends on environmental conditions during the grain-filling period (Moral *et al.*, 2002). According to our results, the HI decreased the yield of spring cereals, especially of oat varieties. Nutrient requirements for oats are less than those for wheat or barley, but oat is more sensitive to climatic factors when applying chemicals (Forsberg and Reeves, 1995). The average level of grain yield of the oat varieties turned out to be the highest in the testing years. Wheat and barley are more suitable for soil pH<sub>KCl</sub> up to 6.0, acidic soil reduces their yield due to less assimilable nitrogen, phosphorus, and other elements of nutrients (Loide, 2006). In our trial soil pH<sub>KCl</sub> 5.5 was more suitable for oat cultivation.

The number of productive tillers per plant, the grain number per spike and grain weight are important features of cereals in determining the yield potential (Moral *et al.*, 2002). High air temperature with water deficiency can reduce the final number of productive tillers and lead to the mortality of a part of them. On the other hand soil nitrogen supply is one of the most important environmental factors influencing the number of productive tillers (Lersten, 1987; Brouwer and Flood, 1995). By our trial results barley had higher tiller production than wheat and oats. A minor decrease of the productive tillers of oat and barley occurred in HI condition.

Producing high yield and quality spring cereals in middle latitudes demands favourable temperature and moisture conditions (Forsberg and Reeves, 1995). According to Brouwer and Flood (1995) the decrease of grain weight was determined by unfavourable weather conditions. The most important are the environmental conditions during the grain filling period (Simmons, 1987; Moral *et al.*, 2002). Drought during that period decreased grain size in 2006 in our trial. Input as factor did not have a significant impact on grain size. Barley had the biggest 1000 kernel weight.

The volume weight is mostly dependent on kernel size, density and shape. Drought and heavy lodging considerably reduce the volume weight of spring cereals (Halverson and Zeleny, 1988;

Brouwer and Flood, 1995). HI decreased significantly the volume weight of wheat and oat varieties, but did not have a significant effect on barley varieties.

Growth conditions, genotype and the application of fertilizers, especially N-fertilizers, affected the level of protein content. Wheat contained considerably more protein, on average, than other cereals (Orth and Shellenberger, 1988). In this trial HI increased the protein content of all the spring cereals. Leaf fertilization (application of N-fertilizer) during kernel development, according to Halverson and Zeleny (1988), increased the protein content of grains. Higher fertilizer doses in the both HI and LI conditions caused the rise of the protein content of all the crops.

The length of the growing period was influenced mainly by species and year. The spring oat and wheat develop more slowly than barley (White, 1995) and barley had the shortest growing period in the trial. Drought from heading to ripening (2006) decreased the length of the growing period more than early drought from tillering to stem elongation (2007).

Air temperature is one of the major components of the environmental factors affecting the development of cereals (Moral *et al.*, 2002). Growth regulators affected the height of cereal plants more than temperature (Simmons, 1987). In this trial HI significantly reduced the height of oat and wheat plants. Barley had the shortest average plant height. HI had no influence on the height of barley plants.

### Conclusions

During the drought years HI decreased significantly the grain yield and increased the protein content of spring cereals. There was no significant influence of inputs on the length of growing the estimated period. The plant height of wheat and oat varieties was reduced while using HI. The plant height of barley varieties had no significant decrease. A decline in the volume weight of wheat and oat occurred in HI conditions. HI had no effect on the number of productive tillers of the wheat varieties. The influence to oat and barley was unclear and needs further testing. Significant influence of input to kernel weight of oat was not observed. The effect of HI on barley and wheat depended on the variety.

This study needs to be continued during a longer period to investigate the characteristics of spring cereals cultivated in different input conditions.

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## MĒSLOJUMA UN ĶĪMIKĀLIJU DEVAS IETEKME UZ VASARĀJU LABĪBU AUGŠANU UN KVALITĀTI

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Vasarāju labības ir visnozīmīgākie graudaugi Igaunijas lauksaimniecībā. Minerālmēsļu un ķīmikāliju nodrošinājums saimniecībās ir atšķirīgs. Sekmīgie zemnieki dod priekšroku intensīvai audzēšanai, viņi izlieto lielus minerālmēsļu, herbicīdu, fungicīdu, insekticīdu un augšanas regulatoru daudzumus (Ministry of Agriculture, 2008).

Pētījuma mērķis bija noteikt dažādu minerālmēsļu un ķīmikāliju devu ietekmi uz vasaras kviešu, miežu un auzu agronomiskām un kvalitātes īpašībām.

Izmēģinājums tika ierīkots ar katras vasarāju labības divām šķirnēm Jõgevas laukaugu selekcijas institūtā laikā no 2006.-2007. gadam. Tika izmantoti divi minerālmēsļu un ķīmikāliju foni (augsts un zems). Zemajā fonā (LI) tika pārbaudītas četras minerālmēsļu devas, apstrāde ar herbicīdiem un insekticīdiem. Tās pašas minerālmēsļu normas, apstrādes ar fungicīdiem, insekticīdiem, kā arī lapu mēslojums un augšanas regulatori tika iekļauti augstajā fonā (HI). Tika novēroti labībām nelabvēlīgi klimatiskie apstākļi (sausums). Izmēģinājuma rezultāti parādīja, ka dažādos minerālmēsļu un ķīmikāliju fonos starp vasarāju labību agronomiskajiem un kvalitātes rādītājiem atšķirības bija būtiskas. HI apstākļos tika novērots būtisks ražas samazinājums un proteīnu satura pieaugums, kā arī kviešu un auzu šķirņu augu augstuma samazinājums. Sausos apstākļos neizpaudās pārbaudīto fonu ietekme uz augšanas perioda ilgumu.

## THE INFLUENCE OF CROP ROTATION AND PLANT-PROTECTION-COMPLEX ON BUCKWHEAT AND POTATTO YIELD

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### Abstract

Buckwheat and potato research has been carried out within the long-term crop rotation stationary that was established in 1969 as a part of the Research Institute of Agriculture. The buckwheat proportion within the particular crop rotations went up to 22%, the proportion of potatoes - up to 16%. The highest buckwheat yields were obtained from the buckwheat variants that were cultivated after the winter rye, and within the buckwheat monoculture experimental plots. A considerable yield decrease was observed when cultivating the buckwheat after the potatoes. Weeds in the buckwheat sowings were effectively brought under control by the herbicide Butisane 400 (1,5 l ha<sup>-1</sup>), applied immediately after sowing.

The highest potato yields were obtained when cultivated after winter rye, as compared to potato monoculture growing. A considerable yield decrease was observed within the crop rotations that contained the buckwheat. Herbicide Titus (50g ha<sup>-1</sup>) treatment on the potatoes provided an increase of yield up to 17% and, a fourfold dose of fungicides increased the yield up to 37%. In the potato monoculture experimental plots we observed up to 4 times more perennial weeds, moreover – the use of pesticides did not provide the increase of the yield which could be obtained while growing the potatoes in a proper crop rotation.

**Key words:** crop rotation, monoculture, potatoes, buckwheat, weediness, herbicides.

## Introduction

The market economy is forcing farms to give up wide selection of cultures and specialize on just a few kinds of cultivated plants, which subsequently causes prerequisites of negative phytosanitary conditions for the cultivation technologies of the remaining cultivated plants. When plants of related cultures are being grown in the same place protractedly, usually specific weeds and plant diseases tend to expand there. The significance of the mentioned problems and their possible solutions can be best seen in the stationery crop rotation experimental complex. Considerable experience and research results concerning issues of crop rotation have been gathered in a long-term stationery crop rotation complex of the LAU Research Institute of Agriculture in Skrīveri. The structure of the complex and the main obtained results are summarized in the publications of D.Lapins, B.Lejina (1997), A.Lejins, B.Lejina (2000), A.Lejins, B.Lejina (2002), A.Lejins, B.Lejina (2003), A.Lejins, B.Lejina (2006). The latest results are displayed in this article and should be considered as a continuation of the previous publications.

## Materials and Methods

Complex crop rotation researches at the Research Institute of Agriculture were started in 1969 and gradually expanded in time and space now reaching five different structures of crop rotation sowings. Within the long-term crop rotation stationery there have been researches with buckwheat and potatoes. The proportion of buckwheat in the crop rotation reaches 33%, the proportion of potatoes – 16%. Characterization of soil: turf low podzolic, cultivated with pH 5.4-6.3, contents of organic substance 19 – 22 g kg<sup>-1</sup>, phosphorus 21 –23 mg kg<sup>-1</sup> and potassium 87-99 mg kg<sup>-1</sup>. The granulometric composition of the soil can be evaluated as sandy loam. The following sorts of cultivated plants have been used in the researches: buckwheat '*Anita Beloruskaja*' and '*Aiva*', potatoes '*Priekulu dzeltenie*' and '*Vineta*'. The total area of one plot of the crop rotation is 354.4 m<sup>2</sup>, one divided plot is 59 m<sup>2</sup>. Differences between variants have been rated mathematically with dispersion analysis. Weed count have been done for each variant in 100 spots, defining the distribution of each weed species in % and then converted into number per m<sup>2</sup> (Rasins A., Taurina M., 1982).

## Results and Discussion

In the crop rotation potatoes have been grown after winter rye, spring wheat (forecrop – buckwheat) and potatoes repeatedly. Potato tuber yields are placed in the very same decreasing sequence. The lowest potato tuber yields are from the monoculture plots – an average 15.4 t ha<sup>-1</sup> during 6 years. Growing potatoes after spring wheat gives a remarkable yield increase up to 23.14 t ha<sup>-1</sup>.

Table 1. Effect of preceding crops and complex of pesticide the system on potatoes yield, 2001-2006

Nr of crop rotation	Variants of crop protection	Preceding crops	Yield t ha <sup>-1</sup>	Increase or decrease of yield			
				by crop protection		by crop rotation	
				+/- t ha <sup>-1</sup>	%	+/-t ha <sup>-1</sup>	%
1.	Without herbicides		26.87				
	herbicide Tituss 50 g ha <sup>-1</sup> + Citovet 200 ml ha <sup>-1</sup>	Clover + timothy II, rye	32.74	+5.87	122		
	Average		29.81			+14.40	193
3.	Without fungicides		18.89				
	Ridomil Gold 2,5 kg ha <sup>-1</sup> 2× un Ditan 2.5 kg ha <sup>-1</sup> 2×	Buckwheat, spring wheat	27.38	+8.49	145		
	Average		23.14			+7.73	150
5	Without pesticides		11.98				
	Complex of pesticides*	Potatoes grown repeatedly	18.95	+6.97	158		
	Average		15.41				100

LSD<sub>0.05 crop protection</sub> = 2.87 t ha<sup>-1</sup> LSD<sub>0.05 crop rotation</sub> = 3.06 t ha<sup>-1</sup>

\*treatment by pesticides: herbicide Titus 50 g ha<sup>-1</sup> + Citovet 200 ml ha<sup>-1</sup>; fungicide Ridomil Gold 2,5 kg ha<sup>-1</sup> 2× and ditan 2.5 kg ha<sup>-1</sup> 2×

Table 2. Effect of different preceding crops and pesticides on the number of weeds (pcs/sq. meter) in potatos, 2001-2006

Nr. of crop rotation	Variants	Preceding crops	Number of weeds total		including				
			per m <sup>-2</sup>	%	annual weeds per m <sup>-2</sup>	%	perennial weeds per m <sup>-2</sup>	%	Incl. couch-grass
1.	Without herbicides	Clover + timothy	76	100	57	10	19	100	10
			29	38	20	35	9	47	3
	Herbicides Titus 50g ha <sup>-1</sup> + Citovet 200 ml ha <sup>-1</sup>	II, rye							
Average			53	100	39	74	14	26	6
3.	Without fungicides	Buck-wheat, spring wheat	72	100	31	10	41	100	10
			54	75	23	74	31	76	5
	Fungicides Ridomil Gold 2.5 kg ha <sup>-1</sup> 2× un Ditan 2.5 kg ha <sup>-1</sup> 2×								
Average			63	100	27	43	36	57	7
5	Without herbicides	Potatos grovn repeatedly	112	100	60	10	52	100	5
			47	42	21	35	26	50	1
	Complex of pesticides*								
Average			80	100	40	50	40	50	3

\*treatment by pesticides: herbicide Titus 50 g ha<sup>-1</sup> + Citovet 200 ml ha<sup>-1</sup>; fungicide Ridomil Gold 2.5 kg ha<sup>-1</sup> 2× un ditan 2.5 kg ha<sup>-1</sup> 2×

Obviously the negative influence of buckwheat as a forecrop remains even in the second year. Growing potatoes after winter rye (forecrop – perennial grass) gives almost doubles the yield. Herbicide Titus treatment 50 g ha<sup>-1</sup> after potato germination ensures persistent yield increases of 22%, 3-4 times fungicide treatment 8.5 t ha<sup>-1</sup> ensures yield increases of 45%. Complex herbicide and fungicide treatment in potato monoculture gives a yield increases of 58%, however, that does not reach the yield level as in a proper crop rotation. Weed quantity in potato plantings after herbicide Titus treatment 50 g ha<sup>-1</sup> mostly decreases because of the restriction of quackgrass *Elytrigia repens* (L.) Nevski and different annual weeds (65%).

Summarizing the results of six years research we see that buckwheat can be successfully grown repeatedly as a monoculture, as well as after winter rye. There is a remarkable yield decrease when growing buckwheat after potatoes. This could be explained with the fact that both these cultures require much potassium, and after 2 years the soil starts to experience shortage of potassium resources

If we compare buckwheat nutlet yields in different variants, we see that buckwheat can be successfully grown repeatedly as a monoculture. However, at the final part of the research, buckwheat nutlet yield starts to decrease again, which means that in buckwheat monoculture sowings there still are some conditions with a bad influence upon the yield amount.

 Table 3. Some forecrop influence on the yield of buckwheat nutlets, t ha<sup>-1</sup> (in crop rotation stationery 2001-2006)

Nr. of crop rotation	Preceding crops	Nutlets yield of buckwheat		
		t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%
3-3	Barley - rye	1.26	+0.08	107
3-6	Rye - potatos	0.99	-0.19	84
5-6	Buckwheat, reiterated growth	1.18		100
LSD <sub>0.05</sub>		0.16		

Table 4. The influence of preceding crops on the yield of buckwheat nutlets at some trial periods, t ha<sup>-1</sup>

Number of crop rotation	Preceding crops	Nutlets yield of buckwheat			
		2001-2002		2005-2006	
		t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>
3-3	Barley - rye	1.63	-0.18	0.46	+ 0.19
3-6	Rye - potato	1.55	-0.26	0.28	+ 0.01
5-6	Buckwheat, reiterated growth	1.81		0.27	
LSD <sub>0.05</sub>		0.15		0.04	

 Table 6. Yield of buckwheat nutlets in some variants of herbicide usage, t ha<sup>-1</sup> (average in 2001 – 2006)

Crop rotation, preceding crop	Control without herbicides	Herbicides, dosage of herbicides		
		t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%
3-3 Rye	0.95	1.42	Betanal AM 2.5 l ha <sup>-1</sup> for buckwheat at 2-4 leaf stage	
			+0.47	149
3-6 Potato	0.78	1.16	Butizan 400 1.5 l ha <sup>-1</sup> before shoots	
			+0.38	149
Buckwheat, reiterated growth	0.97	1.37	+0.40	141
Average	0.93	1.34	+0.41	144
LSD <sub>0.05</sub>		0.18		

Evaluating the efficiency of the herbicide treatment we see that both herbicides – Butisan 400 and Betanal AM – ensure similar yield increasement and weed control level. We would give preference to Butisan 400, because it is less sensitive to rain during spraying and moreover the optimal spraying time for Betanal AM is very limited.

Table 7. Number of weeds in some variants of herbicide usage, pcs/sq. meter (average in 2001 – 2006)

Weeds	Control without herbicides	Betanal AM 2.5 l ha <sup>-1</sup> for buckwheat at 2-4 leaf stage	Butizan 400 1.5 l ha <sup>-1</sup> before shoots
Hearteasy – VIOLA ARV	1	1	1
Hempnettle, var. spec.-GALEO spp	6	4	1
Goosefoot, var. spec. – CHENO spp	15	7 (4-10)	6 (4-9)
Chamomile, false – TRIPL INO	7	6	1 (0-1)
Catchweed – GALIU APR	5	5	3
Bindweed, black – FALLO CON	2	1	
Chickweed, common–STELL MED	2		1
Charlock, jointed –RAPHA RAP	4	2	2
The others	3	2	
Annual weeds total	47	28 (21-36)	16 (11-21)
%	100	59	34
Horsetail, common – EQUIS ARV	5	7	6
Sow thistle, field – SONCH ARV	8	5	5
Mint, corn – MENTH ARV	5	6	3
Thistle, creeping – CIRSI ARV	3	1	3
Betony, marsch – STACH POL	33	3(2-6)	8(5-11)
Couch, common – ELYTR REP	20	15(10-20)	10(7-14)
Perennial weeds total	78	40(31-52)	36(27-45)
%	100	51	46
Number of weeds total	125	67(51-84)	52(41-67)
%	100	53	42

## Conclusions

The highest potato tuber yield is in the crop rotation where potatoes are grown after winter rye (forecrop of winter rye – perennial grass).

Potato monocultural growing is not recommended.

Potatoes and buckwheat are incompatible cultures and they shouldn't be grown in the same crop rotation.

The highest buckwheat yields are gathered from monocultural sowings or growing buckwheat after winter rye. A remarkable decrease in the of buckwheat yield is noted when grown after a potato crop.

Betanal AM and Butisan 400 can be used in buckwheat sowings for successful weed control.

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## AUGU MAIŅAS UN AUGU AIZSARDZĪBAS LĪDZEKĻU KOMPLEKSA IETEKME UZ GRIĶU UN KARTUPEĻU RAŽU

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Pētījumi ar griķiem un kartupeļiem veikti ilggadīgā augseku stacionāra ietvaros, kas iekārtots 1969. gadā LLU Zemkopības zinātniskajā institūta. Griķu īpatsvars pētītās augsekās ir līdz 22%, kartupeļu līdz 16%. Augstākās griķu riekstiņu ražas iegūst griķu atkārtotās audzēšanas variantā un pēc ziemas rudziem. Ticams griķu ražas samazinājums ir tur, kur tos audzē pēc kartupeļiem. Griķu sējumos nezāles efektīvi ierobežo herbicīds butizāns 400 1.5 l ha<sup>-1</sup>, kuru izsmidzina tūlīt pēc sējas.

Augstākās kartupeļu ražas iegūst, stādot tos pēc ziemas rudziem, salīdzinot ar kartupeļu bezmaiņas audzēšanu. Būtisks kartupeļu ražas samazinājums ir augsekā, kur audzēti griķi. Kartupeļu stādījumos herbicīda titusa 50g ha<sup>-1</sup> lietošana deva ražas pieaugumu līdz 17 % un 4- riezēja fungicīdu lietošana 37%. Kartupeļos, kur lieto bezmaiņas audzēšanu, nezāļu, it sevišķi daudzgadīgo, ir būtiski vairāk (līdz 4 reizēm) un pesticīdu lietošanu nenodrošina kartupeļu bumbuļu ražas pieaugumu, ko iegūst audzējot kartupeļus augmaiņā.

## INFLUENCE OF VARIOUSLY SPECIALIZED CROP ROTATIONS AND PLANT PROTECTION-MEANS-COMPLEX UPON SPRING CROP YIELD

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## Abstract

The complex crop rotation experiments at the Research Institute of Agriculture started in 1969. The plants, preceding the barley, according to their influence upon the crop yield, can be ranged as follows (in a descending order): clover + timothy; oats; winter rye. A remarkable yield decrease started with the winter rye. Barley monoculture sowings gave a yield decrease up to 1.17 t ha<sup>-1</sup>. Applying the herbicides Grodil and MCPA during tillering provided a constant yield increase on

average 0.76 t ha<sup>-1</sup>. The greatest barley yield increase (1.05 t ha<sup>-1</sup>) was observed within the monoculture experimental plot, after treating them with the herbicides and fungicides. Weediness increased in those barley sowings where the cereal proportion of the whole crop rotation went over 83%. There were observed the perennial weeds, mostly in the monoculture sowings, and, especially with the quick-growing grass *Elytrigia repens*. Within the crop rotations, when the cereal proportion went up to 83%, the oats had a particularly small influence upon the various preceding plants. A remarkable yield decrease was observed within the monoculture oat sowings. The greatest oat yield increase (0.86 t ha<sup>-1</sup>) was observed within the monoculture plots, after treating them with the complex of the herbicides and fungicides nevertheless, it did not reach the best crop rotation variants yield index. Weediness was much lower within the oat sowings, with potatoes as a preceding plant, than in the monoculture plots, or when growing the oats after the winter rye. In general, there was 10 times more weeds within the monoculture oat sowings than in the plots with a proper crop rotation. The highest spring wheat yields were obtained from the plots where the preceding plants were buckwheat, or lupine. Hericides and fungicides were more effective within the monoculture spring wheat sowings than within the crop rotations. Weediness within the spring wheat sowing was higher within those crop rotation variants that had a higher cereal proportion.

**Key words:** crop rotation, barley, oats, spring wheat, weediness, herbicides.

### Introduction

Market economy is forcing farms to give up a wide selection of cultures and specialize on a just few kinds of cultivated plants, which subsequently causes prerequisites of negative phytosanitary conditions for the cultivation technologies of the remaining cultivated plants. When plants of related cultures are being grown in the same place protractedly, usually specific weeds and plant diseases tend to expand there. The significance of the mentioned problems and their possible solutions can be seen best in the stationery crop rotation experimental complex. Considerable experience and research results concerning issues of crop rotation have been gathered in a long-term stationery crop rotation complex of LAU Research Institute of Agriculture in Skriveri. The structure of the complex and main obtained results are summarized in publications of D.Lapins, B.Lejina (1997), A.Lejins, B.Lejina (2000), A.Lejins, B.Lejina (2002), A.Lejins, B.Lejina (2003), A.Lejins, B.Lejina (2006). The latest results are displayed in this article and should be considered as a continuation of the previous publications.

### Materials and Methods

Complex crop rotation research in the Research Institute of Agriculture started in 1969 and gradually expanded in time and space now reaching five different structures of crop rotation sowings. The proportion of cereals in the crop rotation in different combinations reaches 50-100%, perennial grass 16.5-33.3%. Characterization of the soil: turf low podzolic, cultivated with pH 5.4-6.3, contents of organic substance 19 – 22 g kg<sup>-1</sup>, phosphorus 21 –23 mg kg<sup>-1</sup> and potassium 87-99 mg kg<sup>-1</sup>. Granulometric composition of soil can be evaluated as sandy loam. The following sorts of cultivated plants have been used in the research: winter rye 'Voshod', barley 'Rūja', oat 'Selma', spring wheat 'Planeta', buckwheat 'Anita Beloruskaja' and 'Aiva'. The total area of one plot of the crop rotation is 354.4 m<sup>2</sup>, one divided plot is 59 m<sup>2</sup>. The yield of each plot has been gathered with a harvester Sampo 500, weighted and equated to the humidity level 14%. Differences between variants have been rated mathematically with dispersion analysis. Weed counts have been done for each variant in 100 spots, defining the distribution of each weed species in % and then converted into number per m<sup>2</sup> (Rasins A., Taurina M., 1982).

### Results

From all the researched cereals the highest yields have been gathered from spring wheat, barley and oats. The lowest yields in all cases are gathered from monocultural sowings. The highest barley yields we got from the crop rotation with a cereal proportion of 50%, barley crop oats (Table 1).



Table 1. The effect of the preceding crop and crop rotation on the grain yield of barley t ha<sup>-1</sup> in long-term trials, 2001-2006

No of crop rotation	Proportion of cereals in crop rotation	Preceding crop	Yield 2001-2005			Yield 2006		
			t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%	t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%
1	50*	oats	4.09	+ 1.27	145	2.44	+1.32	218
2	66	rye	3.52	+ 0.70	125	2.26	+1.14	202
3	66 incl.33% buckwheat	buckwheat	4.05	+ 1.23	144	2.76	+1.64	246
4	83	rye	3.58	+ 0.76	127	2.12	+1.00	189
4	83	oats	3.69	+ 0.87	131	2.32	+1.20	207
5	100	oats	3.95	+ 1.13	140	1.98	+0.86	177
5	100	barley	3.64	+ 0.82	129	1.78	+0.66	159
5	100	barley grown repeatedly	2.82		100	1.12		100
LSD <sub>0.05</sub>			0.29			0.32		

\*Including potatoes 16 %

Same barley yield also from the crop rotation with buckwheat proportion 33% and potato 16%. In crop rotations with a high cereal proportion the barley yield increase (in comparison with monocultural sowings) can be obtained with correct cereal species rotation. By evaluating the barley forecrop it was found that higher barley yields can be gathered when growing them after oats or barley repeatedly. We do not recommend growing barley after winter rye.

In 2006, due to aridity in the vegetation period, there generally were very low cereal yields, especially in monocultural sowings. Barley yield gathered from sowings after buckwheat, oat and winter rye was twice as large as that from barley monoculture. It means that proper crop rotation is even more important during unfavourable meteorological weather conditions.

The best forecrop (from examined) for spring wheat is buckwheat, which gives 1.28 t ha<sup>-1</sup> yield increase if compared to spring wheat monocultural sowings. (Table 3 and 4) Also good foreplants for spring wheat are clover and lupine.

The best forecrop for oats are potatoes. (Table 2)

 Table 2. Effect of preceding crop and crop rotation on the yield of oats t ha<sup>-1</sup> in the long-term trial, 2001-2006

No of crop rotation	Proportion of cereals in crop rotation	Preceding crop	Yield 2001-2005			Yield 2006		
			t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%	t ha <sup>-1</sup>	+/-t ha <sup>-1</sup>	%
1.	50	Potatoes	3.86	+1.28	150	2.12	+0.98	186
2.	66	Rye	3.25	+0.67	126	1.50	+0.36	132
4.	83	Barley	3.56	+0.98	138	1.89	+0.75	166
5.	100	Rye	3.75	+1.17	145	1.67	+0.53	146
5.	100	Oats grown repeatedly	2.58		100	1.14		100
LSD <sub>0.05</sub>			0.25			0.29		

In crop rotations with high cereal proportions (66-100%) the forecrop's forecrop is also very important. For example, growing oats in a crop rotation with a cereal proportion of 66% after winter rye (which have been grown there repeatedly) we get for 0.5 t ha<sup>-1</sup> lower yield than growing the same oats in a crop rotation with the cereal proportion 100% after winter rye whose forecrop is a legume-cereal mix.

Herbicide Monitor treatment in spring wheat sowings was absolutely successful, especially in crop rotations with a high cereal proportion due to the intense distribution of common couch-*Elytrigia repens* (L.) Nevski.

Table 3. Effect of preceding crop and crop rotation on the grain yield of spring wheat  $t\ ha^{-1}$  in the long-term trial, 2001-2006

No of crop rotation	Proportion of cereals in crop rotation	Preceding crop	Grain yield $t\ ha^{-1}$ 2001-2005			Grain yield $t\ ha^{-1}$ 2006		
			$t\ ha^{-1}$	+/- $t\ ha^{-1}$	%	$t\ ha^{-1}$	+/- $t\ ha^{-1}$	%
3	66, incl. buckwheat 33%	Buckwheat	4.27	+1.28	143	2.90	+1.10	161
4	83	Early clover or lupine	4.00	+1.01	134	2.41	+0,61	133
5	100	spring wheat grown repeatedly	2.99		100	1.80		100
LSD <sub>0.05</sub>			0.35			0.38		

The herbicides Grodil and MCPA treatment in barley sowings ensures a remarkable yield increase, mostly – in monocultural sowings. (Table 5) One-time fungicide treatment in barley sowings provided a little bigger yield increase than herbicide treatment.

 Table 4. Effect of crop rotation and herbicides on the grain yield of spring wheat  $t\ ha^{-1}$  (in the long-term trial 2001-2006)

No of crop rotation and proportion of cereals in crop rotation	Preceding crop	Without herbicides		Herbicide Monitor 0.015 $kg\ ha^{-1}$ at tillering		
		$t\ ha^{-1}$	%	$t\ ha^{-1}$	+/- $t\ ha^{-1}$	%
3-83*	Buckwheat	3.66	100	4.37	+0.71	119
4- 83*	Early clover or lupine	3.21	100	4.27	+1.06	133
Average		3.44	100	4.32	+0.89	126
5 – 100	Spring wheat grown repeatedly	2.14	100	3.45	+1.31	161
LSD <sub>0.05</sub>		0.39				

\*The efficiency of herbicides was tested in the background of fungicides Tango 1.0  $l\ ha^{-1}$  or Folikur 250 e.k. 1  $l\ ha^{-1}$

 Table 5. Grain yield of oats as effected by pesticide application and different crop rotations,  $t\ ha^{-1}$  (2001-2006)

No of crop rotation and proportion of cereals in crop rotation	Preceding crop	Control		Treatment with pesticides as compare to control	
		yield, $t\ ha^{-1}$	yield, $t\ ha^{-1}$	+/- $t\ ha^{-1}$	%
2 – 66 4 – 83 Average	Rye Barley	Without herbicides	Grodil 30 $g\ ha^{-1}$ + MCPA 11 $ha^{-1}$ at tillering		
		2.66	3.22	+ 0.56	121
		2.90	3.63	+ 0.73	125
	Average	2.78	3.43	+ 0.65	123
1 – 50 5 - 100 Average	Potatoes Rye	Without fungicides	Tango 1.0 $l\ ha^{-1}$ or Folikur 1 $l\ ha$ at stalk-shooting		
		3.13	4.13	+1.0	132
		2.92	3.59	+0.67	123
	Average	3.03	3.59	+0.83	127
5 -100	Oats grown repeatedly	Without pesticides	Complex of pesticides*	+ 0.83	145
LSD <sub>0.05</sub>		0.31			

\* Grodil 30  $g\ ha^{-1}$  + MCPA 11  $ha^{-1}$  at tillering and Tango 1.0  $l\ ha^{-1}$  or Folikur 250 e.k. 1  $l\ ha^{-1}$  at stalk-shootin

Also fungicide treatment in oat sowings provided a bigger yield increasement than herbicide treatment, mainly due to the wide distribution of orange leaf rust (*Puccinia coronata var.avenae*) in some years of research. (Table 6)

Table 6. Grain yield of barley as effected by pesticide application and different crop rotations, t ha<sup>-1</sup> (2001-2006)

No of crop rotation and proportion of cereals in crop rotation	Preceding crop	Control	Treatment with pesticides		
		yield, t ha <sup>-1</sup>	yield, t ha <sup>-1</sup>	as compare to control +/- t ha <sup>-1</sup>	%
		Without herbicides	Grodil 30 g ha <sup>-1</sup> + MCPA 11 ha <sup>-1</sup> at tillering		
2 - 66	Rye	2.66	3.22	+ 0.56	121
4 - 83	Barley	2.90	3.63	+ 0.73	125
Average		2.78	3.43	+ 0.65	123
		Without fungicides	Tango 1.0 l ha <sup>-1</sup> or Folikur.1 l ha at stalk-shooting		
1 -50	Potatoes	3.13	4.13	+1.0	132
5 - 100	Rye	2.92	3.59	+0.67	123
Average		3.03	3.59	+0.83	127
5	Barley grown repeatedly	Without pesticides	Complex of peticides*		
		1.86	2.70	+ 0.83	145

LSD<sub>0.05</sub> treatment of herbicides = 0.31 t ha<sup>-1</sup>

LSD<sub>0.05</sub> treatment of fungicides = 0.34 t ha<sup>-1</sup>

\*Grodil 30 g ha<sup>-1</sup> + MCPA 11 ha<sup>-1</sup> at tillering and Tango 1.0 l ha<sup>-1</sup> or Folikur 250 e.k. 1 l ha<sup>-1</sup> at stalk-shooting

## Conclusions

Farms which specialize in cereal growing and have sowings with a very high cereal proportion should follow these suggestions: avoid monocultural sowings of any cereal species; introduce a correct crop rotation plan; provide correct plant protection methods; seed material should be acquired from specialized seed breeding farms.

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## AUGU MAINĀS UN AUGU AIZSARDZĪBAS LĪDZEKĻU KOMPLEKSA IETEKME UZ VASARĀJU LABĪBU RAŽU DAŽĀDAS SPECIALIZĀCIJAS AUGSEKĀS

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Kompleksie augseku izmēģinājumi LLU Zemkopības zinātniskajā institūtā uzsākti 1969.gadā. Izmēģinājumā tiek salīdzinātas piecas dažādas sešlauku augsekas, kur graudaugu īpatsvars ir no 50-100%, daudzgadīgās zāles – āboliņš + timotiņš no 0-33%. Miežu priekšaugi pēc ietekmes uz graudu ražu lejujupslidošā secībā sarindojami sekojoši: āboliņš+ timotiņš, auzas, ziemas rudzi. Būtisks ražas samazinājums sākās ar ziemas rudziem. Mieži atkārtotā audzēšanā dod būtisku ražas samazinājumu līdz 1.17 t ha<sup>-1</sup>. Herbicīda grodila un MCPA lietošana miežos cerošanas fāzē

nodrošina stabili graudu ražas pieaugumu- vidēji par  $0.76 \text{ t ha}^{-1}$ . Vislielāko ražas pieaugumu efektu –  $1.05 \text{ t ha}^{-1}$  dod kompleksa fungicīdu un herbicīdu lietošana miežu atkārtotas audzēšanas variantā. Miežu sējumos nezāļu daudzumus palielinās augsekās ar graudaugu īpatsvaru virs 83%. It sevišķi izteikti vairāk ir daudzgadīgo nezāļu, galveno kārt ložņu vārpas *Elytrigia repens*, ir miežu atkārtotas audzēšanas variantos. Auzas, augsekas ar graudaugu īpatsvaru līdz 83%, maz reaģēja uz to izvietojumu pēc dažādiem priekšaugiem. Būtisks ražas samazinājums ir variantos, kur tās audzē atkārtoti. Auzu atkārtotas audzēšanas variantos, lietojot herbicīdu un fungicīdu kompleksu smidzinājumu, panākts vislielākais auzu ražu pieaugums ( $+0.86 \text{ t ha}^{-1}$ ), bet tas nenodrošināja tik augstu auzu ražu kā labākajos augseku variantos. Auzu sējumos nezālainība, kur tās sētas pēc ziemas rudziem, kā arī atkārtotas audzēšanas variantos, ir ievērojami lielāka kā pēc kartupeļiem. Tā atkārtotas audzēšanas variantos nezāļu skaits ir 10 un vairāk reizes lielāks kā auzās, kuras audzētas augmaiņā. Augstākās vasaras kviešu ražas ir pēc griķiem un lupīnas. Graudaugu bezmaiņas sējumos herbicīdu un fungicīdu efektivitāte ir lielāka kā augsekās. Nezālainība vasaras kviešos pieaug augseku variantos, ar lielāku graudaugu īpatsvaru

## EFFECT OF LONG-TERM APPLICATION OF DIFFERENT FERTILIZATION ON THE FERTILITY OF GLEYIC CAMBISOL

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### Abstract

Long-term stationary trials were conducted on a clay loam *Gleyic Cambisol* medium in phosphorus supply ( $0.057 \text{ g kg}^{-1}$ ) and very high in potassium supply ( $0.174 \text{ g kg}^{-1}$ ) in a five-course crop rotation. The trials were designed to study the effects of different fertilisation systems – mineral, organic – mineral and organic on the changes in soil agrochemical properties, nutrient balance and crop productivity. It was found that in the mineral fertilisation system (annual avg.  $\text{N}_{56}\text{P}_{48}\text{K}_{60}$ ) soil humus and phosphorus contents per rotation remained close to the initial level, while potassium content inappreciably declined. In the organic – mineral fertilisation system the incorporation of different farmyard manure rates (40; 60 and  $80 \text{ t ha}^{-1}$ ) and NPK as much as in the mineral fertilisation system resulted in a significant increase in humus (11.5-14.5 %), available phosphorus (22.4-36.3 %) and potassium did not change significantly over two rotation. In all organic – mineral fertilisation systems positive changes in humus and other agrochemical indicators had a considerable effect on soil structure. The content of metabolisable energy of the crop rotation plants was directly dependent on the contents of phosphorus ( $r=0.97^{**}$ ) and nitrogen ( $r=0.86^{*}$ ) incorporated with organic and mineral fertilisers.

**Key words:** clay loam, crop rotation, fertilization systems, humus, phosphorus, potassium, metabolisable energy.

### Introduction

When crops are intensively fertilised with only mineral fertilisers, they are unevenly supplied with nutrients at different stages of development, since due to the uneven distribution of rainfall, part of the nutrients are leached during the growing period, and the shortage of nutrients causes stress to plants (Jenkinson, 2001; Edmeades, 2003). However, when fertilising with organic fertilisers, they mineralise and create a nutrient reserve which can secure more consistent plant nutrition throughout the whole growing period, therefore the plants form vegetative and generative organs more intensively (Helander and Delin, 2004; Tripolskaja, 2005). In fine-textured soils due to the high abundance of clay particles and poorer aeration, the mineralization of organic matter occurs more slowly and this determines the specific action of organic fertilisers and a longer period of nutrient release, compared with coarser-textured soils. This determines the specific organic fertiliser action to maintain soil potential productivity and to increase crop rotation productivity

(Maiksteniene, 2002). The data obtained by many authors the yield-increasing effect of stable manure was also felt in the second and third years, especially when lower N rates were applied (Kismanyoky and Kiss, 1998).

Experiments designed to compare mineral and organic–mineral fertilisation systems were carried at the Lithuanian Institute of Agriculture's Joniskelis Experimental Station with 40 t ha<sup>-1</sup> farmyard manure (FYM) once per six-course rotation, 6-7 t ha<sup>-1</sup> FYM per year and the application of optimal mineral NPK fertiliser rates did not secure the stability of humus (Kristaponyte, 2001). As a result, since 1995 the experiment has been reconstructed aiming at a positive humus and nutrients balance, in which organic – mineral fertilisation systems with increased organic fertiliser rates 60-80 t ha<sup>-1</sup> and organic system with 80 t ha<sup>-1</sup> FYM are investigated. The objective of the experiment was to estimate the effect of fertilisation systems differing in intensity, on the dynamics of nutrients and humus in heavy soils.

### Material and Methods

The soil of the experimental site is characterised as an *Endocalcari-Endohypogleyic Cambisol* (*CMg-n-w-can*), and according to its texture – clay loam on silty clay. The agrochemical characteristics of the topsoil were as follows: humus (Tyurin's method) 1.98 %, available phosphorus (A-L method) 0.020 g kg<sup>-1</sup>, available potassium (A-L method) 0.147 g kg<sup>-1</sup> of the soil. The following fertilisation systems were investigated: mineral, organic, and organic-mineral. The experiment was replicated four times, the size was 105.0-112.5 m<sup>2</sup>, of record plots 45-50 m<sup>2</sup>. The trial involved the following five - course crop rotation: sugar beet, spring barley, first year (1<sup>st</sup>) perennial grasses, second year (2<sup>nd</sup>) perennial grasses, winter wheat. Organic fertilisers (farmyard manure) at a rate of 40; 60 and 80 t ha<sup>-1</sup> were applied sugar beet once per rotation. The average composition was as follows: total N (according to Kjeldahl) – 0.34 %, phosphorus (calorimetric method) 0.20 %, potassium (flame photometer) – 0.65 %. Ammonium nitrate, granulated superphosphate and potassium chloride were used as mineral fertilisers. Mineral fertiliser rates were applied in relation to the crops grown: sugar beets received N<sub>120</sub>P<sub>90</sub>K<sub>120</sub>, spring barley - N<sub>30</sub>P<sub>60</sub>K<sub>60</sub>, first year (1<sup>st</sup>) perennial grasses – P<sub>50</sub>K<sub>60</sub>, second year (2<sup>nd</sup>) perennial grasses – N<sub>60</sub>, winter wheat – N<sub>70</sub>P<sub>40</sub>K<sub>60</sub>.

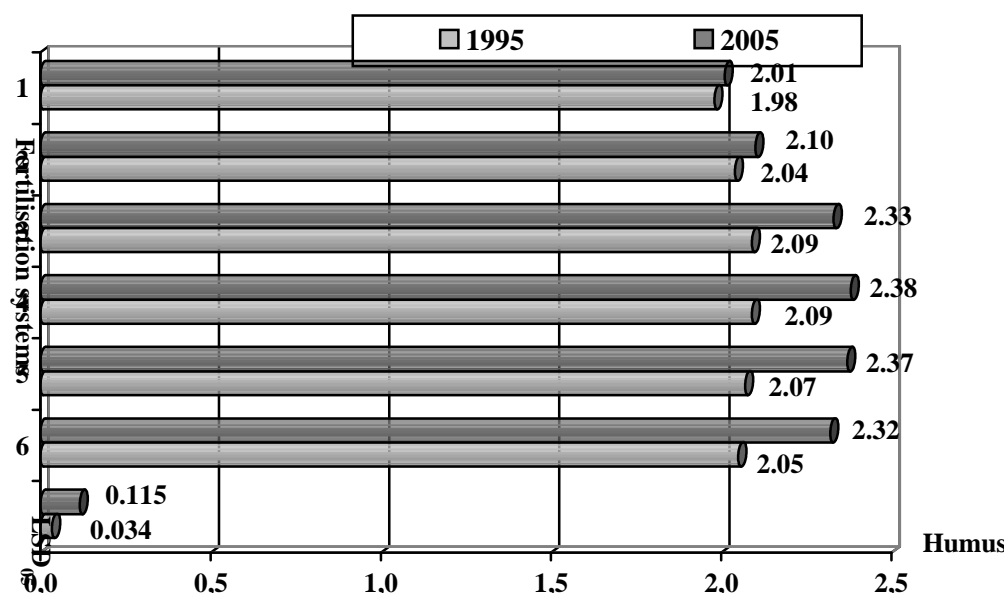
The productivity of the crop rotation crops was re-calculated into metabolisable energy according to the coefficients of yield chemical composition set for separate crops yield (Jankauskas, 1999).

*Meteorology.* The weather conditions during the crop growing seasons were evaluated according to hydrothermal coefficient (HTC). Having summarised the weather conditions during the crop growing season it was found that during the experimental period, the plant growing seasons in 1996; 1997; 1999; 2003 and 2004 were normally wet (HTC 1.12-1.26 and 1.27; 1.07 and 1.20), in 1998 and 2001 there was excessive moisture (HTC – 2.08 and 2.14). In 2000 and 2005 the growing season was dry (HTC – 0.90 and 0.97) and only in 2002 the droughty season (HTC-0.60).

*Statistical analysis.* Experimental findings were processed by the analysis of variance and correlation regression methods (Tarakanovas, 1999). The credibility of equations was determined according to the Fisher criterion ( $r$  of  $\eta$ ) at a 95 % probability level (marked as\*), 99 % (marked as\*\*).

### Results and Discussion

**Humus.** In the crop rotation with two fields of perennial grasses after two crop rotation (ten experimental years) the content of humus in the control treatment (without fertilisers) in the 0-20 cm depth did not decline (figure 1). In the mineral fertilisation system which received on average N<sub>56</sub>P<sub>48</sub>K<sub>60</sub> annually (treatment 2) the content of humus was increase by 0.06 percentage units compared with the initial level. Organic – mineral fertilisation systems of various intensity had a different effect on the build up of humus in the soil. In the organic – mineral fertilisation system having incorporated 40 t ha<sup>-1</sup> FYM, the content of humus in the topsoil significantly increased by 0.24 percentage units, having incorporated 60 t ha<sup>-1</sup> FYM by 0.29 percentage units and having incorporated 80 t ha<sup>-1</sup> FYM by 0.30 perc units, compared with the initial level.



Fertilisation systems: 1. Without fertilisers; 2.  $N_{56}P_{48}K_{60}$ ; 3.  $40 \text{ t ha}^{-1}$  of farmyard manure +  $N_{56}P_{48}K_{60}$ ; 4.  $60 \text{ t ha}^{-1}$  of farmyard manure +  $N_{56}P_{48}K_{60}$ ; 5.  $80 \text{ t ha}^{-1}$  of farmyard manure +  $N_{56}P_{48}K_{60}$ ; 6.  $80 \text{ t ha}^{-1}$  of farmyard manure.

Figure 1. The effect of different fertilisation systems on the changes of humus in the soil

In different organic – mineral fertilisation systems, compared with the mineral fertilisation system content of humus, increased by 0.23; 0.28; 0.27 percentage units, however, there were no significant differences between different FYM rates. In the organic fertilisation system having incorporated  $80 \text{ t ha}^{-1}$  FYM only, the humus content increased by 0.27 percentage units over two rotations, compared with its initial level and 0.22 percentage units with mineral fertilisation system. This suggests that in the organic – mineral fertilisation system more favourable conditions occurred for organic matter humification and humus accumulation in the soil than in solely organic or mineral system. The correlation – regression analysis shows that soil humus content in the plough layer was strongly dependent on the nutrients incorporated with organic and mineral fertilisers: with potassium  $r=0.90^{**}$ , with nitrogen  $r=0.68^*$ , and a slightly weaker relationship with phosphorus  $r=0.50$ .

*Available phosphorus.* After crop two rotation with no fertilisation to all crops the amount on available phosphorus declined by 37.9 % (Table 1).

Table 1. The effect of different fertilisation systems on the agrochemical properties of heavy-textured soils

Fertilization systems	P			K		
	1995	2005	±	1995	2005	±
	g kg <sup>-1</sup> soil					
Without fertilizers	0.029	0.018	-0.011	0.155	0.143	-0.012
Mineral NPK	0.039	0.040	0.001	0.161	0.161	0
Organic-mineral (I) FYM $40 \text{ t ha}^{-1}$ +NPK	0.051	0.062	0.011	0.172	0.168	-0.004
Organic-mineral - (II) FYM $60 \text{ t ha}^{-1}$ +NPK	0.055	0.071	0.016	0.181	0.178	-0.003
Organic-mineral- (III) FYM $80 \text{ t ha}^{-1}$ +NPK	0.055	0.074	0.019	0.174	0.182	0.008
Organic FYM $80 \text{ t ha}^{-1}$	0.055	0.055	-	0.178	0.181	0.003
LSD <sub>05</sub>	0.005	0.013		0.013	0.0180	

In the mineral fertilisation system which received on average  $N_{56}P_{48}K_{60}$  annually, the content of available phosphorus inappreciably increased by 2.6 %, compared with its initial level. In the organic – mineral fertilisation systems of different intensity having incorporated  $40 \text{ t ha}^{-1}$  FYM and optimal NPK fertiliser rates, the content of phosphorus, compared with its initial level, increased by 21.6 % and having incorporated 60 and  $80 \text{ t ha}^{-1}$  FYM and NPK fertilisers, phosphorus content significantly increased by 29.1 % and 34.5 %, respectively. Having incorporated higher FYM rates

60 and 80 t ha<sup>-1</sup> (treatments 4 and 5) soil phosphorus content significantly increased, compared with the mineral fertilisation system (treatment 2). In the organic fertilisation system having incorporated 80 t ha<sup>-1</sup> FYM once per rotation (treatment 6) the soil phosphorus content, compared with the mineral fertilisation system, increased by 37.5 %. Such results were determined by the fact that in the case of mineral fertilisation the yields of the crop rotation plants were relatively high and higher contents of phosphorus were removed with the yields.

Available potassium. The content of available potassium in the unfertilised treatment also inappreciably declined, compared with its initial level, since crops utilising much potassium were cultivated in the crop rotation, especially sugar beet which removed 126-226 kg ha<sup>-1</sup> of potassium with by-production (Table 1). In the organic – mineral fertilisation system having incorporated 40 and 60 FYM and optimal NPK amounts, the content of available potassium in the soil decreased by 2.3 and 1.7 % over two rotations. Having incorporated 60 and 80 t ha<sup>-1</sup> FYM, the content of available potassium in the plough layer was significantly (by 5.9 and 8.4 %) higher compared with the treatments that had received the lower 40 t ha<sup>-1</sup> FYM rate. In the organic fertilisation system the content of available potassium did not change significantly per rotation, however, compared with the mineral fertilisation system it significantly increased by 12.4 %.

Metabolisable energy. For the final assessment of the effects of the fertilisation systems on the changes in soil agrochemical properties, the productivity of the crop rotation crops was recalculated into metabolisable energy. While estimating fertilisation systems it was found that in the mineral fertilisation system which received on average N<sub>56</sub>P<sub>48</sub>K<sub>60</sub> annually, the metabolisable energy of the crops increased by 70.2 %, compared with the unfertilised treatment; a more significant increase 101.2 % was identified for by-production than for primary production 62.9 % (Table 2).

Table 2. Metabolisable energy accumulated by the crop rotation crops in different fertilisation systems 1995-2005.

Fertilization systems	For rotation, GJ ha <sup>-1</sup>			Average in the years, GJ	Increase	
	primary production	by-production	common		GJ	%
Without fertilizers	1987.6	465.1	2452.7	490.5	-	-
Mineral NPK	3237.1	935.7	4172.8	834.6	344.6	70.2
Organic – mineral 40 t ha <sup>-1</sup> FYM+NPK	I 3491.0	1067.9	4558.9	911.8	421.8	85.9
Organic – mineral 60 t ha <sup>-1</sup> FYM+NPK	II 3625.1	1142.4	4767.5	953.4	463.4	94.4
Organic – mineral 80 t ha <sup>-1</sup> FYM+NPK	III 3592.8	1114.4	4707.2	941.4	451.4	91.9
Organic 80 t ha <sup>-1</sup> FYM	3022.8	748.6	3771.4	754.3	264.3	53.8
LSD <sub>05</sub>	167.45	172.88	118.84	23.77	-	-

In the mineral fertilisation system one kilogram of NPK gave an increase in metabolisable energy of 2.10 GJ ha<sup>-1</sup>. In the organic – mineral fertilisation systems the metabolisable energy of the crop rotation crops increased by 85.9; 94.4 and 91.9 %, compared with the unfertilised treatment. A significant increase of 9.3 and 14.3 % in metabolisable energy occurred in the organic – mineral fertilisation systems that received 40 or 60 t ha<sup>-1</sup> FYM, compared with the mineral fertilisation system. In the organic-mineral fertilisation system having increased FYM rate to 80 t ha<sup>-1</sup>, the amount of metabolisable energy increased by 106.8 GJ or 12.8 %, compared with the mineral fertilisation system. In the organic fertilisation system involving 80 t ha<sup>-1</sup> FYM, with which N<sub>55</sub>P<sub>32</sub>K<sub>104</sub> was incorporated per year, the metabolisable energy of the rotation crops increased by 53.8 %, compared with the unfertilised treatment. Although in this system the amount of potassium incorporated with organic fertilisers was 73.3 % higher than in the mineral fertilisation system, the amount of phosphorus incorporated was 33.3 % lower. In the organic fertilisation system the amount of metabolisable energy accumulated was significantly lower by 9.6 % than in the mineral fertilisation system. More over, this was determined by lower plant nutrient utilisation coefficients (N-51.4; K-59.8 %), compared with mineral fertilisers, as well as by the shortage of phosphorus in low-phosphorous heavy-textured gleyic cambisols (their genetic characteristic). One kg of NPK present in farmyard manure increased the metabolisable energy content by 1.38 GJ, and this is by 34.3 % less than by 1 kg of mineral fertilisers.

The total metabolisable energy of the crop rotation crops ( $y$ ) directly depended on the content of nitrogen ( $x_1$ ) and phosphorus ( $x_2$ ) incorporated with mineral and organic fertilisers:  $y=3195.092+2.912x_1$ ;  $r=0.885^*$ ;  $y=3135.079+4.221x_2$ ;  $r=0.974^{**}$ .

In the soils high in potassium there was no significant relationship between the productivity of the crop rotation crops and the potassium content introduced with fertilisers  $y=3601.953+1.361x$ ;  $r=0.64$ .

Making direct comparisons between mineral and organic farming systems is fraught with difficulty. Many authors suggest that on clay loam soils the organic - mineral fertilisation system is the best choice (Zarina, 2000; Tripolskaja, 2005). Such a system creates conditions for the balance between mineralization and the humification processes, since due to the poor aeration, the destructive process of organic matter is longer, and it is promoted by the incorporation of mineral nitrogen (Kurnisieva *et al.*, 1996; Perucci *et al.*, 1997). This suggests that in the organic – mineral fertilisation system more favourable conditions occur for organic matter humifications and humus accumulation in the soil than in solely organic or mineral systems. This confirms some of authors' conclusions that in order to maintain positive humus balance, it is necessary to incorporate on average 8-16 t ha<sup>-1</sup> FYM annually during the five-course crop rotation (Kristaponyte, 2005). According to many authors (Niggli *et al.*, 1999), systematic soil fertilisation with moderate farmyard manure rates (30-60 t ha<sup>-1</sup>) has a positive effect on almost all soil properties. Farmyard manure application improves agrochemical soil properties: soil acidity declines, the contents of major nutrients (nitrogen, phosphorus, potassium) and trace nutrients increase their plant availability improves, and humus content increases (Kiss and Kismanyoky, 1998). However, the results do offer support to the argument that organic manure is mining reserves of P and K built up by conventional management. Changes in the content of available phosphorus and potassium in the soil depend on the fertilisation intensity (Smith *et al.*, 2001), however the variation of the potassium content at the same rates as phosphorus is lower (Schneider, 1997; Magid *et al.*, 1999). Zarina (2000) reported that the content of phosphorus and potassium changed the minerals in the fertilisation system with stable manure 20 t ha<sup>-1</sup>, but increased essential in other studied fertilisation systems. The correlation – regression analysis of our experiments shows that the soil phosphorus content (g kg<sup>-1</sup>) was strongly dependent on the nutrients incorporated with mineral and organic fertilisers and their combinations: with nitrogen  $r=0.91^*$ , with phosphorus  $r=0.76^*$ , and with potassium  $r=0.98^{**}$ .

## Conclusions

On a fine-textured *Gleyic Cambisol* in the mineral fertilisation system (with average annual application of N<sub>56</sub>P<sub>48</sub>K<sub>60</sub>) the humus content per five-course rotation with two fields of perennial grasses did not change, compared with the initial level. In the organic – mineral fertilisation systems, having applied to sugar beet 40; 60 and 80 t ha<sup>-1</sup> of FYM and the same amount of NPK as in the mineral system, the humus content increased by 11.0; 13.3 and 12.9 %, respectively, and in the organic fertilisation system (80 t ha<sup>-1</sup> FYM) by 10.5 %, compared with the mineral fertilisation system.

In the organic – mineral fertilisation systems the incorporation of different farmyard manure rates (40; 60 and 80 t ha<sup>-1</sup>) and NPK much as in the mineral fertilisation system, resulted in a significant increase in available phosphorus (21.6; 29.1 and 24.5 %) over two rotations. The higher FYM of 60 and 80 t ha<sup>-1</sup>, increased the content of available phosphorus in the soil by 14.5 and 19.4 %, compared with the lower FYM rate (40 t ha<sup>-1</sup>). In the organic – mineral fertilisation systems the content of available potassium significantly increased by 4.3; 12.4 and 13.0 %, compared with the mineral fertilisation system and by 6.0 and 8.3 %, compared with the lower FYM rate (40 t ha<sup>-1</sup>).

With improving agrochemical properties of heavy-textured soils in the organic – mineral fertilisation systems having applied 40; 60 and 80 t ha<sup>-1</sup> FYM, the metabolisable energy of the crop rotation plants was 9.3; 14.3 and 12.8 % higher, compared the mineral fertilisation systems however, there were no significant differences between separate FYM rates. In the organic fertilisation system having incorporated 80 t ha<sup>-1</sup> FYM, the content of metabolisable energy accumulated by the crops was 53.8 % higher, compared with unfertilised, but 9.6 % lower compared with the mineral fertilisation system.



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## DAŽĀDA MĒSLOJUMA ILGLAICĪGAS PIEMĒROŠANAS IETEKME UZ GLEYIC CAMBISOL AUGLĪGUMU

**Maiksteniene S., Kristaponyte I., Masilionyte L.**

Ilglaicīgi stacionāra izmēģinājumi tika veikti māla augsnē *Gleyic Cambisol*, vidēji bagātā ar fosfora krājumiem ( $0.057 \text{ g kg}^{-1}$ ) un ļoti augstiem kālija krājumiem ( $0.174 \text{ g kg}^{-1}$ ), piecu-lauku augu sekā. Izmēģinājumi tika plānoti, lai izpētītu dažādu mēslošanas sistēmu – minerālās, organo-minerālās un organiskās – ietekmes uz augsnes agroķīmisko īpašību, barības vielu līdzsvara un laukaugu ražīguma izmaiņām. Tika atklāts, ka minerālajā mēslošanas sistēmā (vidēji gadā  $\text{N}_{56}\text{P}_{48}\text{K}_{60}$ ) augsnes humusa un fosfora saturs augsekā saglabājās tuvs sākotnējam līmenim, bet kālija saturs nedaudz samazinājās. Organo-minerālajā mēslošanas sistēmā dažādu kūtsmēsļu normu ( $40$ ;  $60$  un  $80 \text{ t ha}^{-1}$ ) un NPK tikpat, cik minerālajā mēslošanas sistēmā, tika iegūts nozīmīgs humusa un pieejamā fosfora pieaugums, attiecīgi  $11.5$ - $14.5 \%$  un  $22.4$ - $36.3 \%$ , bet kālijs divās pilnās augsekas rotācijās mainījās maz.

Visās organo-minerālajās mēslošanas sistēmās pozitīvajām humusa un citu agroķīmisko rādītāju izmaiņām bija vērā ņemama ietekme uz augsnes struktūru. Augu sekas augu saistītās enerģijas

daudzums bija tieši atkarīgs no fosfora ( $r=0.97^{**}$ ) un slāpekļa ( $r=0.86^*$ ) saturs, kas iekļauts ar organisko un minerālmēslojumu.

## THE SOIL ACIDITY PARAMETERS OF SOILS WITH STOPPED LIMING

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### Abstract

The present problem is mass soil acidification in the whole of Lithuania, especially in the eastern and western regions and soil variability in the acidification stages. The results of the last stage (2002-2006) of the long-term experimental work carried out at the LIA Voke branch since 1972, demonstrate that the effect of primary soil liming is statistically significant only for mobile Al. Changes in the periodically limed soil pH, hydrolytic acidity and mobile Al were more intense and their restoration to previous levels is slower compare to primary limed. In such soils even after 14-23 years since last the lime application the productivity of crop rotation is still higher than in unlimed soils.

Our conclusions clarified questions about the use of agricultural soils with stopped liming – what soil parameters can identify soil acidity and what is the lime requirement of previously limed soils? It suggest further investigations into the effects of stopping liming on soil profile chemical properties changes, especially the dynamic of mobile Al restoring. The selection of new criteria for lime requirements and the optimal lime rate determination concerning ecological and economical efficiency are the most important questions for maintaining the productivity of these acidifying soils. Only the further monitoring of the soils will enable a more detailed evaluation of the liming effect on the environment and make accurate predictions about the restoration of soil acidity possible.

**Key words:** acidifying soils, primary liming, mobile Al

### Introduction

The territory of Lithuania is located in the transitional climatic zone between maritime West European and continental East European and Asian. According to the hydrothermal conditions, Lithuania is located in the zone of excessive precipitation and moderate organic matter decomposition. (Buzas *et al.*, 1966). The mean annual air temperature is  $+6.2^{\circ}\text{C}$ , and the mean annual atmospheric precipitation constitutes 661 mm.

Areas with acid soils are mainly distributed in the eastern and western parts of Lithuania (Eidukevičienė, 1993; Eidukeviciene, Vasiliauskienė, 2001). Agricultural soils with a pH value below 5.5 have been regularly limed for several decades (1965-1990), resulting in a decrease in the total area of acid soils from 40.7 to 18.6 percent (Mazvila *et al.*, 2004).

With the period of economical changes, since 1991 the practice of soil liming significantly declined and has practically stopped at the present time. This led to the secondary acidification of limed soils and resulted in a negative effect upon crop production and the economics of Lithuania. The dynamics of agrochemical soil properties have changed in the 35 y. period passed since the primary liming analyzed in the current work. The restoration of soil acidity after regular liming and the stopping of naturally acid soils and the methods of evaluation of limed soil acidity are discussed.

### Materials and Methods

Experiments were conducted at the Lithuanian Institute of Agriculture's Vokė Branch (located in latitude  $54^{\circ}37'$  North and longitude  $25^{\circ}08'$  East.) during 1972-2006. The experimental plots were established in sandy loam on carbonaceous fluvial-glacial gravel soil, *Haplic Luvisols* (LVh) according to the FAO-UNESCO classification. The depth of carbonate effervescence was 60–80 cm. It was a typical arable soil in the eastern part of Lithuania characterized by low organic matter

content (1.6-2.0 % of humus), very acid soil reaction ( $\text{pH}_{\text{KCl}} < 4, 5$ ) and a low content of available plant phosphorus and potassium ( $100\text{-}150 \text{ mg kg}^{-1}$ ). Dust limestone was used as liming agent, with 95-98% of  $\text{CaCO}_3$ . Lime requirements were calculated according to the soils hydrolytic acidity (H). Primary soil liming was executed in 1972. Primary lime rates were 1,0 r from H ( $7,18 \text{ t ha}^{-1} \text{ CaCO}_3$ ). Regular liming was repeated in 5-10 year cycles, with various dust limestone rates in various treatments. In this paper we discuss the efficiency of moderate intensity liming when during 4 cycles (20 years) 1,0-3,5 rates of  $\text{CaCO}_3$  according H have been applied (table 1). Liming efficiency was tested in a crop rotation that was composed of 50% cereals. The average rates of mineral fertilizers were:  $\text{N}_{30-60} \text{ P}_{30-60} \text{ K}_{60} \text{ kg ha}^{-1}$  active matter.

Table 1 The intensity of liming in experiment of periodical liming

Treatment	Date of liming and lime rates									
	1973		1978		1983		1988		total	
	rate	t ha <sup>-1</sup>	rate	t ha <sup>-1</sup>	rate	t ha <sup>-1</sup>	rate	t ha <sup>-1</sup>	rate	t ha <sup>-1</sup>
Regularly limed 1,0 r $\text{CaCO}_3$ every 10 year	1,0	7,86	0	0	1,0	7,86	0	0	2,0	15,72
Regularly limed every 5 year : 0,5r-0,5r+1,5r+1,5r $\text{CaCO}_3$	0,25	1,96	0,25	1,96	1,5	11,78	1,5	11,78	3,5	27,48

Methods of analyses. Soil samples from the arable layer (0-20 cm) for the determination of agrochemical properties were collected at the beginning of the experiment and after every rotation (1972, 1977, 1982, 1987, 1992, 1996, 2002, and 2006). After 24 years since the beginning of regular liming (in 1996), soil samples from individual profiles (to 1m depth, every 20cm) were collected, for the evaluation of dust limestone effect on subsoil layer properties.

Agrochemical analyses of the soil samples were performed applying the following methods: soil  $\text{pH}_{\text{KCl}}$  by the method of potentiometry (1 M KCl suspension), hydrolytic acidity (H) – by Kapen, exchangeable acidity and mobile aluminum (mob. Al) – by Sokolov, the amount of absorbed bases (S) – by Kapen–Hilkovic methods.

For the statistical analysis, the ANOVA procedure was used. The data of the plant and soil agrochemical analyses were processed applying the analysis of variance and the LSD test (Little, T.M., Hills, F.J., 1978).

## Results and Discussion

The analysis of the changes in soil acidity characterizing properties ( $\text{pH}$ , hydrolytic acidity, absorbed bases, mobile Al) during the 34 year period show that natural soil acidity is determined by such abiotic factors as the  $\text{pH}$  of precipitation, the soil moisture regime, the development of the root system of crops and the errors of laboratory analyses. Altogether these factors led to variations in  $\text{pH}$  and mobile Al in unlimed soil during the experimental period (Figure 1).

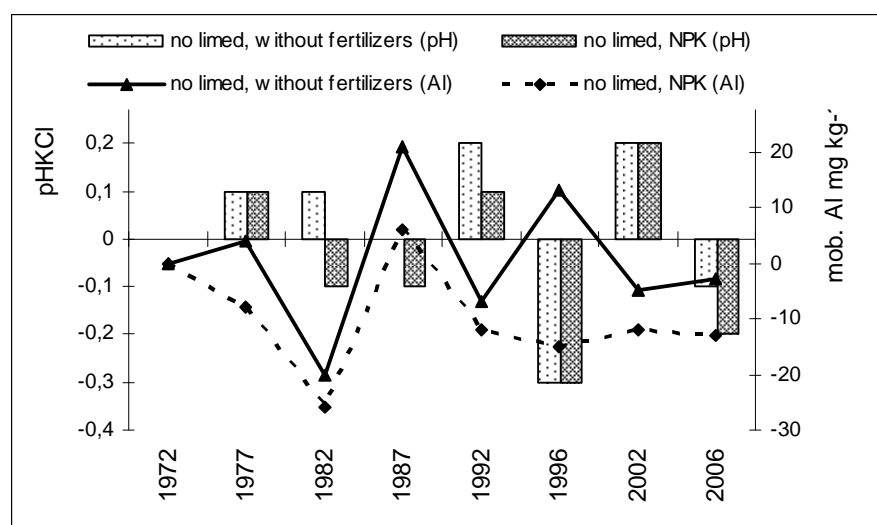


Figure 1. Changes of  $\text{pH}_{\text{KCl}}$  and mobile aluminum in unlimed soil (Voke, 1972-2006)

The total experimental period variation of pH was from -0.4 to +0.2 counted from the primary level (4.4 pH), the variation of the mobile Al from -20 to +19 mg kg<sup>-1</sup>, (counted from primary 63 mg kg<sup>-1</sup> Al). The application of average mineral fertilizers 30-60 kg ha<sup>-1</sup> N, 30-60 kg ha<sup>-1</sup> P and 60 kg ha<sup>-1</sup>

K didn't stimulate soil acidification. Soil pH and mobile Al levels did not differ significantly between unlimed unfertilized soils and unlimed fertilized soil. At the 4.4 pH level the removing of Ca with the yield was negligible (7-8 kg ha<sup>-1</sup> Ca per year) and didn't decline the soil pH. Because higher yield from fertilized plots respectively increased the removal of Ca (10-12 kg ha<sup>-1</sup> Ca), but it was partly compensated by the input of Ca with superphosphate. During the experimental period about 18 kg ha<sup>-1</sup> P was applied.

Primary liming with an optimal dust limestone rate (1,0 r CaCO<sub>3</sub> according H ) decreased soil acidity from pH 4,4 to 5,2 (Fig. 2). After five years liming efficiency started to decline and during the tenth year after liming the pH was just 0.2 of a unit higher compare to the unlimed plots. pH change was still significant after 15 year, but after 20 year the soil return to its primary acidity level. Intensive regular liming for 20 years (in total 2,0 to 3,5 rates according H) had a more significant effect for pH changes and led to different dynamic changes. Every new cycle of liming compared to the primary was more effectively increasing soil pH. Application of 2 rates of limestone in total allow one to achieve a 5.7 pH level (in 1992), application of 3.5 rates – 6.4 pH. With stopped regular soil liming acidification started. The speed of the acidification was similar to acidification in the primary limed soil. The speed of the acidification is expressed by the logarithmic equation -  $y_1(\text{pH}_{\text{KCl}}) = -0,413\text{Ln}(x)+1,466$ . The correlation of pH changes with time passed after liming is very strong (R 0.95). Respectively acidification after regular liming is described in  $y_2(\text{regular, 2,0 rates}) = -0,612\text{Ln}+3,12$  (R 0,24) and  $y_3(\text{regular, 3,5 rates}) = -0,779\text{Ln}+4,40$  (R 0.45).

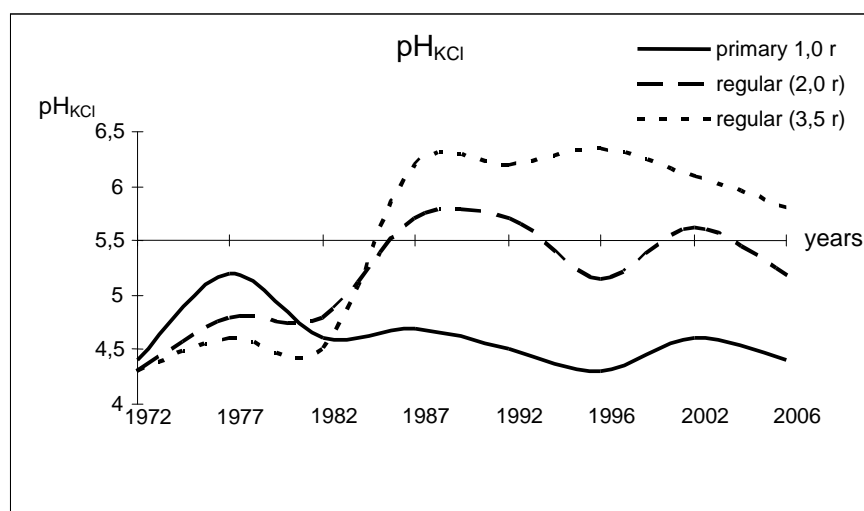


Figure 2 The effect of primary and periodical liming on soil pH<sub>KCl</sub> in Luvisol

Primary liming declined hydrolytic acidity by 200 cmol kg<sup>-1</sup>, regular more – 290 cmol kg<sup>-1</sup> if 2.0 rates were applied, and by 430 cmol kg<sup>-1</sup> if 3,5 rates were applied (Fig. 3.). In the case of primary liming hydrolytic acidity returns to its initial levels slightly latter then pH, and after 20 year was similar to unlimed soil. The effect of regular liming on hydrolytic acidity was more intense. Five year since liming stopped hydrolytic acidity continuing to decrease and later signs of restoration appeared.

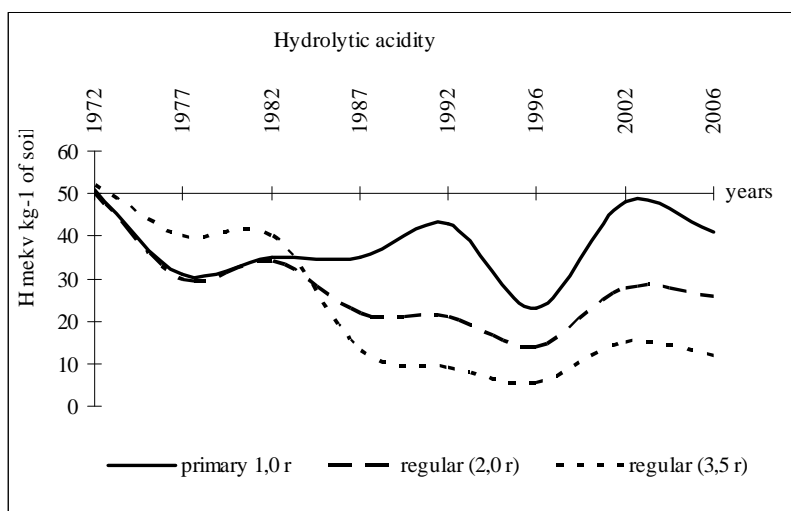


Figure 3 The effect of primary and periodical liming on hydrolytic acidity in Luvisol

The 20 year period after liming shows an increase in hydrolytic acidity 120-70  $\text{cmol kg}^{-1}$  depending on the limestone rate, but stays 2-4 times lower than the primary level. The third and one of the main soil acidity characterizing parameters is mobile aluminum. Before liming the mobile Al level was 70  $\text{mg kg}^{-1}$ . To apply 1 rate of limestone was enough to bind mobile Al to the immobile compounds. Primary liming kept Al immobilized for the 5 years - at the level 4  $\text{mg kg}^{-1}$  (Fig 4). A significant increase was registered after 15 years and continued to increase slightly with time. But even after 34 years the mobile Al concentration in soil was twice as low (34  $\text{mg kg}^{-1}$ ) compared to the primary status (66  $\text{mg kg}^{-1}$ ). Regular liming also immobilized almost all of the mobile Al, but not after a first single application of 0.25 rates  $\text{CaCO}_3$ . Minimal mobile Al levels in the soil after the whole regular liming cycle were similar to those of primary liming - 2-4  $\text{mg kg}^{-1}$ . The restoration of that parameter after liming had stopped and was slow compared to others. The amount of mobile Al was negligible - 1-3  $\text{mg kg}^{-1}$  even 20 years after the last liming.

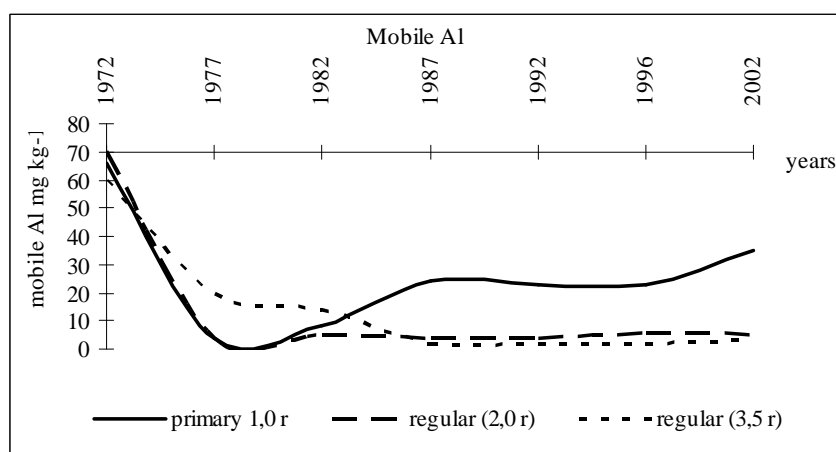


Figure 4 The effect of primary and periodical liming on mobile aluminium concentration in Luvisol

Another important soil chemical property is the amount of absorbed bases. At the beginning of the experiment the background level was as low as 200-300  $\text{cmol kg}^{-1}$  (Fig. 5). Primary liming increased the amount to 480  $\text{cmol kg}^{-1}$ , but because of decalcification processes and continuing decline after 20 years, the amount was similar to the primary state. Regular liming increased the amount of absorbed bases to higher levels - by 560  $\text{cmol kg}^{-1}$  if 2.0 rates  $\text{CaCO}_3$  were applied, and by 690  $\text{cmol kg}^{-1}$  if 3.5 of  $\text{CaCO}_3$  were applied. A significant decline in regularly limed soil was registered 10 years after the last liming, but even after 20 years it was 2,5-3,4 times higher than before the first liming.

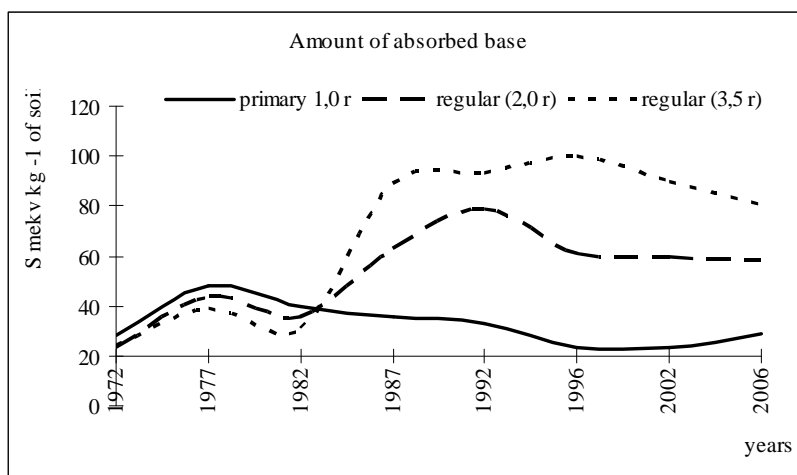


Figure 5 The effect of primary and periodical liming on amount of absorbed base in Luvisol

Productivity evaluation of the last crop rotation (2002-2006) shows that the primary liming effect continued to stimulate yield 30-34 years after (1.0 rate) liming – the total productivity of crop rotation compared to the unlimed soil was 8% higher. The regular liming affected productivity even more, because after the last liming a shorter period passed (20 years) and more limestone was used in total. From the plant production point of view, most effective was soil liming every 10 year with 1 rate of limestone, which resulted in pH changes from 4.4 to 5.7 and the immobilizing of mobile Al. More intensive neutralizing – up to  $\text{pH}_{\text{KCl}}$  6.4, didn't stimulate plant production and even had a negative effect compared to pH 5.7 soil. Nevertheless, the productivity of crop rotation compare to unlimed soil was still 18% higher in the 20<sup>th</sup> year.

The results of long-term experiments shown that primary liming efficiency in various Lithuanian regions and foreign countries relate to the liming rate, lime material (type and particle size) as well as on soil properties (genesis, initial chemical, hydrothermal) (Ahern, Weinand, 1995; Motowicka-Terelak, 1985). Regular liming compared to primary is characterized by a lower economic effect, but stabilize soil acidification processes, improve soil physical properties and the microbiologic background (Plesevičius, 1995; Tripolskaja, Marcinkonis, 2005; Bernotas *et al.*, 2005).

The results of the experiment proved that in Lithuanian climate conditions for light textured soils formed on calcareous parent materials liming is having a long-term effect. The liming effect on very acid soils ( $\text{pH} \leq 4.5$ ) with 1.0 rate  $\text{CaCO}_3$  according H persist for 34 years. The fastest restoration (after 17-20 y) to previous levels is shown by pH, slower (20-25 y) for hydrolytic acidity and absorbed bases. The mobile Al restoring dynamic is slowest. 34 years after limestone (1.0 rate accord. H, or  $7.18 \text{ t ha}^{-1}$ ) application, the mobile Al concentration in soil was half that before liming. Analogical results of liming publicized by Shilnikov *et al.* (2002) summarizing long-term experimental results in Russia: the dynamic of soil acidity characterizing parameter changes are similar to our soils dynamics. A lower concentration of mobile Al after primary liming had a positive effect on plant production. After 30-34 years because of the primary liming effect crop production was still 8% higher compared to unlimed soil, even pH, hydrolytic acidity and absorbed bases were restored to primary levels. According to Plesevicene (2000) data, the effect of primary liming depends on the lime rates and persists up to 50 years for light loam acidity properties. It should be noticed that pH level restoration to the primary state during the shortest period (20 y.) where slaked lime was applied, but persisted for 50 years where tufa had been applied. Similar to other experiments the slowest was mobile Al restoring.

Periodical soil liming changed soil the reaction from pH 4.4-4.5 to pH 5.7 or 6.4 depending on the lime rates. Crop production evaluation concludes that neutralizing to pH 6.0 or higher is not productive because it does not stimulate production increase. A similar opinion was published by Russian scientists, who registered that during a 14 year period highest crop production was from soil limed with 0.5 or 1 rates (Шильников *et al.*, 2002).

Our research on soil acidification in regularly limed soil was conducted for a shorter time than the primary limed one – just 20 years. During this period the soil pH in soils above 5.5 declined by 0.5 unit and the downgrade was close to those in primary limed soil. But the amount of absorbed bases and mobile Al increased much slower. Practically, after 20 years since the last liming which led to the decline of mobile Al to  $2 \text{ mg kg}^{-1}$  it was stabilized. Nevertheless after primary liming the mobile Al started to increase after 10 years, and increased significantly after 15 years ( $32 \text{ mg kg}^{-1}$ ). There is a substantial difference between the slower restoration of mobile Al. Regular liming is a more intensively changing structure of exchangeable acidity, consisting of hydrogen and aluminum ions concentration in the soil liquid phase. For instance in 34th year of the experiment at same pH level ( $\text{pH}_{\text{KCl}} 4.5$ ) in unlimed and 1.0 rate limed the soil proportions between  $\text{H}^+$  and  $\text{Al}^{3+}$  ions was different - 4,10 and 3,39 respectively. This proportion becomes less with the increase in the number of liming cycles. After 20 years since the last liming  $\text{H}^+$  and  $\text{Al}^{3+}$  ratio was 2,09 in 2,0 limed soil and 0,85 – in 3,5 rates limed soil. Significant changes of acidity structure in the periodically limed soil limit options to evaluate the real soil acidity of me pH value.

Studies of soil acidity characterizing properties in the soil profile of the 25th year of the experiment suggest that long-term liming acidity declined not only in the arable layer but also in the subsoil (Eidukeviciene *et.al.*, 2001). In relation to liming rates, the duration and soil texture neutralizing effect can cover up to 100 cm of the soil profile. If the calcareous layer is laying at 80-100 cm depth the liming effect getting in touch with the calcareous layer has a neutralizing effect. It means that the parent material relieve the soil acidity optimization in the whole profile. Other researchers had to similar evaluations (Ivanov, 2000; Bernotas *et.al.*, 2005).

Field studies of soil acidity changes in 1998-2002 proved the conclusions of the experimental studies. With stopped liming since 1991 acidification is slower than the prognosis predicted. The comparison of results from 1995-2000 and 1986-1990 assumes an increase in the area of acid soils ( $\leq 5.5$ ) only by 1.7 % (Mazvila *et al.*, 2004). The most intensive acidification was registered in Western Lithuania soils where soils are naturally very high in acidity and the depth of the calcareous layer is quite deep (~300 cm). During the period of the study the acid soils in western Lithuania increased by 3.6%, in Eastern Lithuania – 1.3%, in Central Lithuania – 0.8%. Soil acidification is a natural cross border phenomenon and can be regulated by agrochemical means.

## Conclusions

Modern environmental protection requirements and soil protection among them also as economic factors stimulate investigations into new criteria for the determination of ecologically and economically based lime rates. Changes in the agrochemical properties listed above in regularly limed soils (structure of exchangeable acidity, mobile Al, absorbed bases, subsoil acidity) warning from use of same principles of lime requirement determination as in unlimed soils – pH and hydrolytic acidity. To answer the question – what is the optimal liming strategy of regularly limed soils, further investigation is required. At the Voke branch of LIA studies of various lime requirement determination methods (to immobilize Al, neutralizing a part of the hydrolytic acidity suggested by Nebolsin, according to base saturation or modified Adam-Evans method) have started since 2000. The first phases do not have a clear advantage over any other method, but proved that the increasing of pH in sandy soils above 6.0 is not needed because in earlier regularly limed soils at pH above 5.0 mobile Al is almost completely immobilized and the negative effect on crop production is minimal.

The experience of Western Europe countries, where long-term liming practice and soil acidity optimization suggest that in Lithuanian conditions in soils with mobile Al below  $10 \text{ mg kg}^{-1}$ , for the stabilizing of the acidification processes a balance method can be applied. It means lime requirement calculation to cover Ca leaching and removal with crops. The calculated amount can be corrected according to the soil texture which determines sorption capacity and the depth of the calcareous layer.

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## AUGSNES SKĀBUMA PAKĀPES RĀDĪTĀJI AUGSNĒM AR PĀRTRAUKTU KAĻĶOŠANU

**Marcinkonis S., Tripolskaja L.**

Lielākās daļas augšņu paskābināšanās un augsnes paskābināšanās pakāpes dažādība ir mūsdienu problēma visā Lietuvas teritorijā, īpaši austrumu un rietumu reģionos. Kopš 1972. gada Lietuvas Zemkopības institūta Voķe nodaļā veikto ilglaicīgo izmēģinājumu pēdējā posma (no 2002.-2006. gadam) rezultāti parādīja, ka augsnes sākotnējās kaļķošanas ietekme statistiski nozīmīga tikai kustīgajam Al. Salīdzinot ar sākotnēji kaļķotām augsnēm, periodiski kaļķotu augšņu pH, hidrolītiskā skābuma un kustīgā Al izmaiņas bija intensīvākas un to atjaunošanās iepriekšējā līmenī lēnāka. Šādās augsnēs pat pēc 14-23 gadiem kopš pēdējās kaļķošanas augu sekas produktivitāte joprojām ir augstāka nekā nekaļķotās augsnēs.

Pētījumā iegūtajos secinājumos tika noskaidroti šobrīd aktuālie jautājumi lauksaimniecības augšņu ar pārtrauktu kaļķošanu izmantošanai – kādi augsnes rādītāji raksturo augsnes skābumu un kāda ir kaļķošanas nepieciešamība agrāk kaļķotām augsnēm? Nepieciešama tālāka kaļķošanas pārtraukšanas ietekmes uz augsnes profila ķīmisko īpašību izmaiņām, it īpaši kustīgā Al dinamikas atjaunošanos, izpēte. Jaunu kritēriju izvēle kaļķošanas nepieciešamības un optimālās kaļķu normas noteikšanai attiecībā uz ekoloģisko un ekonomisko efektivitāti ir visnozīmīgākais jautājums, lai saglabātu šo skābo augšņu auglīgumu. Tikai tālāks augšņu monitorings dos iespēju detalizēti novērtēt kaļķošanas ietekmes uz vidi un precīzāk prognozēt augsnes optimālā skābuma līmeņa atjaunošanos.



## PERSPECTIVES OF WINTER TURNIP RAPE (*Brassica rapa* L. var *oleifera* subvar. *Biennis*) FOR BIOFUEL IN ESTONIA

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### Abstract

The utilization of biofuels is a possible alternative to fossil-based liquid fuels. Rapeseed oil is the most exploited raw material for biodiesel production in Europe. In Estonian growing conditions winter turnip rape (WTR) is most suitable for this purpose. Good resistance to pests and plant diseases is the advantage of WTR compared to spring rapeseed. Thus, the cultivation of WTR is inexpensive and environmentally friendly because of the minimised usage of pesticides and herbicides.

The oil yield of WTR per hectare is the most important trait for biodiesel production. WTR has proved itself to Estonian farmers as an oil crop with high yield and very good quality.

Raw fat content, seed yield and raw fat yield of the two varieties and seven perspective breeds were estimated in a two-year experiment at the Jõgeva Plant Breeding Institute. As a result, the most promising breed for biodiesel production was JSv 01-13051: seed yield in 2004 – 3,179\*\*\* kg ha<sup>-1</sup> and in 2006 – 3,756\*\*\* kg ha<sup>-1</sup>; raw fat content in 2004 – 449 g kg<sup>-1</sup> and in 2006 – 446 g kg<sup>-1</sup>; raw fat yield in 2004 – 1,427\*\*\* kg ha<sup>-1</sup> and in 2006 – 1,675\*\*\* kg ha<sup>-1</sup> (all the data represented on moisture content 75 g kg<sup>-1</sup>).

**Key words:** seed yield, raw fat content, raw fat yield

### Introduction

Global energy demand increases rapidly. According to the International Energy Agency World Energy Outlook reference scenario, economic growth and increasing population will lead to an increase in global energy consumption of 18% between 2000 and 2030. Utilization of reproductive sources of energy is a possible alternative to fossil-based liquid fuels. One opportunity is using biofuels made from biomass (Clini *et al.*, 2005).

Liquid biofuels can decrease the pollution level caused in some microenvironments such as urban centres by cars and other motor vehicles fuelled by fossil fuels. At present about 30 billion litres per year of biofuels are commercialised in North and South America, Europe and South Africa (Clini *et al.*, 2005). Vegetable oils have the potential to serve as a substitute for petroleum diesel fuel. Of the more than 350 known oil bearing crops, those with the greatest production potential are sunflower, safflower, soybean, cottonseed, rapeseed, canola, corn and peanut oil. The development of vegetable oil as an alternative fuel would make it possible to provide energy for agriculture from renewable sources located in the area close to where it could be used (Peterson, 2002).

Europe currently imports 50% of its total energy needs. In transport, which relies heavily on oil, 80% of the energy is imported. In 2003, the European Parliament and the Council have adapted the Directive 2003/30/EC aiming to promote the use of biofuels for transport. Biodiesel is one possible alternative fuel options that has the potential to help to reduce oil dependence and global warming pollution (Union of...).

Unlike the US where biodiesel is produced from soybean, the EU uses mainly rapeseed and to some extent sunflower seed. Production of biodiesel in Europe jumped by in 2006 – to 4.89 million tonnes up from 3.184 million tonnes in 2005 according to the European Biodiesel Board. This follows a 65% record growth in 2005 over 2004 (European Biodiesel...).

Although natural fats and oils are among the main biological raw material for chemical and technical application (the estimated annual consumption in Europe is about 2.6 million tons), only a limited fraction (according to different estimations between 15 and 25%) of the total vegetable oil supply in the world are used in the industrial sectors, in non-food or technical applications (Riva and Calzoni, 2003). First generation biofuels like ethanol from corn and biodiesel from rapeseed have effects on food prices (Krenn, 2007).

Cultivation of oil crops has increased in Estonia from 45.500 ha in 2003 up to 72.500 ha in 2007 (Statistics Estonia, 2007). Spring oilseed rape is the main cultivated oil crop in Estonia; winter oilseed rape and winter turnip rape (WTR) are more used as alternatives (Narits, 2006 a). The success of the cultivation of spring oilseed rape depends on plant protection, because rape is attractive to a large number of insect species, both beneficial and pests (Winfered, 1986). The winter hardiness of winter rape is very low in Estonian climatic conditions (Norman, 1994); therefore the cultivation of winter rape is not economically beneficial. WTR as an oil crop that has achieved popularity among Estonian farmers. The raw fat content and seed yield of WTR are equal or in some years even exceed spring oilseed rape (Narits 2006 b). WTR needs less plant protection against pests and diseases, compared to spring oilseed rape, which is its definite economical advantage, both financially and environmentally. It is also of great importance that oil cake used as an animal feed has no residuals of plant protection chemicals.

The Jõgeva Plant Breeding Institute (Jõgeva PBI) started a cooperation with the Svalöf Weibull AB in WTR breeding in 1993. The WTR varieties Prisma and Largo obtained in the cooperation are registered in the Estonian Variety List. In the beginning of the cooperation winter hardiness was the main breeding objective but currently high and stable raw fat yield is defined to be a more important breeding task. The Svalöf Weibull AB transferred all its WTR breeding material to the Jõgeva PBI in 2002. Samples of each breeding line are maintained in the genebank of the Jõgeva PBI. Looking for alternatives to fossil fuels is an important activity all over the world, thus researchers from Sweden, Norway, Finland and Canada have requested the WTR varieties and breeds from the Jõgeva PBI for testing in their climatic conditions.

The aim of this study was to estimate the seed yield, raw fat content and raw fat yield of WTR varieties and perspective breeds and to determine the most promising as raw material for biodiesel production.

### Materials and Methods

Experiments were conducted at the Jõgeva PBI in the growing seasons of 2003-2004 and 2005-2006. The field trial with the WTR varieties Prisma and Largo and seven perspective breeds was established on 10 m<sup>2</sup> plots using NNA (nearest neighbour adjustment) randomised design in three replications. Herbicide 'Triflurex 480' (trifluralin) at 2 l ha<sup>-1</sup> and complex fertilizer 'Kemira Skalsa' N5-P10-K25, of 300 kg ha<sup>-1</sup> were applied before sowing. The sowing rate was 6 kg ha<sup>-1</sup>; seeds were not treated. The trial was top-fertilized with 'Kemira Power' N30-P6-S2, of 200 kg ha<sup>-1</sup> (N 60 kg ha<sup>-1</sup> on agent) in spring. The winter hardiness of all varieties and breeds of both the trial years was very good (9 points). No pesticides and fungicides were applied during the growing period. Weather conditions of the growing seasons of the trial years were different: 2004 was wet and 2006 dry. Seeds were dried to the moisture content of 75 g kg<sup>-1</sup> and cleaned/separated after the harvest. The seed yield of all varieties and breeds from each replication was estimated. Seeds of all the three replications were mixed and an average sample of 200 g of each variety and breed was analysed at the laboratory of the Jõgeva PBI. Raw fat, raw protein and glucosinolates content in seeds were analysed by Free and Open Source Software for Near Infrared Reflectance Spectroscopy (FOSSNIRS) system at the laboratory of the Jõgeva PBI. NIRS is widely used for the analysis of bulk rapeseed samples for oil, protein and glucosinolate content (Sato *et al.*, 1998; Velasco *et al.*, 1999; Velasco *et al.*, 2004, Font *et al.*, 2004, Aulric and Böhm, 2007). Raw fat yield per hectare was estimated.

The Least Significant Difference (LSD) procedure was used when the F-test was significant (P=0,001) and correlation was analysed (p< 0,005). The results were processed by the statistical program STATISTICA 4.5 ([www.statsoft.com](http://www.statsoft.com)).

### Results and Discussion

The following traits of each variety and breed were determined: seed yield as a main crop characteristic; raw fat yield as a main characteristic for oil production; raw fat content as a main quality characteristic of the oil crop; protein content and glucosinolate content as a characteristics of cake as an animal feed.

The results of 2004 are presented in Table 1. The average (Control) seed yield of tested varieties and breeds was 2,890 kg ha<sup>-1</sup>. The seed yield of the variety Largo (3,326 kg ha<sup>-1</sup>) and the breed JSv

01-13051 (3,179 kg ha<sup>-1</sup>) exceeded significantly the Control. Significantly lower was the seed yield of the variety Prisma (2,473 kg ha<sup>-1</sup>) and the breed JSv 01-13084 (2,537 kg ha<sup>-1</sup>).

Table 1. Seed yield, raw fat yield (kg ha<sup>-1</sup>), raw fat and protein content (g kg<sup>-1</sup>) and glucosinolate content (µmol g<sup>-1</sup>) of the varieties and breeds of winter turnip rape in 2004

Variety/breed	Seed yield, kg ha <sup>-1</sup>	Raw fat content, g kg <sup>-1</sup>	Raw fat yield, kg ha <sup>-1</sup>	Protein content, g kg <sup>-1</sup>	Glucosinolate content, µmol g <sup>-1</sup>
Prisma	2,473***	454	1,123***	306***	132***
Largo	3,326***	437***	1,455***	327	98
JSv 00-13426	2,937	462***	1,357	328	89
JSv 00-15588	2,716	450	1,222	348	73
JSv 01-11403	2,912	448	1,308	314***	80
JSv 01-11449	2,919	442***	1,290	342	93
JSv 01-13051	3,179***	449	1,427***	355***	53***
JSv 01-13102	2,537***	452	1,147***	353***	78
JSv 01-13102	3,007	444	1,335	333	101
Average of 2004 (Control)	2,890	449	1,296	334	89

\*\*\* - significance at 0.001 probability level

The average raw fat content was 449 g kg<sup>-1</sup>. The breed JSv 00-13426 (462 g kg<sup>-1</sup>) showed significantly higher raw fat content compared to the Control; the variety Largo (437 g kg<sup>-1</sup>) and the breed JSv 01-11449 (442 g kg<sup>-1</sup>) had significantly lower raw fat content.

The average raw fat yield was 1,296 kg ha<sup>-1</sup>. The raw fat yield of the variety Largo (1,455 kg ha<sup>-1</sup>) and the breed JSv 01-13051 (1,427 kg ha<sup>-1</sup>) was significantly higher; the raw fat yield of the variety Prisma (1,123 kg ha<sup>-1</sup>) and the breed JSv 01-13084 (1,147 kg ha<sup>-1</sup>) was significantly lower compared to the Control.

The average protein content was 334 g kg<sup>-1</sup>. Significantly higher was the protein content of the breeds JSv 01-13051 (355 g kg<sup>-1</sup>) and JSv 01-13084 (353 g kg<sup>-1</sup>). Significantly lower was the protein content of the variety Prisma (306 g kg<sup>-1</sup>) and the breed JSv 01-11403 (314 g kg<sup>-1</sup>).

The average glucosinolate content was 89 µmol g<sup>-1</sup>. Significantly lower was the glucosinolate content of the breed JSv 01-13051 (53 µmol g<sup>-1</sup>) and higher of the variety Prisma (132 µmol g<sup>-1</sup>).

The results of 2006 are presented in Table 2. The average seed yield was 3,512 kg ha<sup>-1</sup>. The seed yield of the breeds JSv 01-11449 (3,832 kg ha<sup>-1</sup>) and JSv 01-13051 (3,756 kg ha<sup>-1</sup>) was significantly higher compared to the Control. Significantly lower was the seed yield of the variety Prisma (3,274 kg ha<sup>-1</sup>) and the breed JSv 01-13102 (3,177 kg ha<sup>-1</sup>).

Table 2. Seed yield, raw fat yield (kg ha<sup>-1</sup>), raw fat and protein content (g kg<sup>-1</sup>) and glucosinolate content (µmol g<sup>-1</sup>) of the varieties and breeds of winter turnip rape in 2006

Variety/breed	Seed yield, kg ha <sup>-1</sup>	Raw fat content, g kg <sup>-1</sup>	Raw fat yield, kg ha <sup>-1</sup>	Protein content, g kg <sup>-1</sup>	Glucosinolate content, µmol g <sup>-1</sup>
Prisma	3,274***	453***	1,483	351	175***
Largo	3,307	448	1,482	359	94
JSv 00-13426	3,445	444	1,530	351	90
JSv 00-15588	3,381	439***	1,484	356	123
JSv 01-11403	3,707	443	1,642	347***	95
JSv 01-11449	3,832***	435***	1,667***	368***	108
JSv 01-13051	3,756***	446	1,675***	358	79***
JSv 01-13102	3,732	442	1,650***	365	106
JSv 01-13102	3,177***	448	1,423***	365	97
Average of 2006 (Control)	3,512	444	1,556	358	107

\*\*\* - significance at 0.001 probability level

The average raw fat content was  $44.4 \text{ g kg}^{-1}$ . Significantly higher was the raw fat content of the variety Prisma ( $453 \text{ g kg}^{-1}$ ) and lower of the breeds JSv 01-11449 ( $435 \text{ g kg}^{-1}$ ) and JSv 00-15588 ( $439 \text{ g kg}^{-1}$ ) compared to the Control.

The average raw fat yield was  $1,556 \text{ kg ha}^{-1}$ . Significantly higher was the raw fat yield of the breeds JSv 01-13051 ( $1,675 \text{ kg ha}^{-1}$ ), JSv 01-11449 ( $1,667 \text{ kg ha}^{-1}$ ) and JSv 01-13084 ( $1,650 \text{ kg ha}^{-1}$ ). Significantly lower was the raw fat yield of the breed JSv 01-13084 ( $1,423 \text{ kg ha}^{-1}$ ).

The average protein content was  $358 \text{ g kg}^{-1}$ . Significantly higher was the protein content of the breed JSv 01-11449 ( $368 \text{ g kg}^{-1}$ ). Significantly lower was the protein content of the breed JSv 01-11403 ( $347 \text{ g kg}^{-1}$ ).

The average glucosinolate content was  $107 \mu\text{mol g}^{-1}$ . Significantly lower was the glucosinolate content of the breed JSv 01-13051 ( $79 \mu\text{mol g}^{-1}$ ) and higher of the variety Prisma ( $175 \mu\text{mol g}^{-1}$ ).

In 2006, the seed yield of all the tested WTR varieties and breeds was higher than three tons per hectare. Only three trial components exceeded the three-ton level in 2004. The breed JSv 01-13051 showed good seed yield in both the years. The breeds JSv 01-11449, JSv 01-13051 and JSv 01-11403 had better seed yield stability compared to the other varieties and breeds. Correlation between seed yield and weather conditions was significant ( $r=0,80$ ).

The raw fat content of the tested material was good in both the years (higher than  $440 \text{ g kg}^{-1}$ ). JSv 00-13426 had the highest raw fat content in 2004 ( $462 \text{ g kg}^{-1}$ ). A negative correlation was observed between raw fat content and seed yield ( $r=-0,51$ ). Seed yield was higher in 2006 compared to 2004; raw fat content was, in opposite, higher in 2004 and lower in 2006. This tendency particularly appeared in the variety Largo in 2004 and the breed JSv 01-11449 in 2006.

To describe better the varieties and breeds as potential raw material for oil producers, the raw fat yield per hectare was calculated (Figures 1, 2). A high correlation between raw fat yield per hectare and seed yield was revealed ( $r=0,99$ ), but the correlation between raw fat yield and raw fat content was not significant ( $r=0,44$ ). The breed JSv 01-13051 showed high raw fat yield in both years. Due to the coincidence of better than the average results of all the quality characteristics, such as seed yield, raw fat yield and raw fat content, the outcome of the mentioned breed was the best in 2006. High level of raw fat yield content and its stability are necessary features for oil production (Narits, 2007 a).

To show WTR varieties and breeds as the potential material for animal feed, raw protein yield per hectare was calculated (Figures 1, 2). The protein content of the WTR varieties and breeds was higher in the dry year of 2006. Year effects on protein content and seed yield were significant ( $r=0,73$  and  $r=0,65$ , respectively). The breed JSv 01-13051 had high protein content in both the years, thus the oil cake of this breed has good nutritive value.

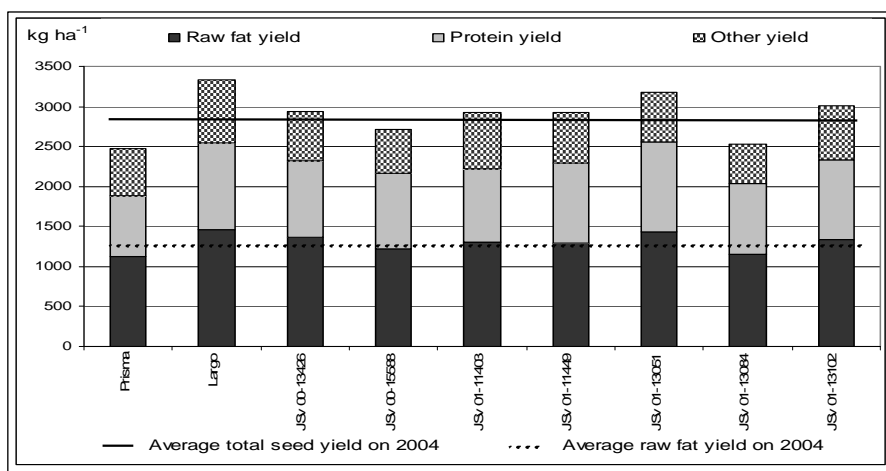


Figure 1. The distribution of total seed yield of WTR varieties and breeds in 2004

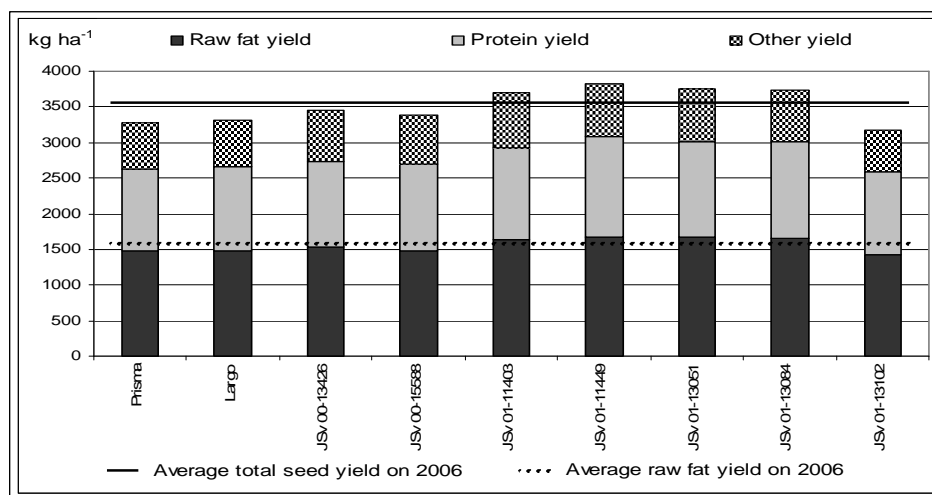


Figure 2. The distribution of total seed yield of WTR varieties and breeds in 2006

The glucosinolate content of the WTR varieties and breeds differed in 2004 and 2006. The effect of the variety in glucosinolate content was significant ( $r=0,52$ ). It was the only indicator having a significant effect to the variety. The breed JSv 01-13051 had good results in both the years when the glucosinolate content was lower than that of the other varieties and breeds. The content of glycosinolates is a variety specific characteristics. For example, Prisma has always a high content of glycosinolates, but concerning the breeds, this characteristic is yet unstable. Correlation between year interaction and glucosinolate content was low ( $r=0,38$ ), thus glucosinolate content depended on weather conditions to a small extent. In drought stressed plants, glucosinolate content increases in all plant parts, including seeds (Narits, 2007 b).

### Conclusions

WTR has good seed yield and raw fat yield potential. Summarizing all data we can conclude that the most promising breed for biodiesel production among nine tested varieties and breeds was JSv 01-13051 with a seed yield of  $3,179^{***}$  kg ha<sup>-1</sup> in 2004 and of  $3,756^{***}$  kg ha<sup>-1</sup> in 2006; and a raw fat yield  $1,427^{***}$  kg ha<sup>-1</sup> in 2004 and  $1,675^{***}$  kg ha<sup>-1</sup> in 2006. Furthermore, this breed had low glucosinolate content and high protein content, which results in healthy oil cake as an animal feed. WTR has good potential for growing as the raw material of biodiesel production in Estonian climatic conditions.

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### **ZIEMAS RIPŠA (*Brassica rapa* L. var *oleifera* subvar. *Biennis*) PERSPEKTĪVAS BIODEGVIELAS RAŽOŠANAI IGAUNIJĀ**

**Narits L., Annamaa K.**

Biodegvielu izmantošana ir iespējama alternatīva fosilajām šķidrām degvielām. Eiropā biodīzeļa ražošanai visvairāk izmantotā izejviela ir rapšu eļļa. Igaunijas augšanas apstākļos šim mērķim vispiemērotākais ir ziemas ripsis (WTR). Tam ir labāka rezistence pret kaitēkļiem un augu slimībām nekā vasaras rapsim. Tādējādi, samazinātas pesticīdu un herbicīdu lietošanas rezultātā WTR kultivēšana ir lēta un videi draudzīga.

Biodīzeļa ražošanai visnozīmīgākais rādītājs ir WTR eļļas raža uz hektāru. Igaunijas zemnieki atzinuši WTR kā eļļas kultūru ar augstu ražu un ļoti labu kvalitāti.

Jēltauku saturs, sēklu raža un jēltauku raža divām pamatšķirnēm un septiņām perspektīvām šķirnēm tika novērtēta divu-gadu izmēģinājumā Jõgeva laukaugu selekcijas institūtā. Rezultātā, visdaudzsološākā šķirne biodīzeļa ražošanai bija JSv 01-13051: sēklu raža 2004. gadā – 3,179\*\*\* kg ha<sup>-1</sup> un 2006. gadā – 3,756\*\*\* kg ha<sup>-1</sup>; jēltauku saturs 2004. gadā – 449 g kg<sup>-1</sup> un 2006. gadā – 446 g kg<sup>-1</sup>; jēltauku raža 2004. gadā – 1,427\*\*\* kg ha<sup>-1</sup> un 2006. gadā – 1,675\*\*\* kg ha<sup>-1</sup>.

### **SIMPLIFICATION OF WINTER RYE (*SECALE CEREALE* L.) GROWING TECHNOLOGY**

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#### **Abstract**

The paper presents the data on winter rye cultivation technology studies conducted during the period 1994-2006. The field trials carried out on a *Haplic Luvisol* were designed to estimate the effects of soil preparation, seed rate and nitrogen fertilization on winter rye grain yield and on the

number and weight of weeds. It was determined that the number and mass of weeds when winter rye was treated with a higher rate of nitrogen and reduced soil tillage system, which do not include ploughing.

With increasing the sowing rate of winter rye from 2.7 million seed ha<sup>-1</sup> to 5.7 million seed ha<sup>-1</sup> the weed dry mass decreased from 27.7 g m<sup>-2</sup> to 19.8 g m<sup>-2</sup> in the disk – cultivated ploughed soil and from 36.5 g m<sup>-2</sup> to 23.6 g m<sup>-2</sup> in the ploughed soil. Nitrogen fertilization had a greater effect on the yield than the seed rate. Having used nitrogen, winter rye yield was by 70.4% higher in the ploughed soil and by 66.6% higher in the disked soil compared with the yield of rye crops that unfertilised.

**Key words:** winter rye, soil cultivation, nitrogen fertilisation, seed quantity, yield

### Introduction

Of all cereal species grown on light soils winter rye is the most important and most-widely grown crop. Its roots are capable of penetrating into deeper soil layers and can utilise nutrients more efficiently compared with other winter or spring cereals. Furthermore, winter rye is very good at utilising the moisture accumulated during winter, is less affected by drought and is tolerant of acid soils (Lazauskas, 1999). Of all cereal crops grown at the Lithuanian Institute of Agriculture's Vokė Branch during the 1997-2000 period winter rye was found to be best at suppressing weeds. The number of weeds in the winter rye crop was on average 87 per m<sup>2</sup> and the air-dry weight amounted to 19-24.2 g. Winter rye is currently grown and in the future will be grown mostly in Lithuania's south eastern subzone and Šilutė district, where the area sown with winter cereals accounts for about 45-50% of the total area under cereals (Nedzinskas, 2001).

With the current increase in the prices of fuel, lubricants, fertilisers and other agricultural inputs, simpler and cheaper soil tillage practices have become increasingly popular and attempts are being made to abandon the most difficult and costly technological process inversion of the whole ploughlayer. Conventional deep ploughing is being replaced by a shallower ploughing or loosening without inversion of the ploughlayer, and by sowing the seed into completely untilled or only in surface-loosened soil; however, in combination with better fertilisation these practices can result in a high yield (Budzynski *et al.*, 2003). Other authors indicate that deeper ploughing forms a deeper ploughlayer, which secures a higher grain yield compared with shallow ploughing (Kouwenhoven *et al.*, 2002; Chmielewski, Kohn, 2000; Kessavalou, Walters, 1999). It was found that in shallowly ploughed sandy loam soil cereal yield was by 10-13% lower and in the direct-drilled soil by as much as 16-17 % lower compared with that in conventionally deeply ploughed soil (Kadžienė *et al.*, 2006). Research conducted in various countries confirm the fact that when there is no weed competition, reduced soil tillage does not decrease the yield of agricultural crops and makes the soil physical properties even more favourable for crops (Miedaner *et al.*, 2003; Kahnt, 1995). Experimental evidence obtained at the Lithuanian University of Agriculture's Experimental Station on neutral reaction soil with a medium humus status suggests that with no inversion of the whole arable layer and with ploughing or loosening at the 12-14 cm depth, weed seeds tend to concentrate in the upper soil layer. In the second crop rotation small-seeded weed species became highly prevalent (Pranaitis, 2002). The best weed control on couch-grass infested sandy loam soil was achieved by stubble cultivation after pat harvesting and deep or moderately deep ploughing before sowing: there were 3-4 times fewer couch-grass stems in the rye crop compared with that in the soil without stubble cultivation but with only deep, moderately deep or shallow ploughing. In the soil with a low couch-grass incidence rye grain yield was very similar both in the treatments where the stubble was broken and the soil was deeply ploughed and in the treatments with stubble breaking but no ploughing and in the treatments with only shallow ploughing (Pranaitis, 2002).

The objective of the present study was to identify and compare the effects of conventional and reduced soil tillage on winter rye as affected by different seed rates, moderate (N<sub>45</sub>) and increased (N<sub>90</sub>) nitrogen fertiliser rates

### Materials and Methods

The experiments were conducted at the Lithuanian Institute of Agriculture's Vokė Branch during the period 1994-2006. The soil where winter rye was grown is characterised as sandy loam haplic

luvisol (LVh) according to FAO classification, with a humus content of 1.85-2.06%, total nitrogen 0.127%, available phosphorus 177-193 mg kg<sup>-1</sup> and potassium 198-230 mg kg<sup>-1</sup> soil, and pH<sub>KCl</sub> 5.7-5.9. Oats were a pre-crop to rye. After oat harvesting (on August 8-13) the soil for rye was deeply ploughed (at the 22-24 cm depth) or only disked at the 8-10 cm depth.

The experiments were done observing the following design:

(i) Soil tillage (A factor): 1) soil for rye was ploughed and 2) soil for rye was not ploughed but disked;

(ii) Nitrogen fertilisation (B factor): 1) no nitrogen fertilisation, 2) N<sub>45</sub> fertilisation and 3) N<sub>90</sub> fertilisation;

(iii) Winter rye seed rate (C factor): 1) 2.7 million viable seed (90 kg ha<sup>-1</sup>), 2) 4.2 million viable seed (140 kg ha<sup>-1</sup>) and 3) 5.7 million viable seed (190 kg ha<sup>-1</sup>).

Prior to rye sowing (on September 2-4) the whole experimental field was applied with phosphorus and potassium fertilisers P<sub>60</sub>K<sub>60</sub> and was cultivated at a 5-7 cm depth. In the first ten-day period of September winter rye was sown by the seed drill 'Saxonia'. In spring, after resumption of rye vegetative growth the respective treatments were fertilised with nitrogen. The number of rye seedlings was determined upon the complete emergence and the number of plants was estimated upon resumption of vegetative growth after the winter. The number of weeds, their species composition and airdry mass were identified in the rye crop at the beginning of July. The above-mentioned parameters were estimated in each treatment by counting and/or taking samples from two 0.25 m<sup>2</sup> plots. Rye was harvested by a combine harvester 'Sampo'. While thrashing, 1 kg samples were taken for estimating grain moisture, purity and 1000 kernel weight. Rye grain data were adjusted to 15% moisture.

Initial plot area was 75 m<sup>2</sup>. The harvested plot area 47 m<sup>2</sup>. Experimental treatments were replicated 4 times.

#### *Statistical data analysis*

The results were analyzed statistically for the randomized split-plot design and the Tukey test was used to verify the significance of differences at  $\alpha = 0.05$ . All data were subjected to ANOVA.

### **Results and Discussion**

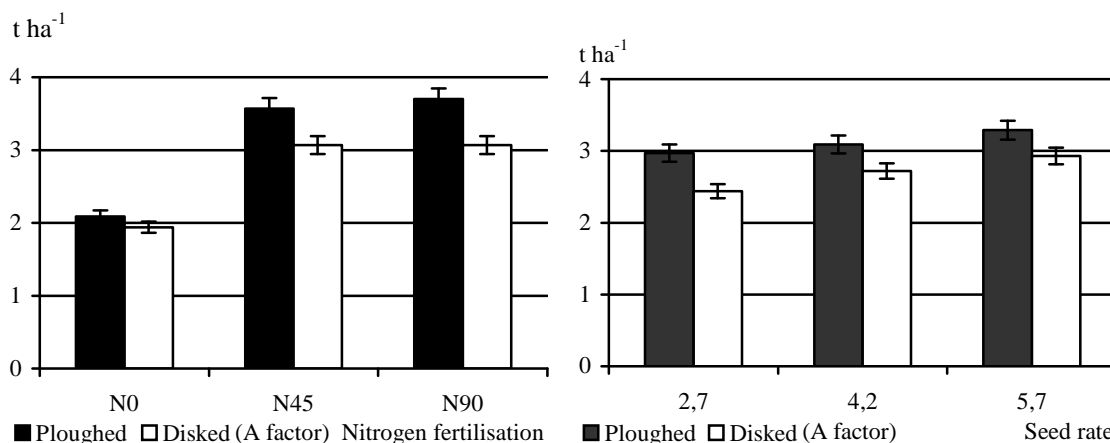
Autumn soil tillage practically had no effect on rye emergence, the number of plants increased in proportion to the seed rate sown both in the ploughed and disked treatments. Rye demonstrated the best winter survival when the soil had been ploughed and fertilised with phosphorus and potassium before sowing. The averaged data suggest that 80-83% of the autumn-emerged plants survived the winter. When the soil disked unploughed and unfertilised, the winter survival of rye plants was 6.0-7.5% lower.

Rye winter survival was also affected by the seed rate. The best winter survival (86-91%) was demonstrated by the rye that had been sown at the lowest sowing rate of 2.7 million seed ha<sup>-1</sup>. Increasing the seed rate to 5.7 million ha<sup>-1</sup> in ploughed and P<sub>60</sub>K<sub>60</sub>-applied soil, 65-73% of autumn-emerged plants survived the winter, and disked unploughed and fertilised with potassium and phosphorus the plants that survived the winter amounted to 62-68%.

#### *Rye grain yield*

Agricultural practices such as soil tillage and nitrogen fertilisation as well as seed rate also had some effect on the rye grain yield. The averaged data indicated that the grain yield amounted to 3.11 t ha<sup>-1</sup> in ploughed soil and was by 13.2% lower in unploughed but disked soil (Fig. 1).





LSD<sub>05</sub>A – 0.19, LSD<sub>05</sub>B – 0.14, LSD<sub>05</sub>C – 0.07, LSD<sub>05</sub> AB – 0.21, LSD<sub>05</sub>AC – 0.16 and LSD<sub>05</sub>ABC – 0.28)

Figure 1. The effect of nitrogen fertiliser (B factor) and seed rate (C factor) on winter rye yield (Traku Voke, averaged data from 1994-2006;

Nitrogen fertilisation had the greatest effect on winter rye grain yield. In the ploughed soil not applied with nitrogen the rye grain yield was low 2.09 t ha<sup>-1</sup>, in the disked soil the grain yield amounted to 1.94 t ha<sup>-1</sup>. Fertilisation of rye with N<sub>45</sub> yield in ploughed soil was 70.4% higher and in the disked soil by 66.6% higher compared with the grain yield of rye that was left unfertilised. When fertilised with N<sub>90</sub> rye was slightly lodged in separate years and the yield increased only inappreciably compared with that from the treatment fertilised with N<sub>45</sub> rate.

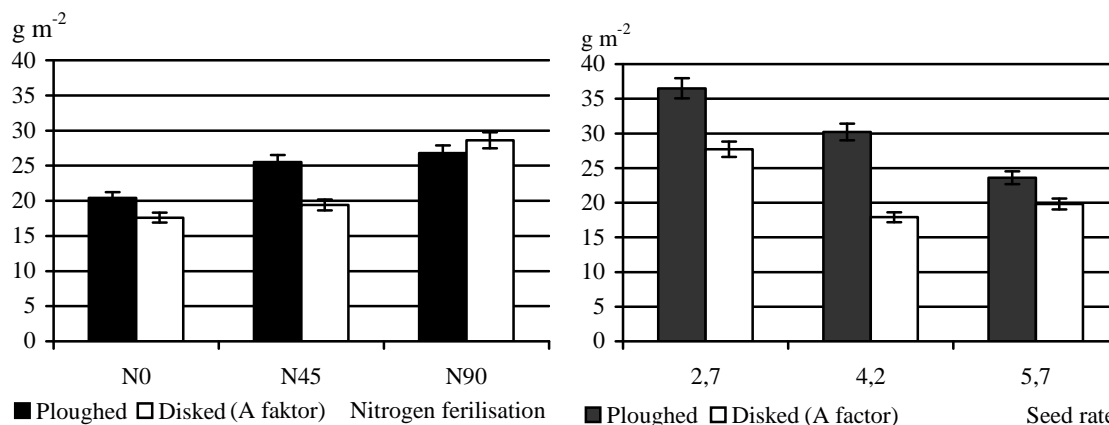
Our tests suggest that the optimal seed rate on sandy loam soil is 5.7 million viable seed ha<sup>-1</sup> or 190 kg ha<sup>-1</sup>. Averaged data from long-term trials show that sowing 2.7 million seeds ha<sup>-1</sup> the grain yield of rye amounted to 2.70 t ha<sup>-1</sup>, the seed rate of 4.2 million ha<sup>-1</sup> generated a grain yield of 2.91 t ha<sup>-1</sup>, and having increased the seed rate to 5.7 million ha<sup>-1</sup>, the grain yield increased to 3.11 t ha<sup>-1</sup>.

Weed incidence in the rye crop

Increasing the seed rate from 2.7 million viable seed ha<sup>-1</sup> to 5.7 million ha<sup>-1</sup> the number of weeds inappreciably declined, however, their air-dry weight consistently declined with an increase in seed rate (Fig. 2). Sowing 2.7 million seeds ha<sup>-1</sup> in the ploughed soil the air-dry mass of weeds per 1 m<sup>2</sup> amounted to 36.5 g, and having sown 5.7 million seed ha<sup>-1</sup> the air-dry mass of weeds declined to 23.6 g and the weeds were small because of the dense rye crop which competed well with weeds.

When the soil for rye had not been ploughed but had been disked, the weed weight was markedly lower, but no consistent reduction with increasing seed rate was observed. As was mentioned, the weed incidence in the rye crop was very diverse, and especially in the unploughed soil, which determined their inconsistent reduction.

With nitrogen application to rye both in the ploughed and unploughed but disked soil weed mass consistently increased. When the soil for rye was ploughed and was not applied with nitrogen weed mass per 1 m<sup>2</sup> amounted to 20.4 g, with the application of N<sub>45</sub> weed mass increased to 25.5 g m<sup>-2</sup>, and with the increase of the nitrogen rate to N<sub>90</sub>, weed mass increased to 26.8 g m<sup>-2</sup>. A similar increase in the weed weight in the rye crop in relation to the nitrogen fertiliser rate occurred in the rye stand where the soil had not been ploughed but disked. In the plots where the soil had not been ploughed but had been disked and the rye had not been fertilised with nitrogen, weed mass was very low 17.6 g per 1 m<sup>2</sup>. Having applied N<sub>90</sub> the weed mass increased to 28.6 g.



LSD<sub>05</sub>A -1.66, LSD<sub>05</sub>B - 2.04, LSD<sub>05</sub>C - 2.28, LSD<sub>05</sub> AB -3.01, LSD<sub>05</sub>AC - 4.16 and LSD<sub>05</sub>ABC - 4.65)

Figure 2. The effect of nitrogen fertilisation (B factor) and seed rate (C factor) on the number of weeds in winter rye and on weed mass (Traku Voke, averaged data from 1994-2006;

When increasing the seed rate of rye from 2.7 million ha<sup>-1</sup> to 5.7 million viable seed ha<sup>-1</sup> in the ploughed soil the number of annual and perennial weeds consistently declined. When the soil had not been ploughed but had been disked, the increasing of the rye seed rate practically did not have any effect on the incidence of perennial weeds, and there was a similar number of weeds as both of the treatments sown with the lowest and the highest seed rates.

### Conclusions

When the soil for winter wheat after the oat pre-crop had been ploughed at the 22-24 cm depth or only had been disked at the 8-10 cm depth the same number of plants emerged, which increased in proportion to the seed rate sown. Rye exhibited the best winter survival (80-83% of autumn-emerged plants) when the soil had been ploughed and the lowest seed rate of 2.7 million ha<sup>-1</sup> was sown.

In ploughed or disked soil the total number of weeds was similar, however in disked soil the number of perennial weeds was higher by 23.7%. By increasing the sowing rate of rye from 2.7 million seed ha<sup>-1</sup> to 5.7 million seed ha<sup>-1</sup> the weed mass consistently increased.

Rye yielded better in ploughed soil where the grain yield was by 13.2% higher than in the unploughed but disked soil. Nitrogen fertilisers had the greatest effect on rye grain yield. Having fertilised rye with N<sub>45</sub> in the ploughed soil the grain yield was 70.4% higher and in the disked soil 66.6% higher compared with the grain yield from fields that were not fertilized. When increasing the sowing rate from 2.7 million seed ha<sup>-1</sup> to 5.7 million seed ha<sup>-1</sup> the rye grain yield increased on average by 15.5%.

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## **ZIEMAS RUDZU (*SECALE CEREALE* L.) AUDZĒŠANAS TEHNOLOĢIJAS VIENKĀRŠOŠANA**

**Nedzinskienė T.L., Asakavičiūtė R.**

Rakstā ietverti dati par ziemas rudzu audzēšanas tehnoloģijas pētījumiem, veiktiem laika periodā no 1994.-2006. gadam. Lauka izmēģinājumi veikti smilšmāla augsnē (*Haplic Luvisol*) ar mērķi novērtēt augsnes sagatavošanas, izsējas normas un slāpekļa mēslojuma ietekmes uz ziemas rudzu graudu ražu un uz nezāļu daudzumu un svaru. Tika konstatēts, ka ziemas rudzos lielāks nezāļu skaits un masa ir pie minimālās augsnes apstrādes (bez aršanas) un pie lielākas slāpekļa normas. Palielinot ziemas rudzu izsējas normu no 2.7 miljoni sēklu ha<sup>-1</sup> uz 5.7 miljoni sēklu ha<sup>-1</sup>, nezāļu sausā masa samazinājās no 27.7 g m<sup>-2</sup> līdz 19.8 g m<sup>-2</sup> augsnē bez aršanas un no 36.5 g m<sup>-2</sup> līdz 23.6 g m<sup>-2</sup> uzartā augsnē. Slāpekļa mēslojumam bija lielāka ietekme uz ražu nekā izsējas normai. Lietojot slāpekli, ziemas rudzu raža bija par 70.4 % augstāka artajā augsnē un par 66.6 % augstāka diskotā augsnē (bez aršanas), salīdzinot ar nemēslotu rudzu graudu ražu.

## **RESULTS OF WEED MONITORING IN THE LONG-TERM EXPERIMENTAL FIELD IN PRIEKULI**

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### **Abstract**

The negative role of weeds in agriculture will be emphasized. They can considerably reduce an amount and the quality of the yield. To make out the influence of different agroecological factors on the weed infestation of arable land, a long-term field experiment was commenced in the Priekuli Plant Breeding Institute (57°19'N, 25°20'E). The investigation was established on a soddy podzolic light loam soil. Data on species and the amount of weeds was collected from the fields of five different fertilization systems. The aim of the research is to show the weed dynamic data, obtained from a 17-year period, in regards to the fertilization system and the management practice in the classical crop rotation: barley – clover/grass – rye – potato.

Results show that the least amounts of the weeds were in unfertilized fields. More weeds were in plots, where stable manure was used as fertilization.

**Key words:** weeds, long-term experiments, fertilization, crop rotation

### **Introduction**

The negative role of weeds in agriculture is well known. Weeds compete with crops for nutrients. An increase in weed invasion, results in a more intensive consumption for soil nutrients by the agricultural community. Intensive nutrient consumption by weeds leads to an increasing drop in crop yield (Lauringson *et al.*, 2000).

The main purpose of crop rotation is to maintain the fertility of the soil, to increase the amount and the quality of the yield. The crop is influenced by soil properties, weeds, the spread of diseases and pests. For this reasons it is not good to grow a crop that was harmed by the same diseases and pests

as previously grown crop. After crops with high weed infestation there should be grown crops that better compete with weeds.

The crop rotation of Norfolk is recognized as a classical and the most successful rotation for the maintenance of soil fertility and reducing weed infestation. This famous light land arable four-course rotation consisting of roots followed by spring barley followed by red clover followed by winter cereals was introduced by Townshend around 1730 (Wibberley, 1996). The rotation consists of 50% cereals, roots 25 % and clover/grasses 25 % and the above mentioned succession allows for the adequate restoration of fertility between corn crops (Rubenis, 1979).

A similar crop rotation was established at the State Priekuli Plant Breeding Institute in 1958. Some results of weed infestation from this experimental field have been published before (Zarina, 1997; 2004; 2006). This investigation covers the period from 1990-2006. The aim of the research was to show the weed dynamic data, obtained from a 17-year period in barley after full crop rotations, in dependence of the fertilization system and the management practice. Our hypothesis is that clover/grasses ley helps to reduce the weed infestation in crop rotation.

### Materials and Methods

The long-term crop-rotation experimental field was established at the State Priekuli Plant Breeding Institute in 1958. It includes crop rotation barley – clover/grass – rye – potato in five different fertilization systems: unfertilized, stable manure 20 t ha<sup>-1</sup> (N-68, P-38, K-58), NPK (N-66, P-90, K-135), stable manure 20 t ha<sup>-1</sup> + NPK (N-134, P-128, K-193), doubled NPK (N-132, P-180, K-270). Sowing time is the 1<sup>st</sup> decade of April for spring crops. The clover in rotation is red clover, which is established as an under-sown crop in barley.

The size of each plot is 100 x 5.9 m. Herbicides and fungicides were not applied during the experiments, but the seeds were treated with fungicides. Weeds have been controlled by ridging in the potatoes and sharing of stubble surface after the harvesting of cereals.

The number of weeds was determined in the first decade of June in ten replicates from the area 0.1 m<sup>2</sup> by the counting method (Rasins, Taurina, 1982). Results were expressed for 1 m<sup>2</sup>. All weed stages were counted (including cotyledons).

### Results and Discussion

The results of weed monitoring show that the highest amount of weeds in the plots was found, where stable manure was used as fertilization (Figure 1). The amount of weeds did not exceed 162 plants per m<sup>-2</sup> in the unfertilized plot – there weeds were small and not vital. In plots, fertilized with NPK and doubled NPK number of weeds was 45-226 plants per m<sup>-2</sup>.

There were identified 29 different weed taxons in total – 21 annual and 8 perennial weed species. The most widely distributed annual weeds were *Vicia spp.*, *Chenopodium spp.*, *Spergula arvensis*, *Viola arvensis* and *Stellaria media*, perennial – *Cirsium spp.*, *Sonchus spp.* and *Equisetum arvense*. The barley crop rotation nine weed taxons were rare – five annual weed species (*Erodium cicutarium*, *Anthemis arvensis*, *Poa annua*, *Galium aparine* and *Veronica spp.*) and four perennial weeds (*Rumex crispus*, *Taraxacum spp.*, *Ranunculus spp.* and *Plantago spp.*).

The dominant species of weeds differ when compared to the years 1990 and 2006 or after four full crop rotations (Figure 2). In the unfertilized plot some species, dominating in 1990 (*Equisetum arvense*), were not registered in 2006 and vice versa (*Viola arvensis* and *Raphanus raphanistrum*). There were even more weeds after four full crop rotations (Figure 1). The same weed species – *Viola arvensis* and *Spergula arvensis* – dominated also in 2006 in the field, where stable manure was used as fertilization. The composition and amount of dominant weeds differ in these years also in plots with other fertilization systems.

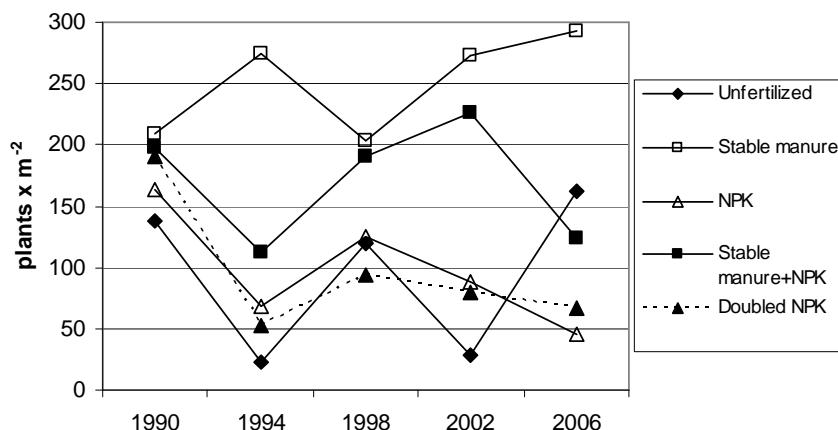


Figure 1. Total amount of weeds in barley under different fertilization systems after full crop-rotations in the 17-year period

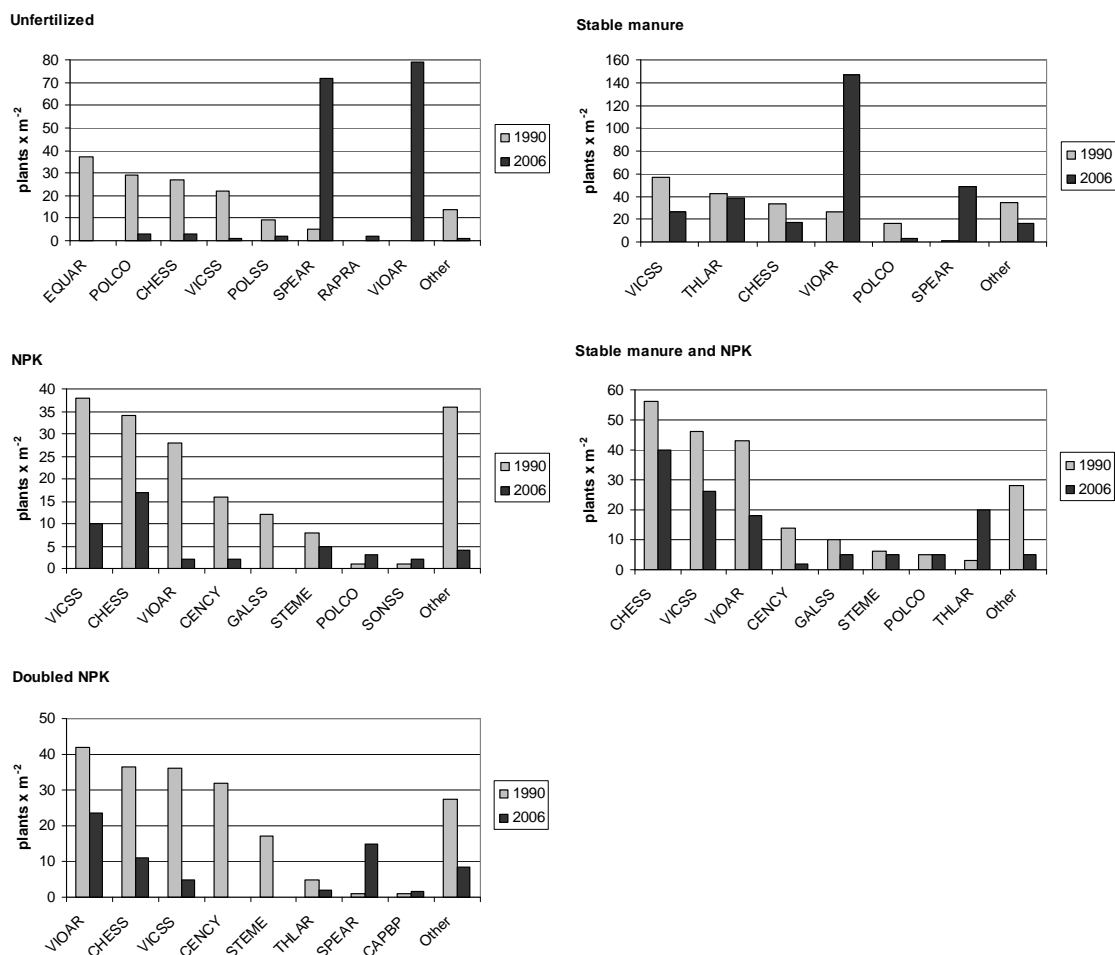


Figure 2. Dominant species of weeds in plots with different fertilization systems  
 CAPBP – *Capsella bursa-pastoris*, CENCY – *Centaurea cyanus*, CHES – *Chenopodium species*, EQUAR – *Equisetum arvense*, GALSS – *Galeopsis species*, POLCO – *Polygonum convolvulus*, POLSS – *Polygonum species*, RAPRA – *Raphanus raphanistrum*, SONSS – *Sonchus species*, SPEAR – *Spergula arvensis*, STEME – *Stellaria media*, THLAR – *Thlaspi arvense*, VICSS – *Vicia species*, VIOAR – *Viola arvensis*

These differences between the years 1990 and 2006 in the composition of weed species do not mean that there are not these weeds in the seed bank. It is probable that the seeds were not

germinated and were waiting for better weather conditions, more favourable temperatures and precipitation. The amount of germinated weeds depend on weather conditions before registration of weeds (on April, when the vegetation period starts, on May and on the beginning of June).

An similar to ones investigation was carried out in Poland (Jaskulska, 2004). In the spring barley *Spergula arvensis* and *Chenopodium album* dominated. Also in our investigation these weed species were among the dominating weeds in spring barley. The lowest dry mass of weeds in spring barley was in the unfertilized fields and in the field where fertilization was used as stable manure. In our investigation weeds were not weighed, but the weeds in the unfertilized fields and in fields fertilized with stable manure were not as vital as weeds in plots fertilized with NPK and doubled NPK.

To understand weed impact on the amount and quality of the yield, it was necessary to compare weed data with the indices of the yield. This was not done in this investigation. The investigation in Canada (Stevenson, Légère, 1998) did not confirm that weed infestation overcomes the quality taken to protect spring barley. The negative influences were by other factors, not weed impact.

As no herbicides were used in our investigation, the number of weeds in the fields of crop rotation was dependent on cultivated crops, the succession of crops in the crop rotation links and crop cultivation technologies as in organic farming systems (Ausmane *et al.*, 2008). Four-field crop rotation: barley – clover/grass – rye – potato, is recognised as the classical and successful crop rotation in regard to soil properties, crop yield and weed infestation. This was affirmed also this by investigation – the amount of weed seedlings did not exceed 293 plants per m<sup>-2</sup> in none of the fertilization backgrounds. This was published also before (Zarina, 2004), that classical crop rotation was the most effectual for the restriction of weed seedlings in a field and the major amount of weeds were found in fertilizing systems with stable manure.

## Conclusions

The results show that the least amounts of the weeds were in unfertilized fields. More weeds were in plots, where fertilization used as stable manure. The use of stable manure probably increased the amount of weeds.

In regards to our hypothesis, the average amount of weeds did not exceed 300 plants per m<sup>-2</sup> in any of the fertilization systems, although the amount of weeds changed over time. The positive impact of clover/grasses ley is visible also in barley after two years, when rye and potato were grown in the field.

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## NEZĀĻU MONITORINGS ILGGADĪGAJĀ IZMĒĢINĀJUMU LAUKĀ PRIEKUĻOS

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Lauksaimniecībā tiek uzsvērtā nezāļu negatīvā loma. Tās var būtiski samazināt ražas daudzumu un kvalitāti. Lai noskaidrotu dažādu agroekoloģisko faktoru ietekmi uz nezāļainību tīrumos, Priekuļos (57°19'Z, 25°20'A) tika uzsākts ilglaicīgs pētījums. Izmēģinājums tika ierīkots velēnu podzolētā viegla smilšmāla augsnē. Nezāļu sugu un daudzuma dati tika iegūti no laukiem piecos atšķirīgos mēslojuma fonos. Pētījuma mērķis ir parādīt ilgtermiņa (17 gadu) nezāļu dinamiku atkarībā no mēslojuma sistēmas un arī laukauga specifikai atbilstošās augsnes apstrādes paņēmieniem klasiskajā augu sekā: mieži – āboliņš/zālaugi – rudzi – kartupeļi.

Vismazāk nezāļu uzskaitītas nemēslotajos lauciņos. Visvairāk nezāļu bija lauciņos, kuros kā mēslojums lietoti kūtsmēsli.

## SOWING TIME INFLUENCE ON THE YIELD OF WINTER WHEAT UNDER THE CLIMATE CONDITIONS OF ZEMGALE

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### Abstract

A 3-year field experiment was conducted at the Study and Research Farm "Peterlauki" on silt loam brown lessive soil. Three varieties of winter wheat (*Cubus*, *Tarso*, *Zentos*) in 4 sowing times with a 10-day interval from the end of August till the end of September with 3 sowing rates – 300, 400, and 500 germinating seeds per m<sup>2</sup> with four replications were included in the experiment. Certified treated seed material was used. The depth of sowing was 3 to 4 cm. Fertilizer treatments: preplant application of P<sub>2</sub>O<sub>5</sub> - 60 kg ha<sup>-1</sup>, K<sub>2</sub>O - 90 kg ha<sup>-1</sup>, split nitrogen topdressing N 150 kg ha<sup>-1</sup> – in spring after renewal of vegetation N 90, and N 60 kg ha<sup>-1</sup> at the beginning of stem elongation stage of plant growth.

The 3-year research results indicate that all the varieties of winter wheat included in the experiment showed higher (8.5 – 9.4 t ha<sup>-1</sup>) and more stable yields between years in treatments with sowing time performed in the second half of September, i.e. in the third and fourth sowing time. Earlier planting dates, particularly at the end of August, regardless of the sowing rate resulted in vigorous tillering, the strong outgrowing of plants and the occurrence of snow mould in spring, causing the death of plants. Winter wheat *Tarso* was more stable regarding sowing time but more sensitive was *Cubus*. The sowing rate was not the yield determinant factor. Only in late sowing (the 4<sup>th</sup> sowing time at around 29 – 30 September), when plants with none or extremely poor tillers were going to overwinter, increased sowing rate showed the tendency of yield increase.

**Key words:** inter wheat, variety, sowing time, sowing rate

### Introduction

Winter wheat is one of the most productive and significant cereal species in Latvia used for food grain production. For that reason, the average grain productivity level in Latvia and grain supply both for food and feed is greatly dependent on the grain yield of winter wheat and yield stability between years. As known, the grain yield and quality of winter wheat are dependent on corresponding agro-technical measures - complex provided for a variety grown in a definite region under variable meteorological conditions. Optimal sowing time and a corresponding sowing rate have a significant role in common agro-technical measures complex to establish in autumn a healthy sward capable of wintering under inconsistent winter conditions. Current assumptions regarding sowing time and sowing rate under conditions of Latvia are based mainly on research results obtained in 70ies-80ies of the last century (Adamovičs, 1978; Bonāts and Sīviņš, 1987; Sēklkopja rokasgrāmata., 1967). As recommended in books (Jurševskis, 1988; Grīnblats, 1985) and

fixed in normative documents (Metodika, 2003), September 1–15 is the optimal sowing time for winter wheat in Zemgale and Kurzeme regions, but August 25 –September 10 in Vidzeme and Latgale regions with sowing rates 450 – 600 germinating seeds per 1 m<sup>2</sup>. Recently, using original breeding methods, new winter wheat varieties characterized by different growth and development indices have been developed and offered to the food industry. Serious climatic changes observed in nature significantly influence the growth, development and productivity of field crops, and particularly winter crops (winter wheat) (Harrison and Butterfield, 1996; Olsen *et al.*, 2000).

Recently, climatic changes have resulted in a prolonged vegetation period of winter crops in autumn which is directly related with the probable sowing time. Recent research, which was mainly devoted to yield quality problems, proved that high grain yields with corresponding food grain quality are possible under conditions of Latvia when corresponding agro-technical measures are used. Yet frequently problems were caused by the inadequate winter-hardiness of plants under the influence of variable meteorological conditions during wintering. The winter-hardiness of wheat sowing is known to be dependent on the sowing status prior to wintering and varietal ability to adapt to variable climatic conditions and inconsistent meteorological situations during wintering. Winter wheat sward of optimal density and not outgrown is a precondition to good winter-hardiness. We can obtain such a sward by choosing the proper sowing time and the number of germinating seeds per unit area suited to a variety. Research results on the time and rate of sowing winter wheat as reported by researchers in other countries (Knapowski and Ralcewicz 2004; Lupu, 2001) are not suited to Latvia and Zemgale due to different climatic conditions. Therefore the goal of this research was to come to new conclusions regarding responses to different sowing times and sward density shown by winter wheat varieties of different origin under field conditions in Zemgale.

### Materials and Methods

Field experiments were conducted at the Study and Research Farm “Peterlauki” on silt loam brown lessive soil (sod calcareous). Three varieties of winter wheat (*Cubus*, *Tarso*, *Zentos*) in 4 sowing times with a 10-day interval from the end of August till the end of September with 3 sowing rates – 300, 400, and 500 germinating seeds per m<sup>2</sup> with four replications were included in the experiment. In total, 36 treatments were included in the experiment. Certified treated seed material was used. The depth of sowing was 3 to 4 cm. Fertilizer treatments: pre-plant application of P<sub>2</sub>O<sub>5</sub> - 60 kg ha<sup>-1</sup>, K<sub>2</sub>O - 90 kg ha<sup>-1</sup>, split nitrogen topdressing N 150 kg ha<sup>-1</sup> – in spring after renewal of vegetation N 90, and N 60 kg ha<sup>-1</sup> at the beginning of stem elongation stage of plant growth. Grain was harvested at the ripening stage from each replication separately. The harvested grain was weighed, and moisture content and grain purity were determined. Yield data were expressed at 100% purity and 14% moisture. Three-factor analysis of variance was used to determine yield significance level. Variation coefficients were calculated between years for each factor.

### Results and Discussion

The average 3-year research results indicate that a comparatively high yield of winter wheat – 8.49 t ha<sup>-1</sup> was obtained. Estimates of the obtained grain yields depending on studied factors show, that different varieties of winter wheat had different responses to sowing time. All investigated winter wheat varieties sown in late August produced significantly lower grain yields compare to later sowing times. The winter wheat variety *Cubus* turned out to be most sensitive to early sowing. For this variety, the 3-year average grain yield obtained in the 1st sowing time was 17% lower compare to the 2nd sowing time (Fig. 1). Vigorous tillering and the strong outgrowing of plants were observed in the variety *Cubus* during the comparatively long after-sowing period till the end of autumn vegetation. In autumn 2006, the length of plant leaves for this variety reached even 20 – 25 cm as a result of which a strange fact was observed when plants already in autumn were in lodging. Regardless of the sowing rate, such thickened and outgrown sowings being the result of tillering frequently suffered from snow mould which caused the death of plants. As regards sowing time, the winter wheat variety *Cubus* proved to be most unstable between the years. Other varieties of winter wheat also showed significant grain yield increase, yet considerably lower – 10 – 12% compare to the 1st sowing time. Winter wheat *Tarso* was less sensitive to sowing time, except early sowing. Grain yield was greatest with the variety *Zentos*, attained in the 3rd and 4th sowing time,



i.e. in the third decade of September (beginning and end). On the whole, all winter wheat varieties included in the experiment produced higher (8.5 – 9.5 t ha<sup>-1</sup>) and between years more stable yields of grain in treatments the sowing time of which was the second half of September – the 3rd and 4th sowing time. Regarding sowing time, more stable was *Tarso* but expressed sensitivity was observed in *Cubus*.

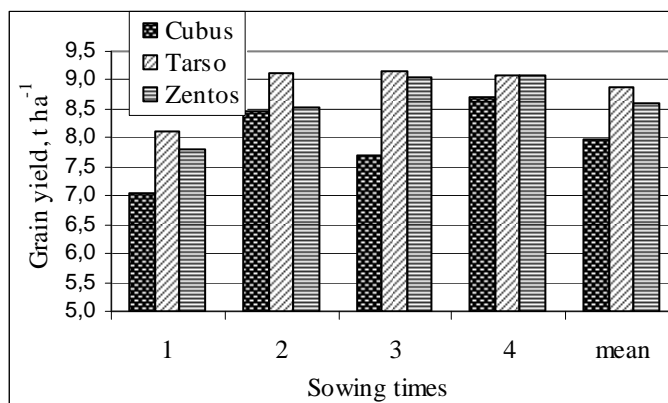


Figure 1. Effect of sowing time on grain yield of winter wheat varieties

The influence of sowing rate on grain yield of winter wheat on average in three years was greatly due to peculiarities of the variety. An increased sowing rate above 300 germinating seeds resulted in the tendency of yield decrease in winter wheat *Cubus* but yield increase in *Tarso* (Fig. 2). However these are comparatively insignificant sowing time yield differences.

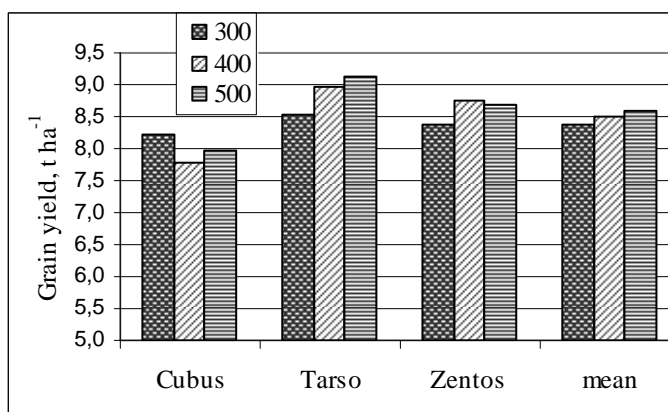


Figure 2. Effect of sowing rates on productivity of winter wheat varieties

In early sowing time, the highest grain yield of winter wheat was reached with the variety *Cubus* using a sowing rate of 300 germinating seeds per 1 m<sup>2</sup>. An increased sowing rate up to 400 or 500 germinating seeds resulted in significant grain yield decrease (Table 1). A similar situation was also observed in the 2nd sowing time. In the 3rd sowing time the grain yield practically did not change under the influence of the sowing rate, and only in the 4th sowing time the increased sowing rate showed the tendency of yield increase. Grain yield fluctuations in the winter wheat varieties *Tarso* and *Zentos* under the influence of sowing rates in most cases were within the limit of error. Only in late sowing (4th sowing time around 29 – 30 September), when plants with none or extremely poor tillers were going to winter on increased sowing rate showed the tendency of yield increase. On the whole, the sowing rate is not a yield determining factor as shown by yield indices for the three experiment years.

Table 1 Grain yield on the three-year average, t ha<sup>-1</sup>

Time of sowing (A)	Variety (B)	Rate of sowing (C)			Mean
		300	400	500	
1st sowing time	Cubus	7,85	6,65	6,61	7,04
	Tarso	7,88	8,05	8,46	8,13
	Zentos	7,54	7,97	7,88	7,80
	Mean	7,76	7,56	7,65	7,66
2nd sowing time	Cubus	8,80	8,15	8,45	8,47
	Tarso	8,80	9,42	9,19	9,14
	Zentos	8,13	8,77	8,65	8,52
	Mean	8,58	8,78	8,76	8,71
3rd sowing time	Cubus	7,79	7,67	7,69	7,71
	Tarso	8,92	9,18	9,38	9,16
	Zentos	8,94	9,11	9,04	9,03
	Mean	8,55	8,65	8,70	8,64
4th sowing time	Cubus	8,41	8,60	9,08	8,70
	Tarso	8,53	9,18	9,52	9,08
	Zentos	8,94	9,09	9,19	9,07
	Mean	8,63	8,96	9,26	8,95
Mean sowing rate		8,38	8,49	8,60	8,49

Factor: A  $\gamma_{0,05} = 0,417$ , Interaction: AB  $\gamma_{0,05} = 0,66$ ; B  $\gamma_{0,05} = 0,366$  AC  $\gamma_{0,05} = 0,72$ ;

C  $\gamma_{0,05} = 0,370$  BC  $\gamma_{0,05} = 0,589$

The 3-year averages of yield indices practically smooth over annual deviations caused by variable meteorological conditions. Under different meteorological conditions the expression of some yield affecting factors is also different as indicated by yield variation coefficients (Table 2).

Table 2 Coefficients of variation, %

Factor	Year 2005	Year 2006	Year 2007
1st sowing time	7,25	7,70	16,06
2nd sowing time	8,95	x	11,04
3rd sowing time	7,34	7,82	3,65
4th sowing time	11,80	8,30	2,73
Cubus	10,65	7,59	x
Zentos	12,76	5,21	7,44
Tarso	7,50	4,69	14,77
300	11,39	7,11	14,00
400	10,05	9,35	11,37
500	9,76	7,46	12,13

Among sowing time between years, the smallest fluctuations in the coefficient of variation - below 8% were observed for the 3rd sowing time (around 20 September). For the rest of sowing time, fluctuations in coefficients of variation between years were considerably greater, particularly in the 1st sowing time. When estimating between separate years, the influence of other factors was different in various sowing times. So in 2005, the coefficient of variation was highest in the 4th sowing time when grain yield differences under the influence of variety and sowing rates were strongly expressed compare to other sowing times. In 2006, variation coefficients are equal for all sowing times. Yet in 2007, in the 1st and 2nd sowing time the grain yield of winter wheat was considerably influenced by the variety and sowing rate, but in the 3rd and 4th sowing time the influence of the mentioned factors was comparatively small – the variation coefficient was below 4%.

The influence of studied factors on grain yield indices of separate winter wheat varieties is greatly due to the meteorological situation of the year. In 2005, the time and rate of sowing had the least effect on the productivity level of the variety *Tarso* (variation coefficient 7.50) but *Zentos* was most sensitive (variation coefficient 12.76). In 2007, the situation between these varieties was entirely contrary.

Variation coefficients between sowing rates within one year are characterized by weakly expressed fluctuations. Coefficients of variation were highest in 2007 – 11.37-14.00 % and lowest in 2006 – 7.11-9.35 %. The sowing rate of 400 germinating seeds per 1m<sup>2</sup> were more stable between years with the range of fluctuations in variation coefficients from 9.35 to 11.37 %.

## Conclusion

The 3-year research results indicate that all winter wheat varieties included in the experiment showed higher grain yields (8.5 – 9.4 t ha<sup>-1</sup>) and were more stable between years in treatments the sowing time of which was the second half of September – the 3rd and 4th sowing time. Sown earlier, particularly in late August, plants of winter wheat strongly tillered and outgrew regardless of the sowing rate. Snow mould occurring in spring caused the death of most plants. The variety of winter wheat *Tarso* was more stable to sowing time but *Cubus* was more sensitive. Sowing rate was not the factor determining yield. Only in late sowing (the 4th sowing time around 29-30 September), when plants with no or very weak tillers were going to winter, increased sowing rates showed a tendency of yield increase.

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## SĒJAS LAIKA IETEKME UZ ZIEMAS KVIEŠU RAŽU ZEMGALES APSTĀKĻOS

### Ruža A., Kreita D.

Mācību un pētījumu saimniecībā “Pēterlauki” puteklaina smilšmāla lesivētā brūnaugsne tika veikts trīs-gadu izmēģinājums. Izmēģinājumā tika iekļautas trīs ziemas kviešu šķirnes (*Cubus*, *Tarso*, *Zentos*), sētas četros atkārtojumos, četros sējas laikos (ar 10-dienu intervālu no augusta beigām līdz septembra beigām), ar trīs izsējas normām – 300, 400 un 500 dīgstošas sēklas uz m<sup>2</sup>. Tika izmantota sertificēta sēkla. Sēšanas dziļums bija 3 līdz 4 cm. Mēslojums: pirmsējas P<sub>2</sub>O<sub>5</sub> - 60 kg ha<sup>-1</sup>, K<sub>2</sub>O - 90 kg ha<sup>-1</sup>, slāpekļa virsmēslojums N 150 kg ha<sup>-1</sup> pa daļām – pavasarī pēc veģetācijas atjaunošanās N 90 kg ha<sup>-1</sup>, un stiebrošanas sākumā N 60 kg ha<sup>-1</sup>.

Trīs-gadu pētījuma rezultāti rāda, ka visām izmēģinājumā iekļautajām ziemas kviešu šķirnēm augstākas (8.5 – 9.4 t ha<sup>-1</sup>) un noturīgākas ražas bija sējot septembra otrajā pusē, t.i. trešajā un ceturtajā sējas laikā. Agrākie sējas datumi, īpaši augusta beigās, neskatoties uz izsējas normu, veicināja spēcīgu asnu dzišanu, stipru augu pāraugšanu un sniega pelējuma parādīšanos pavasarī, izraisot augu bojāeju. Ziemas kvieši *Tarso* bija noturīgākie attiecībā uz sējas laiku, bet jutīgākie bija *Cubus*. Izsējas norma nebija ražu noteicošs faktors. Tikai vēlā sējā (ceturtajā sējas laikā ap 29.-

30. septembri), kad augi pārziemoja bez vai ar ļoti vājiem dzinumiem, palielināta izsējas norma uzrādīja ražas pieauguma tendenci.

## THE PRODUCTIVITY AND PERSISTENCY OF PURE AND MIXED FORAGE LEGUME SWARDS

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### Abstract

The objective of this research was to study long - yielding legume species grown in a pure stand and in a mixture with other legumes and grasses in an organic crop rotation. The experiments were conducted during the period 2001-2006. The swards were cut two and three times during the growing season and were used for five years in total. The highest dry matter yield of swards was produced and the largest amount of metabolisable energy in herbage was accumulated in the third year of use. Pure sainfoin (*Onobrychis viciifolia*) and lucerne (*Medicago sativa*) produced 8.65-9.73 t ha<sup>-1</sup> of the dry matter. Legume swards in the exceptionally dry fifth year of use produced up to 4.09 t ha<sup>-1</sup> dry matter. The best yielding was mixed sward composed of fodder galega (*Galega orientalis*), lucerne and *Festulolium*. In the fifth year of use the sward of pure sainfoin produced a higher dry matter yield than pure fodder galega and pure lucerne. The sown legumes persisted well in the swards. In the fifth year of use they accounted for 73-93 % of the dry matter yield. However, there was little left of *Festulolium* only 1- 2%. When the swards were cut two or three times, a similar herbage yield was produced, however when cut more frequently the swards thinned out more severely and there was a higher infestation of forbs of which *Taraxacum officinale* prevailed. Lucerne responded more sensitively to more frequent cutting.

**Key words:** legumes, swards, forage production, organic farming

### Introduction

In Lithuania, the area of land under certified organic production is rapidly increasing. The growing interest in organic farming has also increased the role of legumes in forage production. Red clover and white clover are the most important legumes in Lithuania (Arlauskienė, Maikštėnienė, 2001). The economic and ecological benefits of forage legumes are well known. Biological nitrogen accumulated by legumes can be useful in various ways: it produces protein rich forage and completes the nutrient balance in soils (Sprent and Mannetje, 1996; Baležentienė *et al.*, 2000; Ledgard, 2001). However, some legumes also have undesirable characteristics, one of which is their short persistence in swards (Frame *et al.*, 1998). Early red clover, which is the most common legume in Lithuania, yields well only in the first – second years of use. In the third year, it often disappears completely. In Lithuania, fodder galega is the most long lived legume exhibiting the best overwinter survival (Baležentienė *et al.*, 2000).

On an organic farm where no mineral nitrogen is applied it is very important that legumes persist as long as possible in the swards, therefore it is necessary to look for more long lived legumes that could at least partly replace red clover (Spruogis, 1999). For the study we selected a mixture of legumes, less commonly used than clover: common sainfoin (*Onobrychis viciifolia*), fodder galega (*Galega orientalis*) and lucerne (*Medicago sativa*). The objective of our tests was to study the productivity and persistence of more long lived legume species on an organic farm in a pure crop and in mixtures with other legumes and grasses under an extensive (2-3 cuts) management regime.

### Materials and Methods

Field experiments were carried out on a sod gleyic loamy soil (Cambisol) in the central part of Lithuania (55° 23' N, 23° 51' E, with a mean annual rainfall of about 600 mm, a mean temperature of 6° C, a growing season of 186-194 days. The soil characteristics were as follows: pH<sub>KCl</sub> 7.0,

soluble P 67-80, K 106-112 mg kg<sup>-1</sup>. In the spring of 2001 pure or mixtures of herb species were sown under barley: 1. *Galega orientalis* 100 %; 2. *Medicago sativa* 100 %; 3. *Onobrychis viciifolia* 100 %; 4. *Galega orientalis* 40 %, *Medicago sativa* 40 %, *Festulolium* 20 %; 5. *Galega orientalis* 40 %, *Onobrychis viciifolia* 40 %, *Festulolium* 20 %; 6. *Galega orientalis* 40 %, *Medicago sativa* 20 % *Trifolium pratense* 20 %, *Festulolium* 20 %; 7. *Galega orientalis* 40 %, *Trifolium repens* 20 %, *Onobrychis viciifolia* 20 %, *Festulolium* 20 %. The seed rate for pure crop was as follows: *Galega orientalis* 30 kg ha<sup>-1</sup>, *Medicago sativa* 15 kg ha<sup>-1</sup>, *Onobrychis viciifolia* 80 kg ha<sup>-1</sup>, *Trifolium pratense* 15 kg ha<sup>-1</sup>, *Trifolium repens* 10 kg ha<sup>-1</sup>, *Festulolium* 18 kg ha<sup>-1</sup>. The experiment was designed as a randomised complete block with 4 replications and a plot size of 2.5×14 m. The swards did not receive any mineral or organic fertilisation. The swards were used for five years and were cut either two or three times per season. The first cut was taken within the first days of June at the beginning (3-cut management) or at mass flowering (2-cut management) stage in the middle of June. The second cut was taken in the middle of July (at the beginning flowering, 3-cut management) or the beginning of August (at mass flowering, 2-cut management). The last cut was taken in the middle of October (3-cut management). Samples for the determination of dry matter (DM) content, botanical and chemical composition were taken from cut herbage mass in 4 replicates. The botanical composition (grasses, legumes, forbs) was measured after separation as DM weight. For an assessment of forage quality, chemical analyses of dry matter were performed for: crude protein, by determining the amount of nitrogen and multiplying by 6.25, crude fibre by the Hennerberg-Stohmann method, crude fat by the Rushkovski method, crude ash, by combustion. The differences in metabolisable energy were calculated on the basis of the chemical composition of DM, using digestibility coefficients and full value coefficients. The experimental data (herb dry matter yield, metabolisable energy, crude and digestible protein) were processed by the method of analysis of variance (two factor experimental design), applying the programme ANOVA.

The weather conditions during the experimental years were unrepresentative. In 2002 the rainfall rate during the growing season in Lithuania was by 48% and in 2003 by 31 % lower than the long-term mean (420mm). The air temperature was by 1-2 °C higher than usual (long-term mean 12.2°C). In 2004 the summer was warm and rainy, conducive to sward growth and development. The weather conditions in 2005 were normal. The year 2006 there was drought. The amount of rainfall that fell during June and July was as low as 11- 55 % of the mean long-term rate. The summer temperature was by 2.1 °C higher than average

## Results and Discussion

While establishing swards on organic farms it is vital that legumes dominate in the sward from the first year of use. In the first year of use when the swards were cut two or three times, the highest dry matter yield 6.70-7.04 t ha<sup>-1</sup> was produced by a mixed sward composed of lucerne, fodder galega and *Festulolium* (Table 1). In terms of herbage yield, pure lucerne herbage yield was similar to that in mixtures or it yielded significantly worse when it had been cut twice per season. Fodder galega due to the weak development in the first year had the worst yield. In the first year of use fodder galega exhibited a low productivity in Estonia also (Raig, 1994; Lillak and Laidna, 2001; Raig and Nommsalu, 2001). Averaged data suggest that in the first two years of use the best-yielding swards were those that were sown with pure lucerne or in a mixture with other herbs. The yield of the first crop of sainfoin was identical to or even higher than that of lucerne. However, due to poorer re-growth, the annual yield of sainfoin and its mixtures was lower than that of lucerne. Fodder galega, due to slow development, produced twice as low dry matter yield of herbage as the swards that included lucerne. The swards with lucerne produced a higher yield when cut three times, and sainfoin-when cut twice. Due to the drought in the first and second years of sward use, the first cut produced the larger share (48-80 %) of the annual dry matter yield of herbage. In years with normal weather conditions the first cut produced 40-60% of the annual yield. Herbage re-growth after cutting depends on plant species and weather conditions during the re-growth period (Mowrey *et al.*, 1992; Frame *et al.*, 1998; Hopkins,2001). The best re-growth was noted for the swards sown with pure lucerne or in mixtures with other herbs. Although sainfoin can withstand droughts, it demonstrated a poor re-growth after being cut. The first cut produced from 60-70 (3-cut management) to 70-80 % (2-cut management) of the annual yield. Fodder galega is not

characterised by a good re-growth (Raig and Nommsalu, 2001). However, in our experiments fodder galega re-grew better than sainfoin.

Table 1. The effect of different legume swards (Factor A) and cutting regime (Factor B) on the annual dry matter yield of herbage t ha<sup>-1</sup>

Sward (Factor A)	2002	2003	2004	2005	2006	Mean	Mean (FA)
Cutting regime (Factor B)							
3 cuts							
1. <i>Galega orientalis</i>	3.50	2.58	4.61	5.08	2.29	3.61	3.76
2. <i>Medicago sativa</i>	6.73	5.25	8.65	5.04	3.13	5.76	5.72
3. <i>Onobrychis viciifolia</i>	4.66	3.52	9.63	4.08	3.83	5.14	5.23
4. <i>Galega</i> + <i>Medicago</i> + <i>Festulolium</i>	7.04	5.92	7.80	5.50	4.09	6.07	6.07
5. <i>Galega</i> + <i>Onobrychis</i> + <i>Festulolium</i>	5.08	3.44	7.96	4.14	3.37	4.80	5.01
6. <i>Galega</i> + <i>Medicago</i> + <i>T.pratense</i> + <i>Festulolium</i>	6.62	5.40	7.02	5.03	3.34	5.48	5.45
7. <i>Galega</i> + <i>T.repens</i> + <i>Onobrychis</i> + <i>Festulolium</i>	5.61	3.75	7.97	4.17	2.83	4.87	4.91
Mean	5.60	4.26	7.45	4.72	3.27	5.06	
2 cuts							
1. <i>Galega orientalis</i>	3.63	2.97	5.15	5.70	2.08	3.91	
2. <i>Medicago sativa</i>	6.09	4.28	9.41	5.06	3.52	5.67	
3. <i>Onobrychis viciifolia</i>	5.44	3.87	9.73	3.86	3.72	5.32	
4. <i>Galega</i> + <i>Medicago</i> + <i>Festulolium</i>	6.70	4.70	9.01	5.82	4.09	6.06	
5. <i>Galega</i> + <i>Onobrychis</i> + <i>Festulolium</i>	6.26	4.18	8.73	4.14	2.78	5.22	
6. <i>Galega</i> + <i>Medicago</i> + <i>T.pratense</i> + <i>Festulolium</i>	6.03	4.68	7.48	5.32	3.53	5.41	
7. <i>Galega</i> + <i>T.repens</i> + <i>Onobrychis</i> + <i>Festulolium</i>	5.86	3.96	8.17	4.14	2.61	4.95	
Mean	5.71	4.09	8.24	4.86	3.19	5.22	
LSD <sub>05</sub> (FA)	0.35	0.24	0.74	0.27	0.16		0.46
LSD <sub>05</sub> (FB)	0.19	0.13	0.30	0.11	0.07	0.19	
LSD <sub>05</sub> F (Trial)	0.49	0.34	1.61	0.40	0.24		0.68

In the third year of use (2004) the swards produced the highest yields. The sward of pure lucerne cut 2 or 3 times per season produced similar yields to those produced by lucerne swards formed with other legumes and grasses. Pure-sown fodder galega produced significantly lower yield than that sown in mixtures. Other researchers have also reported a higher productivity of fodder galega in mixtures (Drikis, 1994; Adamovich, 1998; Adamovich, 2000; Tuori *et al.*, 2000). The swards of pure sainfoin or those mixed with other grasses were similar to those of lucerne or its mixtures. Legume swards that were not fertilized yielded relatively satisfactorily in the fifth year of use. They produced a dry matter yield of up to 4.09 t ha<sup>-1</sup>. The best yielding was found to be fodder galega and lucerne mixed sward with *Festulolium*. In the fifth year of use the sward of pure sainfoin produced higher dry matter yields than pure galega and pure lucerne. Lucerne and sainfoin responded more sensitively to more frequent cutting.

The data averaged over five years suggest that the highest dry matter yield (6.07 t ha<sup>-1</sup>) was obtained when cutting 2 or 3 times in the swards (Factor A), sown with grass mixture composed of fodder galega, lucerne and festulolium, or pure lucerne – 5.72 t ha<sup>-1</sup> (Table 1). Pure sainfoin yielded more poorly than pure lucerne, however the differences were low – 0.49 t ha<sup>-1</sup>.

In the botanical composition (Table 2) only the content of legumes is provided. Forbs and grasses account for the other half. For mixed swards, the percentage of each legume was determined and added together to show the total content of sown legumes in the sward. In our experiments in the first year of use, fodder galega accounted for 51-53 % the dry matter yield of the herbage. Lucerne and sainfoin in pure stands accounted for 83-89 %, respectively. In the first year of use, *Festulolium* dominated by 53-84 % in the mixed swards. Among legumes, the dominant species were lucerne: 36-18 % (swards 4 and 6), sainfoin: 10-18 % (swards 5 and 7), and red clover: 30% (sward 6). Fodder galega grown in mixtures was crowded out and it accounted in the dry matter yield for as little as 1-3 % in the first year. In the second year of use in the pure stand the share of fodder galega increased to 69- 73%. The highest content of sown legumes in the total dry matter yield was in the swards with lucerne or sainfoin, when they were sown pure or in mixtures with

other grasses. In pure lucerne swards lucerne accounted for 73-86 %, and in mixtures - 79-90 %. Forbs accounted for the other half. In pure sainfoin swards the sown herb was prevalent 59-88 %, and in mixtures with other species sainfoin accounted for 72-79 %. Fodder galega due to its slower development in a sward was not able to compete with the more rapidly developing lucerne and sainfoin. It accounted for as little as 1-5%. In a pure sward lucerne accounted for 75-78%. Lucerne and sainfoin choked out red and white clover which accounted for as little as 1 %. Luxuriant legumes Not fertilized with nitrogen also had a great suppressive effect on a sown grass – *Festulolium* (1-2%) and on grass weeds. Couch – grass (*Elytrigia repens*) accounted for up to two percent. Other grasses accounted for up to 1.0 %. Weeds thrived most abundantly. The most prevalent weed species was dandelion (*Taraxacum officinale*). In the sward cut twice weeds accounted for 7-24%, and in those cut three times for 17-26%.

Table 2. The total content of legumes (%) in the dry matter yield for each sward in the first, second and fifth year of use.

Sward	Year of sward use					
	First		Second		Fifth	
	3 cuts	2 cuts	3 cuts	2 cuts	3 cuts	2cuts
<i>Galega orientalis</i>	51	53	69	73	76	75
<i>Medicago sativa</i>	89	87	86	95	73	86
<i>Onobrychis viciifolia</i>	83	83	85	90	59	88
<i>Galega.+Medicago+Festulolium</i>	38	41	68	91	83	90
<i>Galega+Onobrychis+Festulolium</i>	19	24	69	81	73	79
<i>Galega+Medicago+T.pratense +Festulolium</i>	47	45	77	85	81	88
<i>Galega+T.repens +Onobrychis+Festulolium</i>	11	14	60	78	75	74

While investigating less frequent long-lived grasses it is very important to estimate their feeding value. The contents of one or another nutrient accumulated by plants have clearly defined limits that depend on the plant growth stage. The contents of nutrients accumulated in the herbage of mixed-composition swards depend on the species of the prevalent herb and sward use (Frame *et al.*, 1998; Hopkins, 2001).

The amount of metabolisable energy accumulated in the swards varied similarly to that of dry matter. The data averaged over 5 years of use indicate that the swards (Factor A) accumulated different contents of metabolisable energy. The highest metabolisable energy yield was produced by the swards with undersown pure lucerne (57.6 GJ ha<sup>-1</sup>) or grass mixture (60.2 GJ ha<sup>-1</sup>) when lucerne accounted for 40 % of the seed rate of the mixture. When cut twice (Factor B) a similar herbage yield was obtained, however, when cutting less frequently, higher contents of legumes, especially lucerne and sainfoin persisted in the sward. The metabolisable energy concentration accumulated in the dry matter of swards was close to 10 MG kg<sup>-1</sup> (Table 3).

Table 3. The contents of crude and digestible protein and metabolisable energy in swards (factor A) and their concentrations per kg of dry matter

Sward	Crude protein		Digestible protein		Metabolisable energy	
	kg ha <sup>-1</sup>	g kg <sup>-1</sup>	kg ha <sup>-1</sup>	g kg <sup>-1</sup>	GJ ha <sup>-1</sup>	MJ kg <sup>-1</sup>
	<i>Galega orientalis</i>	658	177	444	120	38.46
<i>Medicago sativa</i>	1002	174	654	114	57.56	10.07
<i>Onobrychis viciifolia</i>	763	145	506	96	50.53	9.83
<i>Galega.+Medicago+Festulolium</i>	1004	164	643	105	60.23	9.91
<i>Galega+Onobrychis+Festulolium</i>	736	147	491	98	48.57	9.70
<i>Galega+Medicago+T.pratense +Festulolium</i>	853	155	546	99	53.30	9.77
<i>Galega+T.repens +Onobrychis+Festulolium</i>	716	144	477	96	47.79	9.70
LSD <sub>05</sub>	81.0	5.6	51.0	3.8	4.790	0.172

The highest metabolisable energy concentration 10.29 MJ kg<sup>-1</sup> was identified in the dry matter of pure galega. In a pure lucerne or mixed sward where lucerne prevailed metabolisable energy differed little 9.91-10.07 MG kg<sup>-1</sup>. The lowest energy concentration accumulated in pure sainfoin

or mixed sward where sainfoin prevailed. When the swards were cut three times, the contents of the metabolisable energy accumulated were significantly higher ( $10.03 \text{ MJ kg}^{-1}$ ) than those in the swards cut twice ( $9.76 \text{ MJ kg}^{-1}$ ) (Table 4).

Table 4. The effect of the cutting regime (Factor B) on crude and digestible protein and the metabolisable energy in swards and their concentrations per kg of dry matter. (averaged over the period 2002-2006)

Cutting regime	Crude protein		Digestible protein		Metabolisable energy	
	$\text{kg ha}^{-1}$	$\text{g kg}^{-1}$	$\text{kg ha}^{-1}$	$\text{g kg}^{-1}$	$\text{GJ ha}^{-1}$	$\text{MJ kg}^{-1}$
3 cuts	836	163	554	109	50.86	10.03
2 cuts	801	153	521	100	50.97	9.76
LSD <sub>05</sub>	33.1	2.3	20.8	1.6	1.956	0.070

In our experiments, the annual yields of crude and digestible protein per hectare depended on herbage dry matter yield. The highest crude protein yield was produced in the third year of sward use. The highest average annual crude protein yield was produced by pure lucerne and a mixture with fodder galega with lucerne accounting for 40% of the seed rate (Tables 3, 4). Sainfoin sown both pure and in a mixture with fodder galega and *Festulolium* in crude protein yield significantly ( $783\text{-}762 \text{ kg ha}^{-1}$ ) lagged behind the sward sown with pure lucerne or its mixture with fodder galega and *Festulolium*. Fodder galega sward produced the lowest dry matter yield, therefore the content of crude protein per hectare was also the lowest. When the sward was cut three times, crude protein content was significantly higher than that of the sward cut twice. The difference was not high  $35 \text{ kg ha}^{-1}$  but significant. Having calculated the data and having derived the crude protein concentration per kilo of dry matter, it was revealed that in most cases the crude protein content was sufficient. According to zootechnical standards, 1 kg of forage should contain 140-160g of crude protein.

When the sward was cut three times, the highest crude protein concentration was identified in pure galega and lucerne swards  $185.3$  and  $176.8 \text{ g kg}^{-1}$ , respectively, while the least concentration  $149.9 \text{ g kg}^{-1}$  was found in sainfoin. Having calculated the data according to two factors it was revealed that when the swards were cut three times the concentration of crude protein  $163.4 \text{ g kg}^{-1}$  was significantly higher than in the case of two cuts  $152.9 \text{ g kg}^{-1}$ . The data averaged over five years indicate that the concentrations of digestible protein in swards (Factor A) were varied. The highest content of digestible protein  $120 \text{ g kg}^{-1}$  was accumulated by a pure galega sward, significantly less  $113.3 \text{ g kg}^{-1}$  in lucerne sward, while in the other swards – there was significantly less -  $96.3\text{-}105.2 \text{ g kg}^{-1}$  of herbage dry matter. The relatively low digestible protein concentration can be explained by the extensive use of swards.

Crude fibre concentration in the first cut varied in relation to the sward from  $286 \text{ g kg}^{-1}$  to  $352 \text{ g kg}^{-1}$ , in the second cut from 265 to 376, and in the third cut from 211 to  $329 \text{ g kg}^{-1}$ . In the first cut higher contents were found in a mixed sward where lucerne prevailed and two cuts were taken per season. In the second cut the fibres content was found when the sainfoin sward was cut three times ( $265 \text{ g kg}^{-1}$ ) or twice ( $278 \text{ g kg}^{-1}$ ). This can be explained by the fact that sainfoin, compared with lucerne, re-grows more weakly and has many rosette leaves and relatively few generative stems.

## Conclusions

The highest dry matter yield and metabolisable energy concentrations in long-lived swards accumulated in the third year of sward use. Pure sainfoin and lucerne produced  $8.65\text{-}9.73 \text{ t ha}^{-1}$  of dry matter. Grass mixtures composed of lucerne, sainfoin and galega yielded  $7.80\text{-}9.01 \text{ t ha}^{-1}$ , but the differences were not significant. Legume swards in the fifth year of use yielded satisfactorily well – up to  $4.09 \text{ t ha}^{-1}$  dry matter. Galega and lucerne mixed sward with *Festulolium* was found to be the best yielding. In the fifth year of use pure sainfoin sward produced a higher dry matter yield than galega and pure lucerne. Lucerne responded more sensitively to more frequent cutting.

The data averaged over five years suggest that the highest herbage dry matter yield  $6.07 \text{ t ha}^{-1}$  was produced by the swards composed of galega, lucerne and *Festulolium* or pure lucerne  $5.72 \text{ t ha}^{-1}$ . The pure sainfoin yield was worse than pure lucerne, however the differences were low  $\text{-}0.49 \text{ t ha}^{-1}$ .



Under two-cut and three-cut management the swards produced similar yields, however under management involving less frequent cuts legumes persisted better. Pure galega swards produced significantly lower herbage yield, compared with lucerne and sainfoin.

Lucerne, galega, and sainfoin sown pure persisted well and prevailed in the swards even in the fifth year of use. However, galega due to the weaker development in mixed swards and poorer competitive power against more rapidly developing lucerne, sainfoin and *Festulolium*, thinned out. The highest crude and digestible protein content per 1 kg dry matter was accumulated in pure galega and lucerne under the three-cut management 185-177 and 125-118 g kg<sup>-1</sup>, respectively. Under two-cut management the content of protein was significantly lower than under three-cut management.

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## LOPBARĪBAS TAURINZIEŽU ZELMEŅU PRODUKTIVITĀTE UN STABILITĀTE TĪRSĒJĀ UN MAISĪJUMOS

### Slepetys J.

Šī pētījuma mērķis bija izpētīt ilggadīgās tauriņziežu sugas, audzētas tīrsējā un mistros ar citiem tauriņziežiem un zālaugiem bioloģiskajā augu sekā. Izmēģinājumi tika veikti laika periodā no 2001.-2006. gadam. Zelmenis tika pļauts divas un trīs reizes augšanas sezonas laikā kopā piecus gadus. Visaugstākā zelmeņa sausnas raža tika saražota un vislielākais metaboliskās enerģijas daudzums augos tika akumulēts trešajā izmantošanas gadā. Tīrsējā sēta vīķlapu esparsete (*Onobrychis viciifolia*) un sējas lucerna (*Medicago sativa*) saražoja 8.65-9.73 t ha<sup>-1</sup>sausnas. Ļoti sausajā piektajā izmantošanas gadā tauriņziežu zelmeņu raža sasniedza līdz 4.09 t ha<sup>-1</sup>sausnas. Visražīgākais bija austrumu galegas (*Galega orientalis*), sējas lucernas un auzeņairesnes mistrs. Piektajā izmantošanas gadā tīrsējā sētas vīķlapu esparsetes zelmeņa sausnas raža bija augstāka nekā

tīrsējā sētai austrumu galegai un sējas lucernai. Sētie tauriņzieži zelmenī saglabājās labi. Piektajā izmantošanas gadā tie veidoja 73-93 % no sausnas ražas. Auzeņairene bija palikusi tikai 1-2 %. Kad zelmenis tika pļauts divas vai trīs reizes, tika saražota līdzīga zaļmasas raža. Pļaujot biežāk, zelmenis izretinājās vairāk un bija novērojama lielāka nezāļu invāzija, no kurām dominēja *Taraxacum officinale*. Visjutīgāk uz biežāku pļaušanu reaģēja sējas lucerna.

## OPTIMIZATION OF SOIL TILLAGE AND WEED CONTROL IN WINTER WHEAT

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### Abstract

Weed infestation in cereals is still one of the problems for growers despite of many tools and possible methods both chemical and non-chemical to control them. This article describes the results obtained in three years experiments (2001 – 2003) carried out at farm Dobeles Agra SIA located in Dobeles region of Latvia. Trials established in two different crop rotations (Factor A): 1. winter wheat sown after winter wheat, 2. winter wheat sown after winter rape. Three different soil tillage and sowing methods compared (Factor B): 1. – minimal conservation soil tillage in 10 – 15 cm deep with mixing of soil, 2. – direct sowing into stubble without any soil cultivation before, 3. – traditional soil tillage with ploughing on 25 cm with cultivation before sowing. Additionally the impact of those soil tillage methods on weed infestation in winter wheat was compared (Factor C): 1. - used herbicide Secator 0.3 kg ha<sup>-1</sup>, 2. – without herbicide treatment. A significantly smaller total number of weeds were observed in treatments where winter wheat was grown in recurrent sowing. Mainly it was caused by differences in number of oil seed rape plants in this treatment. Also significantly smaller number of weeds was observed after traditional soil tillage with ploughing. The data analysis show a significant linear negative correlation between winter wheat yield and the amount of total weed infestation in several weed species - *Stellaria media* (L.) Vill., *Sinapis arvensis* L., *Matricaria perforata* Merat. and *Lamium purpureum* L. The highest impact to changes of winter wheat grain yield was made from herbicide use – 64.1 %.

**Key words:** winter wheat, minimal soil tillage, direct drilling, ploughing, weed infestation

### Introduction

Conventional ploughing at the depth of 22 – 24 cm is expensive and labour – consuming work. In the world's soil tillage practice attempts have been made to replace ploughing by subsurface cultivation, rototilling, and chisel ploughing as well as reduced depth of ploughing (Rubenis et al., 1995). A big profit from reduced soil tillage is saving resources and working time. In the Rot Amsted research farm (U.K.) it was found that growing cereals working time can decrease two times using reduced tillage methods but using direct sowing – 3 – 4 times (Cannel, 1985).

### Methods

The three years experiments (2001 – 2003) were carried out at farm Dobeles Agra SIA located in The Dobeles region of Latvia. The soil there is sod podzolic loam soil. Trials established in two different crop rotations (Factor A): 1. winter wheat sown after winter wheat, 2. winter wheat sown after winter rape. Three different soil tillage and sowing methods compared (Factor B): 1. – minimal conservation soil tillage in 10 – 15 cm deep with mixing of soil, 2. – direct sowing into stubble without any soil cultivation before, 3. – traditional soil tillage with ploughing on 25 cm with cultivation before sowing. Additionally compared impact of those soil tillage methods on weed infestation in winter wheat (Factor C): 1. - used herbicide Secator 0.3 kg ha<sup>-1</sup>, 2. – without herbicide treatment.

The winter wheat variety 'Zentos' sown on 25.09.2000., 29.09.2001. and 30.09.2002 with pneumatical seed driller Vaderstad Rapid 600P. Soil ploughing was done with 6 furrow conventional plough KwerneLand, cultivation done with equipment Vaderstad Rexus. Minimal soil

tillage was carried out with heavy disc harrows Simba Discs 34C 4.6 together with press Simba double press 4.6. Herbicide Secator with rate  $0.3 \text{ kg ha}^{-1}$  were used during the tillering stage of winter wheat. Yield was harvested with Claas combine harvester.

There were variable weather conditions with large differences from long time averages during the experiments. That caused large differences in the results between three years with different impact of various factors.

Autumn 2000 were long and warm with a significant amount of precipitations. Those conditions were favourable for the active growth and development of crops and weeds. In the variant where winter wheat were sown after winter rape using minimal soil tillage and direct sowing, there was a significant invasion of winter safety weeds like *Matricaria perforata* Merat. Those conditions effected the growth and the development of the winter wheat because of high weed competitiveness. In the spring, those weeds were already big in size and the effectiveness of herbicide was low. Also a high amount of precipitations during June and July of 2001 (115 un 118 mm, SIA Dobeles Agra weather station data) caused additionally active growth of weeds and that factor affected the productivity of winter wheat yield results. The trials of second year were established under conditions with high soil moisture, caused by high amount of precipitations during the summer 2001 (309 mm, SIA Dobeles Agra weather station data). Those conditions were not favourable for quality of soil tillage work, in comparison with the previous year. Winter wheat crop growth and development were different from previous year. Spring 2002 was favourable for winter wheat crop growth and development, but the summer months were sunny and very dry (June 37 mm, July 30 mm, August 0 mm, Dobeles Agra weather station data). In those conditions efficiency of herbicides were high in variants, where was used traditional soil cultivation with ploughing. The third year experiments were established under very dry soil conditions, caused by the dry summer 2002. There was drought period of 60 days. Under such conditions there were difficulties to do qualitative direct sowing. Cold winter with a big amplitude of temperatures negatively effected winter hardiness of the winter wheat. Hot temperatures in July and heavy rainfalls in August effected harvesting results. Those variables in the whether conditions gave wide experience of using of different soil tillage methods in different weather and soil conditions.

Three factor ANOVA, correlation and regression analysis were used for data analyse.

## Results

The averages of the three years results shows significantly highest received yields of winter wheat grown in crop rotation after winter rape (1<sup>st</sup> wheat). Herbicide use in both cases of crop rotation gave significant yield increases compare with untreated case (Fig. 1).

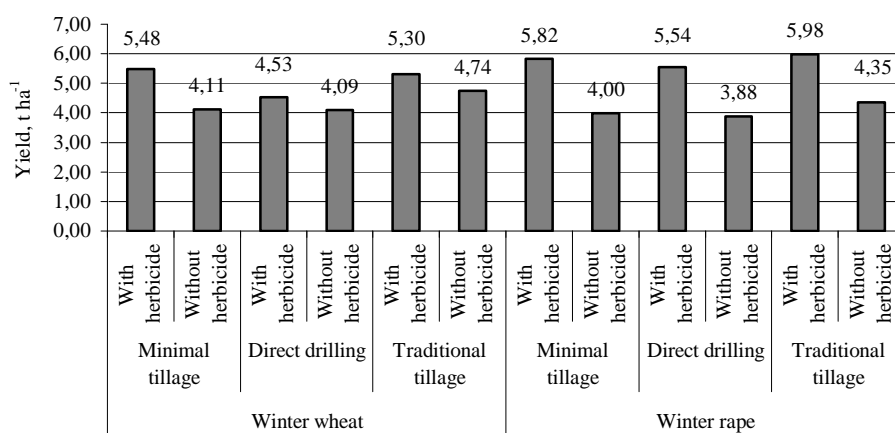


Figure 1. The yield of winter wheat depending on soil tillage and weed control technologies in the years 2001-2003:  $\gamma^A_{0.05} = 0.130$ ;  $\gamma^B_{0.05} = 0.159$ ;  $\gamma^C_{0.05} = 0.130$

In trials of 2<sup>nd</sup> wheat (wheat after wheat in crop rotation) together with herbicide use the highest yield was in variant with minimal soil tillage, which was significantly higher than yield in variants with direct drilling and traditional soil tillage.

Highest winter wheat yield in variants where herbicide was not used in case of traditional soil tillage. Yield differences with minimal tillage and direct drilling were lower than critical difference.

The highest yield of 1<sup>st</sup> wheat together with herbicide use was in the variant with traditional soil tillage, which was significantly higher than yield in the rest of the variants. The same tendencies were in the variants without herbicide use. The lowest winter wheat yield was in the variants where used direct drilling. In case of herbicide use it was significantly lower, in the case without herbicide – not significantly.

When comparing the influence of different factors on the yield of winter wheat (Fig. 2) we show that the highest impact was from herbicide use – 64.1 %.

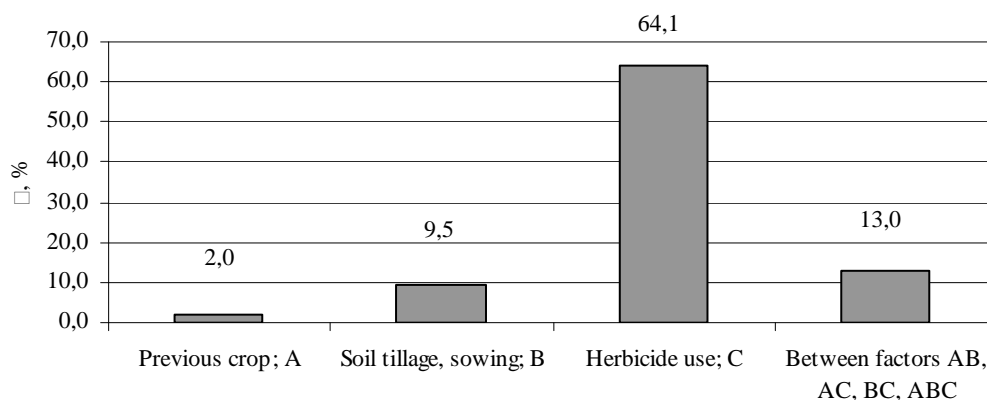


Figure 2. Impact of different factors on winter wheat yield, average 2001-2003

The impact from soil tillage was 9.5 %, but from previous crops just 2.0 %. This indicates the demand of use of different weed control methods and technologies when using different soil tillage technologies. The noxious effect of weeds has the main impact on formation of winter wheat yield. The previous statement certifies strong correlation between number of weeds per m<sup>2</sup> and winter wheat grain yield (Fig. 3).

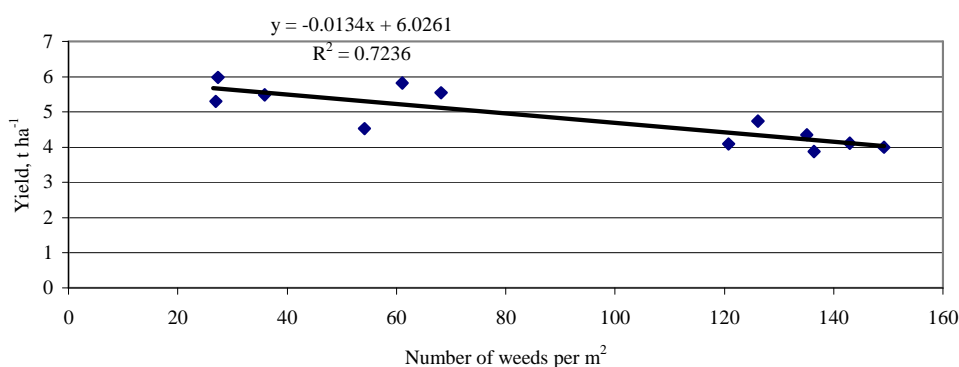


Figure 3. Coherence between the number of weeds per m<sup>2</sup> and the winter wheat yield, average 2001-2003

Analysing the impact of different factors to separate weed species we find that herbicide application has a significant effect against all dominant species. An especially high impact was observed for total weed infestation in winter wheat – 80.8 % (Table 1). The distribution of oil seed rape as weed mainly was affected by the previous crop.

Table 1 Impact of different factors to changes of the number of some weed species (average 2001-2003),  $\eta$  %

Weed species	Factors						
	Previous crop (A)	Soil tillage (B)	Herbicide application (C)	Interaction between factors			
				AB	AC	BC	ABC
Oil seed rape	37.7	10.3	6.5	11.5	3.0	0.5*	1.0*
<i>Stellaria media</i> (L.) Vill.	2.3	0.3*	41.7	1.9*	0.8*	2.5*	0.7*
<i>Sinapis arvensis</i> L.	0.0*	3.4	59.4	0.4*	0.1*	3.8	1.9
<i>Galium aparine</i> L.	0.2*	0.2*	34.2	2.7*	1.6*	4.9	0.1*
<i>Matricaria perforata</i> Merat.	0.3*	5.5	34.5	1.4*	0.5*	3.2	1.7*
<i>Lamium purpureum</i> L.	0.0*	1.5*	51.8	0.7*	0.4*	1.6*	2.3*
Others	0.0*	5.1	41.0	2.3*	0.5*	5.5	1.3*
All weed species	1.4	2.7	80.8	0.3*	0.0*	2.5	0.3*

\*-impact is not significant at 95 % probability level

We will exclude herbicide application as factor in the next calculation because it has the main affect on changes in the weed flora. Soil tillage has shown a higher impact on weed infestation in winter wheat compared to previous crop. Such coherence was observed for the total number of weeds and for *Sinapis arvensis* L., *Matricaria perforata* Merat. and other weed species what were observed in the trial. Soil tillage had a higher effect to changes of the number of *Galium aparine* L. and *Lamium purpureum* L. compared to the effect of the previous crop, but this impact was insignificant at the 95 % probability level (Table 2).

 Table 2 Impact of previous crop and soil tillage to thr number of weeds per m<sup>2</sup> (average 2001-2003),  $\eta$  %

Weed species	Previous crop (A)	Soil tillage (B)	Interaction (AB)
Oil seed rape	49.9	13.7	15.2
<i>Stellaria media</i> (L.) Vill.	7.1	1.1*	6.1*
<i>Sinapis arvensis</i> L.	0.0*	19.1	2.2*
<i>Galium aparine</i> L.	0.4*	0.6*	7.9*
<i>Matricaria perforata</i> Merat.	0.7*	11.8	3.1*
<i>Lamium purpureum</i> L.	0.1*	7.6*	3.7*
Others	0.0*	16.9	7.6*
All weed species	11.7	22.7	2.1*

\*-impact is not significant at 95 % probability level

A significantly smaller total number of weeds was observed in treatments where winter wheat was grown in recurrent sowing. Mainly it was caused by differences in the number of oil seed rape plants in these treatments – many rape seed were left on the surface of the soil in treatments with reduced soil tillage after winter rape. That mainly caused significant differences between soil tillage treatments – significant smaller number of weeds was after the traditional soil tillage with ploughing. Previous crop had significant impact to number of *Stellaria media* (L.) Will. also. A smaller amount of this weed was observed in treatments after winter rape (Table 3).

Data analysis show a significant linear negative correlation between winter wheat yield and the number of some weed species. There are no significant differences for coefficient  $b_1$  among separate weed species and total weed infestation. Coefficient  $b_0$  are significantly smaller for the total number of weeds at 95 % confidence level. That means the slope describing this coherence is more flat than others and weed species what had an insignificant correlation with winter wheat grain yield.

Table 3. Number of some weed species, p m<sup>-2</sup> (average 2001-2003)

Previous Crop (A)	Soil tillage, sowing (B)	Oil seed rape	<i>Stellaria media</i> (L.) Vill.	<i>Sinapis arvensis</i> L.	<i>Galium aparine</i> L.	<i>Matricaria perforata</i> Merat.	<i>Lamium purpureum</i> L.	Others	Total
Winter wheat (A1)	Minimal tillage (B1)	0.93	13.27	15.53	8.80	11.33	17.73	16.27	89.40
	Direct drilling (B2)	1.20	16.80	9.53	9.93	13.27	16.40	11.72	87.45
	Traditional tillage (B3)	1.53	16.20	10.78	12.77	9.33	12.27	8.80	76.55
	Average, A1	1.22	15.42	11.95	10.50	11.31	15.47	12.26	84.47
Winter rape (A2)	Minimal tillage (B1)	21.60	13.60	14.07	11.20	9.40	15.87	13.00	105.13
	Direct drilling (B2)	22.60	8.53	9.47	9.73	17.33	16.33	12.00	102.27
	Traditional tillage (B3)	4.87	12.73	12.87	8.67	10.73	15.07	11.53	81.20
	Average, A2	16.36	11.62	12.13	9.87	12.49	15.76	12.18	96.20
Average, B1		11.27	13.43	14.80	10.00	10.37	16.80	14.63	97.27
Average, B2		11.90	12.67	9.50	9.83	15.30	16.37	11.86	94.86
Average, B3		3.20	14.47	11.83	10.72	10.03	13.67	10.17	78.88
$\gamma_{0.05}^A$		2.691	3.607	2.402	2.482	3.506	2.578	2.121	7.456
$\gamma_{0.05}^B$		3.296	4.418	2.942	3.039	4.294	3.158	2.598	9.132

Equations describing significant coherences between winter wheat yield and separate weed species are the same at 95 % confidence level (Table 4).

Table 4. Equations of regression between winter wheat yield and number of some weed species

Weed species	$y = b_0x + b_1$	b <sub>0</sub> 95 % confidence interval		b <sub>1</sub> 95 % confidence interval	
		Upper limit	Lower limit	Upper limit	Lower limit
		<i>Stellaria media</i> (L.) Vill.	$y = -0.0634x + 5.676$	-0.0267	-0.1002
<i>Sinapis arvensis</i> L.	$y = -0.06x + 5.5409$	-0.0277	-0.0923	6.0410	5.0407
<i>Matricaria perforata</i> Merat.	$y = -0.0813x + 5.785$	-0.0353	-0.1273	6.4213	5.1492
<i>Lamium purpureum</i> L.	$y = -0.067x + 5.865$	-0.0303	-0.1038	6.5205	5.2093
All weed species	$y = -0.0134x + 6.026$	-0.0076	-0.0192	6.6182	5.4341

## Conclusions

A significantly smaller total number of weeds was observed in treatments where winter wheat was grown in recurrent sowing.

A significantly smaller number of weeds was observed after traditional soil tillage with ploughing. The data analysis show significant linear negative correlation between winter wheat yield and the number of total weed infestation and several weed species - *Stellaria media* (L.) Vill., *Sinapis arvensis* L., *Matricaria perforata* Merat. and *Lamium purpureum* L.

The highest impact to changes of winter wheat grain yield made herbicide use – 64.1 %.

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## AUGSNES APSTRĀDES UN NEZĀĻU KONTROLES OPTIMIZĀCIJA ZIEMAS KVIĒŠOS

Stašinskis Ē.

Neraugoties uz daudzām iespējām nezāļu ierobežošanā, to invāzija vēl joprojām ir liela problēma. Rakstā atspoguļoti trīs gadu (2001.-2003.) izmēģinājumu, kuri veikti Dobeles SIA Agra laukos, rezultāti. Izmēģinājumi iekārtoti divās atšķirīgās augu sekās (faktors A): 1) ziemas kvieši sēti pēc ziemas kviešiem, 2) ziemas kvieši sēti pēc ziemas rapša. Tika salīdzinātas trīs augsnes apstrādes un sējas metodes (faktors B): 1) minimālā augsnes apstrāde 10-15 cm dziļi ar augsnes sajušanu, 2) tiešā sēja rugainē bez augsnes iepriekšējas apstrādes, 3) tradicionālā augsnes apstrāde ar aršanu 25 cm. Papildus tika pētīta šo paņēmieni ietekme uz nezāļainību ziemas kviešos (faktors C): 1) ar herbicīda lietošanu, 2) bez herbicīda lietošanas.

Būtiski mazāks nezāļu daudzums tika konstatēts variantos ar atkārtotu kviešu augšanu un pēc tradicionālās augsnes apstrādes. Datu analīze uzrāda būtisku negatīvu lineāru korelāciju starp ziemas kviešu ražu un kopējo nezāļainību un atsevišķām nezāļu sugām- *Stellaria media* (L.) Vill., *Sinapis arvensis* L., *Matricaria perforata* Merat. un *Lamium purpureum* L.

## PHYSIOLOGICAL ASPECTS OF THE WHEAT YIELD OBTAINED FROM SEEDS TREATED WITH PHOSPHORUS

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### Abstract

The Germination of the spring wheat 'Triso' seeds treated with phosphorus and the seeds of their next generation were investigated during the vegetation experiments. The germination process of seeds treated with phosphorus decreased compared to the control seeds. The concentration of chlorophyll increased in the first leave of shoots from seeds treated with phosphorus. In the next generation of seeds treated with phosphorus, a positive post-effect on the germination quality (germination dynamics, germination energy) and the concentration of chlorophyll in the leaves was observed.) The field experiments were carried out with spring wheat 'Triso' seeds treated with phosphorus, using various fertiliser applications. The grain yield, obtained from seeds treated with phosphorus, using various fertiliser applications, increased by 2.6-102 per cent. The vegetation

experiments were carried out at the Department of Plant Physiology, Faculty of Biology of the University of Latvia, and field tests at the Scientific Agriculture Centre of Latgale in the during 2002 and 2003.

**Key words:** spring wheat, mineral nutrition, seed germination, pigments of green plastids, yield.

### Introduction

Phosphorus is essential in increasing the capacity of wheat seed yield. Phosphorus is second to nitrogen as the most limiting element for plant growth. The amount of phosphorus in plants ranges from 0.05 to 0.30 % of the total dry weight. It is a component of key molecules such as nucleic acids, phospholipids, and ATP, and, consequently, plants cannot grow without a reliable supply of this nutrient. Phosphorus is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways. Thus, although bound phosphorus is quite abundant in many soils, it is largely unavailable for uptake. Phosphorus is unavailable because it rapidly forms insoluble complexes with cations and is incorporated into organic matter by microbes. In order to have a good yield, for the phosphorus needs mineral nutrition. However, even under adequate phosphorus fertilisation, only 20 % or less of the applied phosphorus is removed by the 1<sup>st</sup> year's growth (Vance, 2001). This results in the phosphorus loading of prime agricultural land. Runoff from phosphorus loaded soils is a primary factor in the eutrophication and hypoxia of lakes and marine estuaries in the developed world. As noted by Abelson (1999), a potential phosphate crisis looms for agriculture in the 21<sup>st</sup> century. The low availability of phosphorus in the bulk soil limits plant uptake. More soluble minerals such as potassium move through the soil via bulk flow and diffusion, but phosphorus is moved mainly by diffusion. Since the rate of diffusion of phosphorus is slow ( $10^{-12}$  to  $10^{-15}$  m<sup>2</sup> s<sup>-1</sup>), high plant uptake rates create a zone around the root that is depleted of phosphorus (Schachtman *et al.*, 1998).

Therefore, in order to use phosphorus nutrition more rationally in the sowings of cultivated plants, specifically phosphorus treated seeds are used (Stramkale *et al.*, 2004). The usage of phosphorus treated seeds improves the utilisation of mineral nutrition, thus uncontaminating the environment (Gravalos *et al.*, 2000).

The aim is to determine how the phosphorus treatment of seeds influences wheat seed germination, the amount of pigments of green plastids in the shoots, and the yield.

### Materials and Methods

Spring wheat 'Triso' selected in Germany was used in the vegetation and field tests (in 2002 and 2003). The variety provides high yield with excellent quality, demonstrates good resistance to different plant diseases and has high lodging consistence.

In the tests, the seeds treated with phosphorus powdered nutrition were introduced. The nutrition is fixed to the seeds with the help of a binding agent. The patent holder for this treatment method (iSeed<sup>TM</sup>) is the Finnish company *Kemira Grow How*, but the patent holders for the binding agent are *Kemira Grow How* and *Fortum Oil and Gas*.

During the tests, the phosphorus treated seeds were used as well as the ones originating from the plants grown from seeds treated with phosphorus. This helped to determine whether the phosphorus treatment is visible also during the next generation.

The seeds of all variants were pickled with Kemikars T ( $2.5 \text{ l t}^{-1}$ ).

Vegetation tests. During the vegetation tests the seeds were germinated at + 20°C in a Petri dish. The length of experiment depended on the germination speed. Germination of the seeds was set up every 4-8 hours. The number of repetitions – 5. The number of seeds in every repetition – 50. As soon as seed lobes appeared, the Petri dish was placed under light in order to see the pigments of green plastids.

The vegetation tests determined:

- 1) The germinating power and energy of wheat seeds (Hartmann *et al.*, 1997).
- 2) Contents of the pigments of green plastids in seven days old shoots – spectrophotometrically the common pigment acetone extract, by determining the optical density of solutions (D) in light wave length, which matches chlorophyll a, chlorophyll b, and the carotenoid absorption maximum. The concentration of pigments ( $C - \text{mg l}^{-1}$ ) was calculated according to the following formulas



(Vikmane, 2002):  $C_{hla} = 9.784 D_{662} - 0.990 D_{644}$ ;  $C_{h1b} = 21.426 D_{644} - 4.650 D_{662}$ ;  $C_k = 4.695 D_{440.5} - 0.268 C_a + C_b$ .

Field tests. In the year 2002, wheat was sown on April 24 and was harvested on the 20<sup>th</sup> of August. In the year 2003, wheat was sown on May 8 and was harvested on September 6. The field tests were organised according to the method of random blocks with 4 repetitions. The total field space was  $2\text{ m} \times 10\text{ m} = 20\text{ m}^2$ . The total space of the test was  $1259\text{ m}^2$ . The soil – humus podzolic gley soil. The contents of organic substances in the soil –  $38\text{-}52\text{ g kg}^{-1}$ ,  $\text{pH}_{\text{KCl}} = 7.3$ ,  $\text{P} = 63\text{-}99\text{ mg kg}^{-1}$ , and  $\text{K} = 98\text{-}147\text{ mg kg}^{-1}$ . The pre-plant was barley. Basic fertiliser – "Kemira Grow How complex mineral nutrition 18:9:9, plant-feeder – ammonium nitrate (dosage according to the methodology). The field tests demonstrated the yield of wheat. The wheat was cropped according to the seed ripening phase by the seed combine harvester Sampo-130.

The mathematical data processing (mean unrepresentation of errors) and Figures were done by *MS Excel*.

May of 2002 was warm but dry (in the 3<sup>rd</sup> decade there was no rainfall at all). The average temperature in June was  $16.2\text{ }^\circ\text{C}$ , rainfall was from  $3.4\text{ mm}$  (in the 1<sup>st</sup> decade) to  $16.6\text{ mm}$  (in the 3<sup>rd</sup> decade). July was warm and sunny. The average temperature was  $20.1\text{ }^\circ\text{C}$  and exceeded the norm by  $3.6\text{ }^\circ\text{C}$ . The rain was insufficient – only  $38.7\%$  of the norm. Hot and dry weather favoured grain ripening. May of 2003 was warm and wet. The humidity the level influenced wheat seed germination. June was hot and dry. In July rainfall exceeded the average norm 1.4 times. During the yield harvest (in the 1<sup>st</sup> decade of August), the rain exceeded the norm 4.2 times. Unfortunately it negatively influenced the yield.

## Results and Discussion

The seed germination process is related to seed quality as well as to the influence of endogenous factors.

Germination of phosphorus treated seeds. The germination of control wheat seeds started after 22 hours, but the shoots of phosphorus treated seeds appeared after 44 hours. The wheat germination dynamics of the control seeds was 15-25 % more intensive than that of seeds treated with phosphorus (Figure 1).

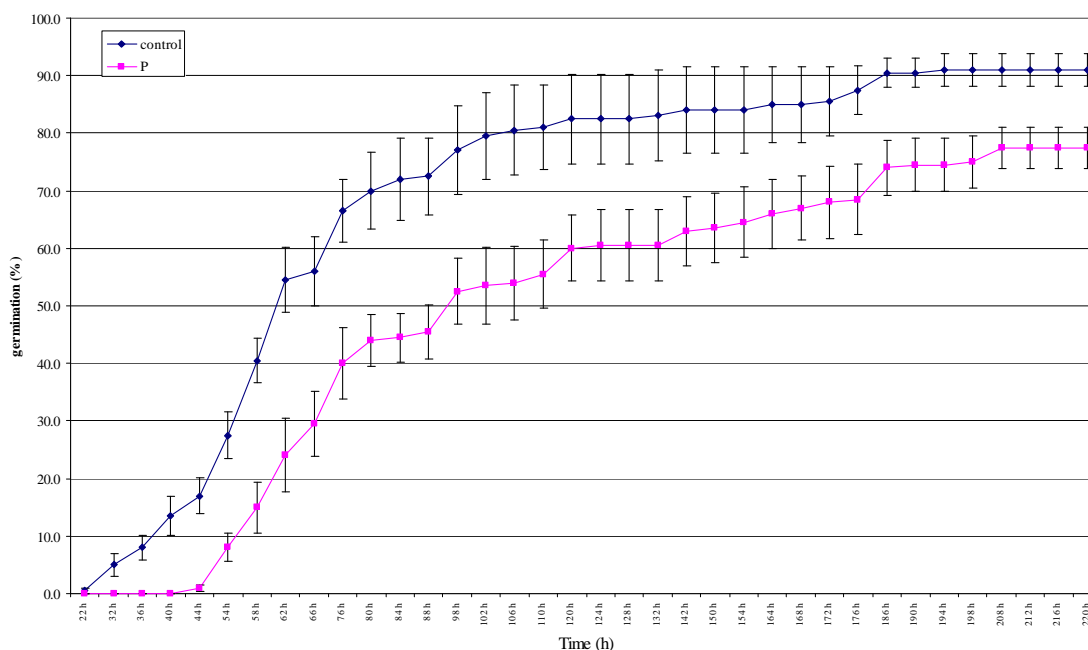


Figure 1. The germination dynamics of phosphorus treated wheat seeds

It is possible that the phosphorus concentration used for plants during the experiments surpassed the optimal. Bieleski (1973) and Ragothama (2001) indicate that the concentration gradient from the soil solution to the plant cell exceeds 2000 – fold. In the soil solution free phosphorus

concentration is on the average 1  $\mu\text{M}$ . This concentration is well below the  $K_m$  for plant uptake (Ткачук *et al.*,1991; Schachtman *et al.*; Marschner, 1999). The germination decrease of phosphorus treated seeds may be explained by the increased environmental inhibiting impact of the osmotic potential. An increased non-organic phosphorus ( $P_n$ ) concentration in the direct vicinity of the seeds may delay water intake (by an increased environmental inhibiting impact of osmotic potential that is essential in the germination process).

The results shown in Figure 2 demonstrate that phosphorus concentration in the binder capsule of treated seeds increases  $P_n$  concentration in the soil.

The germination of the second generation obtained from phosphorus treated seeds is considerably more intensive than that for the control seeds (Figure 2).

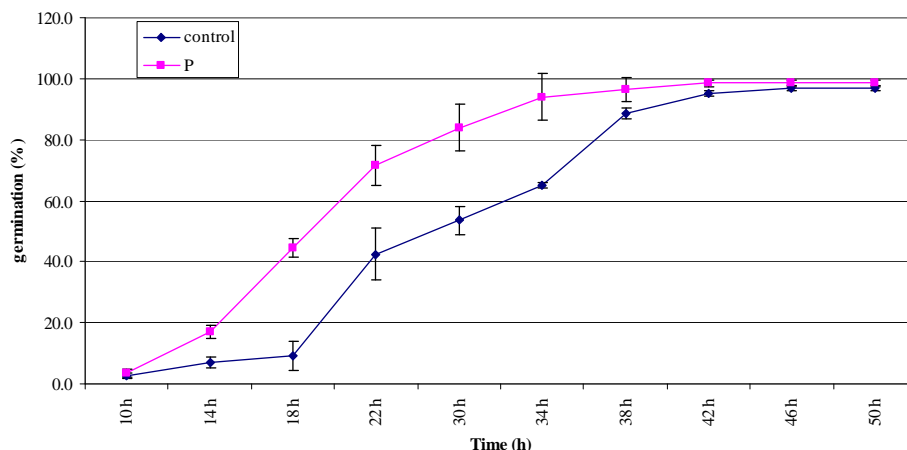


Figure 2. The germination dynamics of wheat obtained from phosphorus treated seeds.

**The germination energy** of control wheat seeds is almost twice as high as the germination energy of the phosphorus treated seeds (Figure 3). However, the germination energy of the second generation obtained from the phosphorus treated seeds is about 5 times higher than the germination energy of the control seeds (Figure 4).

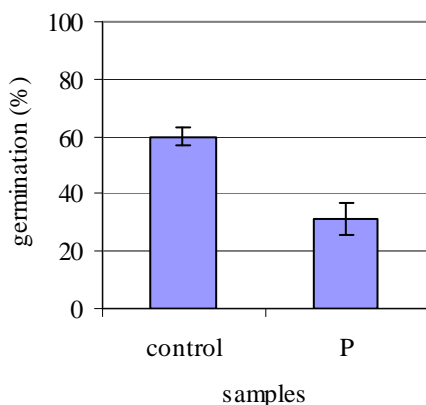


Figure 3. The germination energy of the control and phosphorus treated wheat seeds.

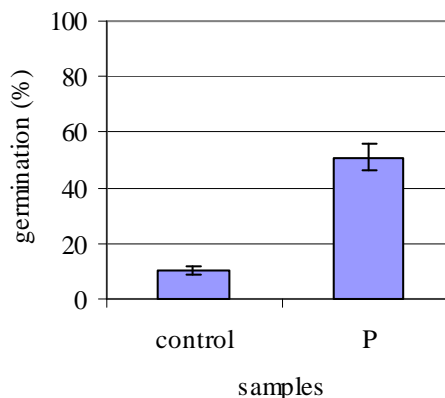


Figure 4. The germination energy of seeds obtained from phosphorus treated seeds with different fertilisation.

According to the indicators of germination power, the essential differences were not visible. Pigments of green plastids. The content of chlorophyll a and chlorophyll b is about 40 % higher in the leaves of the phosphorus treated seeds. Significant changes in the carotenoid content were not determined (Figure 5).

The content of chlorophyll a and carotenoids is higher in the shoots of the second generation than in the control shoots (Figure 6).

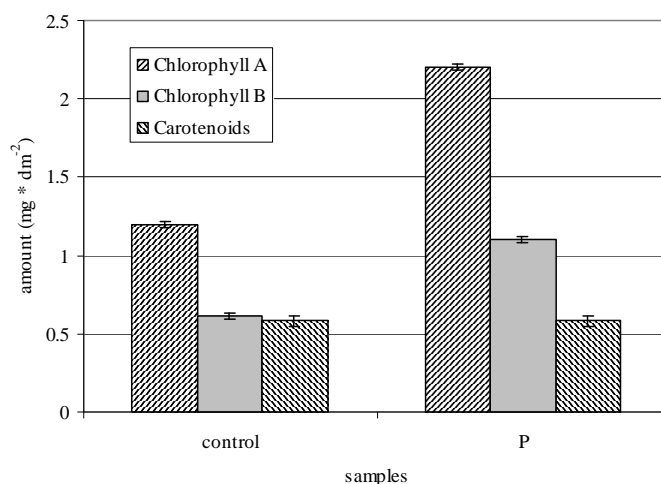


Figure 5. The total amount of pigments of green plastids in wheat leaves.

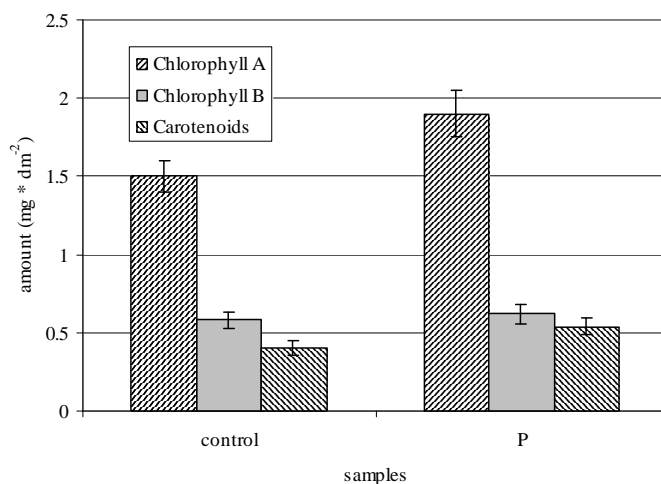


Figure 6. The total amount of pigments of green plastids in wheat leaves of the second generation of wheat obtained from phosphorus treated seeds.

As phosphorus is directly or indirectly related to the synthesis process of the pigments of green plastids, wheat seed treatment with phosphorus has positively influenced the biosynthesis of the pigments, as well as during its further development it has stimulated the formation of types of grain that increase the synthesis potential of these pigments.

The literature indicates (Махроносов, 1981; Brenner, Cheikh, 1995; Malkin, Niyogi, 2000) a positive correlation between photosynthesis and the concentration of chlorophyll in the leaves. It may be that treatment of wheat seeds with phosphorus has positively influenced the plant photosynthesis and energy, as well as that plastic substances are more generated by these plants.

Yield analyses of wheat. The field tests demonstrated that when the wheat 'Triso' was fertilised with different nitrogen, phosphorus and potassium fertiliser, the plants germinated and grew better if the seeds were treated with phosphorus. The yield analyses testified that the yield of wheat seeds increased by 2.6-102 % when the seeds were treated with phosphorus and when nitrogen, phosphorus and potassium fertilisers were applied compared to the control test (Table 1). The greatest yield of the wheat seed 'Triso' (7.78 t ha<sup>-1</sup>) was obtained from the plants grown from the

phosphorus treated seeds and using the mineral nutrition N 120 (N<sub>45</sub> 18:9:9 -250 kg ha<sup>-1</sup> + N<sub>75</sub> - 220 kg ha<sup>-1</sup> ammonium nitrate + KCl).

Table 1. The yield of wheat 'Triso' in 2002 and 2003.

Fertilizer on Variants	Yield, t ha <sup>-1</sup>	
	2002	2003
1. control – without fertilizer	4.18	3.85
2. control + P seeds (phosphorus treated seeds (iSeeds™))	4.29	4.02
3. N <sub>120</sub> (N <sub>90</sub> 18:9:9 – 500 kg ha <sup>-1</sup> + N <sub>30</sub> – 88 kg ha <sup>-1</sup> ammonium nitrate)	4.37	6.71
4. N <sub>120</sub> (N <sub>90</sub> 18:9:9 – 500 kg ha <sup>-1</sup> + N <sub>30</sub> – 88 kg ha <sup>-1</sup> ammonium nitrate) + P seeds	4.68	7.76
5. N <sub>120</sub> (N <sub>45</sub> 18:9:9 – 250 kg ha <sup>-1</sup> + N <sub>75</sub> – 220 kg ha <sup>-1</sup> ammonium nitrate + KCl)	4.76	6.87
6. N <sub>120</sub> (N <sub>45</sub> 18:9:9 – 250 kg ha <sup>-1</sup> + N <sub>75</sub> – 220 kg ha <sup>-1</sup> ammonium nitrate + KCl) + P seeds	4.97	7.78
$\gamma_{0.05}$	0.18	0.20

When comparing the yield of wheat in 2002 and 2003, for the control variants it was greater in 2002 compared to 2003, when meteorological conditions were unfavourable for plant growth and development. Nitrogen, phosphorus and potassium fertilisers had a much greater effect on the grain yield in 2003 compared to 2002, when there was an insufficient amount of rain.

Estimates of reliability deviations of the experimental results (or results of mathematical processing) demonstrate that increase in wheat grain yield is essential, except for the second case, i.e., phosphorus treated seeds without fertiliser.

As phosphorus plays an essential role in all the physiological and biochemical processes of the plant (Hartmann, 1997; Marschner, 1999; Gilroy, Jones, 2000; Abel, 2002), the results of the tests confirm the importance of phosphorus. Phosphorus usage in the seed treatment has ensured its better absorption and involvement in the wheat metabolism processes. It increased yield of the seeds of the plants that were grown from the phosphorus treated seeds.

### Conclusions

The seed germination characteristics of spring wheat 'Triso' treated with phosphorus are the following. Seed germination dynamics and germination power decreases after treatment with phosphorus and phosphorus influences the increase of chlorophyll in the shoots.

The treatment of seeds with phosphorus positively effects the physiological activity of the next generation of seeds: increases seed germination dynamics and germination power and there is a greater content of chlorophyll and carotenoids in the shoots.

The yield of wheat seeds increases by 2.6-102 %, when the seeds are treated with phosphorus, and when different nitrogen, phosphorus and potassium fertilisers are used.

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## AR FOSFORU APSTRĀDĀTU KVIEŠU SĒKLU RAŽAS FIZIOLOĢISKIE ASPEKTI

**Stramkale V., Vikmane M., Zeļonka L.**

Augsnē fosfors lielākoties ir augiem grūti pieejamā formā. Tāpēc kultūraugu sējumos lieto īpašas ar fosforu apstrādātas sēklas, Darba mērķis bija noskaidrot, kā miežu sēklu apstrāde ar fosforu ietekmē to dīgšanu, fotosintēzes pigmentu saturu lapās un graudu ražu; kā arī to, kāda ir sēklu apstrādes pēctiekme uz nākamās paaudzes sēklu dīgšanu. Laboratorijas izmēģinājumos, nosakot vasaras kviešu šķirnes 'Triso' sēklu dīgšanu procentos un pigmentu saturu dīgstos spektrofotometriski, konstatēja, ka sēklu apstrāde ar fosforu kavēja sēklu dīgšanu un samazināja dīgšanas enerģiju, bet hlorofila daudzums dīgstos fosfora ietekmē palielinājās. Sēklu apstrāde ar fosforu pozitīvi ietekmēja nākamās paaudzes sēklu fizioloģisko aktivitāti (sēklu dīgšanas dinamiku, dīgšanas enerģiju un hlorofila saturu dīgļlapās). Kviešu graudu raža, kas iegūta, izmantojot ar fosforu apstrādātas sēklas un lietojot minerālmēslojumu, ir palielinājusies par 2.6 – 102%. Var secināt, ka ar fosforu apstrādāto kviešu sēklu izmantošana ir efektīva: palielinās sēklu fizioloģiskā aktivitāte un graudu raža, tiek uzlabota fosfora izmantošana un notiek vides saudzēšana.

## EXPANSION OF WINTER CROP PROPORTIONS IN ROTATION STRUCTURE: EFFICIENCY AND SUSTAINABILITY

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### Abstract

Investigations to increase the efficiency and sustainability of soil management were carried out on a clay loam *Gleyic Cambisol* at the Joniskelis Experimental Station of the Lithuanian Institute of Agriculture over the period of 1998-2002. The following was investigated: A. Crop rotation with different proportion of winter and spring crops (1. Without winter crops; 2. 25% of winter crops; 3. 50% of winter crops; 4. 75% of winter crops; 5. 100% of winter crops) growing annual and perennial grasses, spring and winter wheat, triticale, and barley; B. Soil tillage systems (1. Conventional – ploughing; 2. Sustainable – ploughing after grasses, ploughless tillage after cereals). The research shows that increasing the winter crop proportion in the crop rotation structure resulted in the reduction of the soil compaction level, the maintenance of higher productive moisture reserves, an improved water to air ratio and humification process in soil, reduction of perennial weeds, and the increase of crop rotation productivity. The application of reduced tillage in a sustainable system determined the reduction of organic substance mineralization and increased the amount of humus in the soil. Winter cereals were more tolerant to reduced soil tillage compared with spring cereals.

**Key words:** clayey *Cambisol*, winter crops, soil tillage, soil physical condition, weeds, crop yield

## Introduction

In contemporary agriculture the soil must be resistant to all degradation factors, and the soil properties must meet the requirements of sophisticated, input-saving and sustainable crop cultivation technologies. Therefore it is vital to improve the soils physical properties, apply proper soil tillage methods, and choose the best-suited crops and their preceding crops and other soil and crop management practices that determine cultivation conditions for the crops grown.

The main type of heavy soils' physical degradation is compaction. The physical degradation of soils considerably deteriorates their physical and technological properties. Clay loam and clay soils, noted for higher susceptibility to compaction, tend to densify more when spring and especially row crops are grown (Maiksteniene, 1997; Horn *et al.*, 2000; Schafer-Landefeld *et al.*, 2004). In soils susceptible to compaction it is recommended to grow more winter crops whose soil preparation, sowing and harvesting operations are usually performed in the dry period, since dry soils are more resistant to compaction. When a field is covered by plants for a longer period, the soil properties are less deteriorated by climatic factors (Etana *et al.*, 1999; Dabney *et al.*, 2001; Palojarvi, Nuutinen, 2002; Mueller *et al.*, 2003). One of the main soil quality indexes, which show its resistance to physical and biological degradation, is the amount of organic matter in the soil (Kristensen *et al.*, 2003; Loveland and Web, 2003). Clayey soil becomes resistant to compaction and suitable for reduced tillage, when the amount of humus exceeds 50 g kg<sup>-1</sup> in the topsoil (Balesdent *et al.*, 2000). The amount of humus in the most of heavy soils in Lithuania doesn't reach 30 g kg<sup>-1</sup>; consequently there is a tendency forward degradation (Lithuanian soils, 2001).

Heavy soils are characterized by a high comparative resistance to mechanical tillage and much energy input is required for this. Energy input for soil tillage is markedly reduced by replacement of ploughing with ploughless tillage (Maiksteniene, 1997; Ausmane *et al.*, 2004). Most authors maintain that ploughless tillage results in improved physical, agrochemical and biological properties of the upper topsoil layer, however, the soil properties tend to deteriorate at the bottom of the topsoil (Etana *et al.*, 1999; Rasmussen, 1999, Ulrich *et al.*, 2006).

The objectives of the current research are to study the expansion of the winter crop proportion in crop rotations under conditions of conventional and reduced soil tillage and to assess the results relative to efficient and sustainable soil management.

## Materials and Methods

Soil and site. Complex research was conducted at the Lithuanian Institute of Agriculture's Joniskelis Experimental Station during the period 1998–2002 on drained clay loam on silty clay with deeper lying sandy loam *Endocalcari-Endohypogleyic Cambisol*, whose parental rock is glacial lacustrine clay. Clay particles <0.002 mm in A<sub>a</sub> horizon (0–30 cm) made up 270.0 g kg<sup>-1</sup>, in B<sub>1</sub> (52–76 cm) – 516.0 g kg<sup>-1</sup>, in C<sub>1</sub> (77–105 cm) – 107.0 g kg<sup>-1</sup> and in C<sub>2</sub> (106–135 cm) – 110.0 g kg<sup>-1</sup>. Topsoil (0–25 cm) according to pH<sub>KCl</sub> is neutral, humus content of 22.0 g kg<sup>-1</sup>.

The experiments were done according to the following design:

Factor A. Crop rotations with different proportion of winter and spring crops: 1. Without winter crops (1). Vetch (*Vicia sativa* L.) and oats (*Avena sativa* L.); (2). Spring wheat (*Triticum aestivum* L.); (3). Spring triticale (*xTriticosecale* Wittm.); (4). Spring barley (*Hordeum vulgare* L.). 2. 25% winter crops (1). Red clover (*Trifolium pratense* L.) and timothy (*Phleum pratense* L.); (2). Spring wheat; (3). Spring triticale; (4). Spring barley). 3. 50% winter crops (1). Red clover and timothy; (2). Winter wheat (*Triticum aestivum* Host.); (3). Spring triticale; (4). Spring barley). 4. 75% winter crops (1). Red clover and timothy; (2). Winter wheat; (3). Winter triticale (*xTriticosecale* Wittm.); (4). Spring barley). 5. 100% winter crops (1). Red clover and timothy; (2). Winter wheat; (3). Winter triticale; (4). Winter barley (*Hordeum vulgare* L.).

Factor B. Soil tillage systems: 1. Conventional (ploughing). 2. Sustainable (ploughing after grasses for wheat, ploughless - after all cereals). All crops were grown every year in 4 replicates. Herbicides of select were applied on the cereals. The soil was ploughed at 23–25 cm depth and in the case of ploughless tillage - loosened by universal stubble breaker at the same depth.

*Measurements and assessments.* Each year the following were estimated: 1) soil bulk density (Kachinski method); 2) air-filled porosity (according to Dolgov), (Rastvorova, 1983); 3) soil moisture content (weighing method) in the 0–15, 15–25 cm layers; 4) total and productive (available to plants) moisture reserves (according to Dolgov); humus content and composition

(Tjurin method modified by Ponomareva and Plotnikova (1980), weed population (species composition, density and biomass), and the yield of all crops. The effects of the tested means on the compaction of clay loam topsoil were assessed as follows: 1) low compaction with the mean topsoil equilibrium bulk density –  $<1.3 \text{ Mg m}^{-3}$ , 2) moderate compaction –  $1.3\text{--}1.5 \text{ Mg m}^{-3}$ , 3) high compaction –  $>1.5 \text{ Mg m}^{-3}$  (Bondarev, 1990). The physical maturity of clay loam soil, when it is most suitable for tillage, occurred at  $175.0 \text{ g kg}^{-1}$  of moisture (Maiksteniene, 1997). The experimental data were processed by ANOVA and STATENG (\* – 95% probability level).

The growing seasons in 1998 and 2001 were characterized by an excess moisture, whereas the years 1999, 2000 and 2002 were droughty.

## Results and Discussion

**Soil physical condition.** We estimated soil bulk density as a major indicator of physical state of the soil and the possible effects of the investigated factors on it. In the first year of the investigation (1999) the soil bulk density in the upper (0–15 cm) topsoil layer in the crop rotations with 75 and 100% winter crops was higher by 6.9 and 5.6%, respectively than in the crop rotation grown with only spring crops, corresponded to a high compaction (Table 1). When the increase of the winter crop proportion in the rotations soil bulk density at the end of the rotation (2002) consistently declined at the bottom (15–25 cm) of the topsoil. Here the soil bulk density in the crop rotations with 100, 75 and 50% winter crops was lower by 5.8, 4.5 and 4.5%, respectively compared with spring crop rotation. Furthermore it corresponded to moderate compaction.

Table 1. Effect of winter crops and soil tillage on soil compaction indices

Treatment	Depth cm	Beginning of rotation (1999)		End of rotation (2002)	
		soil bulk density, $\text{Mg m}^{-3}$	compaction level	soil bulk density, $\text{Mg m}^{-3}$	compaction level
Proportion of winter crops % (factor A)					
0	0-15	1.44	moderate	1.42	moderate
	15-25	1.57	high	1.56	high
25	0-15	1.46	moderate	1.44	moderate
	15-25	1.58	high	1.54	high
50	0-15	1.50	moderate	1.42	moderate
	15-25	1.56	high	1.49	moderate
75	0-15	1.54	high	1.43	moderate
	15-25	1.57	high	1.49	moderate
100	0-15	1.52	high	1.44	moderate
	15-25	1.57	high	1.47	moderate
Soil tillage systems (factor B)					
1. Conventional	0-15	1.49	moderate	1.42	moderate
	15-25	1.56	high	1.50	moderate
2. Sustainable	0-15	1.49	moderate	1.44	moderate
	15-25	1.58	high	1.52	high
LSD <sub>05</sub>		A	B	A	B
	0-15	0.062	0.050	0.062	0.051
	15-25	0.073	0.059	0.068	0.056

The increasing of the winter crop proportion in crop rotation created a better persistence of soil moisture and more abundant reserves of total and productive moisture; however the soil air-field porosity in all topsoil declined in most cases in the second half of the crop growing season. In the rotation with 100 % winter crops the reserves of productive moisture in the topsoil were 37.3% higher, compared with only spring crops rotation (18.4 mm) (data not shown). While applying reduced soil tillage we observed a reduction in soil air-filled porosity in the topsoil. In the upper topsoil layer this phenomenon exhibited a slight decline, and in the bottom layer it declined by 8.0%, compared with conventional tillage (air-filled porosity – 20.1 v/v, %) (data not shown).

**Humus indexes.** Averaged data, having increased the proportion of winter crop in the rotation till 100% total content of humic acids, degree of humification and amount of humus in the soil basically increased in comparison with the version without winter crop (Table 2). Applying the

system of sustainable soil tillage these indexes also basically increased in comparison with the conventional soil tillage system.

Table 2. Effect of winter crops and soil tillage on humus indicators in topsoil (0–25 cm) 1999–2002 averaged data

Proportion of winter crops % (Factor A)	Soil tillage systems (Factor B)						Average of factor A		
	1. conventional			2. sustainable			humic acids (total) g kg <sup>-1</sup>	humus content g kg <sup>-1</sup>	humifi-cation degree %
	humic acids (total) g kg <sup>-1</sup>	humus content g kg <sup>-1</sup>	humifi-cation degree %	humic acids (total) g kg <sup>-1</sup>	humus content g kg <sup>-1</sup>	humifi-cation degree %			
0	3.59	20.3	30.1	3.88	21.5	30.8	3.74	20.9	30.5
25	3.59	20.5	30.2	3.88	21.5	31.0	3.74	21.0	30.6
50	3.63	20.6	30.4	3.89	21.6	31.1	3.76	21.1	30.8
75	3.73	20.9	30.7	3.96	21.8	31.2	3.85	21.4	31.0
100	3.85	20.9	31.7	4.07	22.1	31.8	3.96	21.5	31.8
Average of factor B	3.68	20.6	30.6	3.94	21.7	31.2			
LSD <sub>05</sub> A	0.180	0.55	1.10						
LSD <sub>05</sub> B	0.070	0.21	0.40						
LSD <sub>05</sub> AB	0.260	0.80	1.60						

Weed infestation in cereals. At the tilling stage of the spring cereals (wheat, triticale and barley) in the final investigation year (2002), the annuals (*Thlapsi arvense* L. – 17.5%, *Fumaria officinalis* L. – 16.3%, *Galium aparine* L. – 14.6%, *Chenopodium album* L. – 12.3%) were as prevailing weeds in crops; perennial weeds accounted for an average of 14.7% of the total amount (102 weed m<sup>-2</sup>). In winter cereals (wheat, triticale and barley) more annual weeds prevailed: (*Stellaria media* (L.) Will. – 36.0%, *Veronica arvensis* L. – 10.9%, *Viola arvensis* Murray – 8.3%, *Galium aparine* L. – 7.7%; perennial weeds: *Cirsium arvense* (L.) Scop., *Sonchus arvensis* L., *Elytrigia repens* (L.) were less prevalent (6.9%). The smallest (22.8 g m<sup>-2</sup>) weed air-dried biomass in the cereals was in the crop rotation with 75% of winter crops. With reduced soil tillage there were 2.1 times more perennial weeds, but the air-dried biomass of weeds was 42.3% larger, compared with conventional tillage (average during four year period – 24.8 g m<sup>-2</sup>).

Productivity of crop rotations. When increasing the winter crop proportion in the rotation, the total average productivity according to metabolizable energy accumulated of all crops increased, and in the crop rotation with 100% winter crops the increase was by 33.2% higher than in the crop rotation composed of solely spring crops (Table 3).

Table 3. Effect of soil tillage and winter crops proportion on the productivity of rotations according to the metabolizable energy accumulated of all crops and cereal grain yield. Average data of 1999–2002.

Proportion of winter crops % (Factor A)	Soil tillage systems (factor B)				Average of factor A	
	1. conventional		2. sustainable		metaboli-zable energy, GJ ha <sup>-1</sup>	cereal grain, t ha <sup>-1</sup>
	metaboli-zable energy, GJ ha <sup>-1</sup>	cereal grain, t ha <sup>-1</sup>	metaboli-zable energy, GJ ha <sup>-1</sup>	cereal grain, t ha <sup>-1</sup>		
0	46.88	3.66	42.04	3.31	44.46	3.49
25	54.34	3.94	49.78	3.68	50.06	3.81
50	56.01	4.07	53.02	3.87	54.51	3.97
75	58.83	4.36	56.75	4.16	57.79	4.26
100	60.06	4.41	58.36	4.14	59.21	4.28
Average of factor B	55.22	4.09	51.99	3.83		
LSD <sub>05</sub>	A	B	AB			
Metabolizable energy	6.365	4.025	9.002			
Cereal grain	0.403	0.255	0.570			



The expansion of winter crops gave a higher increase in grass yield compared with cereals. Moreover, when increasing winter crops the differences in the crop productivity between sustainable and conventional soil tillage systems declined, and in the crop rotations with 100% winter crops it was as low as 2.8%. Soil tillage did not exert any significant effect on average crop productivity (during the whole 4-year rotation).

With an increasing winter crop proportion in the rotations the total average cereal grain yield consistently increased, however, in the crop rotations with 75 and 100% winter crops it did not differ significantly. When growing cereals according to the sustainable soil tillage system, the total average grain yield per rotation was 6.4% lower, compared with the conventional technology.

Soil compaction can often be prevented or alleviated by the proper choice of cropping systems (Boizard *et al.*, 2002; Riley *et al.*, 2006). The effects of crop rotation with different proportion of winter (wintering) crops on the physical condition of clayey are not well known. Our slows down to decline the harmful technological effect on clayey soil and to reduce its physical degradation. While comparing the data of topsoil bulk density and its correspondence to the compaction level at the end and beginning of the rotation, we can see that in the crop rotations without and with 25% winter crops these indicators were very similar, and when increasing winter crops to 50, 75 and 100% the level compaction declined from high to moderate. In the sustainable soil tillage system due to the application of reduced tillage a high compaction still persisted at the bottom of the topsoil.

The possibilities to maintain adequate moisture parameters in heavy soils are of special relevance both for crop emergence and the establishment of technological measures – soil tillage and the performance of other agricultural practices (Mueller *et al.*, 2003). Our experimental data revealed a moderate and inverse relationship between the productive moisture reserves at the bottom of the topsoil and soil bulk density ( $r = -0.516^*$ ,  $y = 47.475 - 26,506x$ ). Consequently, the soil productive moisture reserves in the topsoil layer ( $r = 0.753^*$ ,  $y = 1.174 + 0.215x$ ) and subsoil layer ( $r = 0.703^*$ ,  $y = 0.791 + 0.556x$ ) directly influence crop yield. It is noteworthy that prior to soil tillage in the spring the moisture content at the bottom of the topsoil, unlike that of the upper layer, on clay loam soil exceeded by 7.1–9.4% physical maturity ( $175.0 \text{ g kg}^{-1}$ ). Therefore the threat for the over compaction of this layer during the pre-sowing soil tillage and sowing remains as long as soil protection requirements are disregarded in the technologies used.

On heavy soils it is vital to maintain proper moisture to air ratio in order to achieve satisfactory plant growth. Experimental evidence suggests that on clay loam soil the best conditions are created when moisture occupies 60% and air 40% of the pores (Maiksteniene, 1997). Therefore for the estimation of the tested means, optimal moisture to air ratio was considered to be 1.5:1. When increasing the proportion of winter crops in rotation, moisture to air ratio consistently improved in the topsoil. The sustainable soil tillage system assured a slightly more favorable moisture to air ratio at the bottom of the topsoil, compared with conventional tillage.

One of the most important aims in sustainable soil management a stable supplement of organic matter in the soil and a balanced transformation of them to humus (Loveland and Web, 2003). The results showed that increasing the proportion of winter crops in the crop structure is beneficial as a biological factor in heavy soil. The systematic growing of the winter crop in the crop rotation in combination with the sustainable soil tillage increase more effectively the accumulation of organic matter and humic substances in the soil and improve the quality parameters of humic substances.

When choosing the structure of crops it is always necessary to evaluate the competition of plants in agrocenoses. The influence of soil tillage on weed incidence is affected by soil conditions, the biological features of crops and other factors (Hatcher *et al.*, 2003). When evaluating the effect of winter crops one could say, that increasing part of them to 75%, as those that have a better choking ability, allowed for the diminishment of the damage done by weeds on crops, especially because of smaller spread of perennial and other harmful weeds. The reduced soil tillage is better for the winter cereals that have better choking power against weeds, than to the spring ones.

Winter crops grow longer period of time and have a stronger root system, make much better use of the potential fertility of the soil and suffer less from extreme climate, than spring crops (Maiksteniene, 1997). Our research obviously corroborates the effectiveness of such influence in

heavy soils. The findings also show that winter cereals on clay loam soils adapted better reduced soil tillage compared with spring cereals.

## Conclusions

Increasing the winter crop proportion in the crop rotation structure resulted in a reduction of the clay loam soil compaction level, the creation of higher productive moisture reserves and an improvement in the water to air ratio and humification process. When growing from 50 to 100% of winter crops during a four-year period it is possible to reduce topsoil compaction from high to moderate. The application of sustainable system with reduced ploughless soil tillage determined the higher compaction in the topsoil and reduced of soil air-filled porosity, however, the moisture to air ratio remained favorable and the amount of humus increased, compared with conventional ploughing.

When increasing the proportion of winter crops in the rotation, annual weeds are growing more in the cereals than the perennial ones. Crop rotations with prevailing winter crops became most effective when choking more damageable and especially perennial weeds. Reduced soil tillage caused a marked outspread of perennial weeds in the cereals and an increase of weed biomass, in comparison with conventional soil tillage. The spreading of perennial weeds, concerning reduced soil tillage, was smaller in winter cereals than in spring cereals.

Increasing winter crops increased the productivity of crop rotation. As a result, the proportion of winter crops in the rotation structure on clay loam soils can be expanded by up to 75–100%. Winter cereals were more tolerant to reduced soil tillage compared with spring cereals.

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## **ZIEMĀJU LABĪBU PIESĀTINĀJUMS AUGU SEKAS STRUKTŪRĀ: EFEKTIVITĀTE UN ILGTSPĒJĪBA**

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Lietuvas Zemkopības institūta Joniškēļu izmēģinājumu stacijā laika periodā no 1998.-2002. gadam māla augsnēs *Gleyic Cambisol* tika veikti sekojoši pētījumi: A. Augu sekas ar dažādām ziemāju un vasarāju kultūru proporcijām (1. Bez ziemāju kultūraugiem; 2. 25% ziemāju kultūraugu; 3. 50% ziemāju kultūraugu; 4. 75% ziemāju kultūraugu; 5. 100% ziemāju kultūraugu), audzējot viengadīgos un daudzgadīgos zālaugus, vasaras un ziemas kviešus, tritikāli un miežus; B. Augsnes apstrādes sistēmas (1. Konvencionālā – aršana; 2. Ilgtspējīgā – aršana pēc zālaugiem, bezaršanas apstrāde pēc labībām). Pētījuma rezultāti parādīja, ka ziemāju labību proporcijas palielināšana augu sekas struktūrā samazina augsnes sablīvēšanās pakāpi, uztur produktīvākas mitruma rezerves, uzlabo ūdens-gaisa attiecību un humifikācijas procesu augsnē, samazina daudzgadīgo nezāļu daudzumu un paaugstina augu sekas produktivitāti. Samazinātas apstrādes izmantošana ilgtspējīgajā sistēmā noteica organisko vielu mineralizācijas pazemināšanos un humusa daudzuma palielināšanos augsnē. Salīdzinot ar vasarāju labībām, ziemāju labības bija tolerantākas pret samazinātu augsnes apstrādi.

## **COMPARISON OF METHODS FOR THE DETERMINATION OF PHOSPHORUS IN CARBONATIC SOILS**

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### **Abstract**

Plant available phosphorus determination by the Egner–Riehm (DL) method (LV ST ZM 82-97) is provided for use in the agrochemical research of sod-podzolic and other soils. However, the method is not suited for characterizing resources of the available phosphorus in carbonatic soils. The research goal was to develop methods for estimating the available phosphorus supply in calcareous soils. By the standard method (Egner-Riehm) the obtained results of phosphorus content was compared with those presented by the Olsen and Mehlich-3 method. The analysis of correlation and regression were employed for comparing the data of available phosphorus content in soil samples determined by different methods, as well as t-test was used for determining phosphorus content change. When analysing Paired Samples T Test (n=145) the most significant correlation ( $r=0.95$ ) was determined between the amounts of phosphorus obtained by the Egner-Riehm and Olsen methods. Changes in the results obtained by the Egner-Riehm and Olsen methods revealed that in alkaline (pH.7.5) soils with  $\text{CaCO}_3$  content above 5%, a certain amount of available phosphorus does not pass into calcium lactate extraction. The Olsen method is the most suited method for phosphorus extraction in carbonatic soils using  $\text{NaHCO}_3$  as an extracting agent.

**Key words:** available phosphorus, Olsen, Mehlich-3, Egner-Riehm

### **Introduction**

Zemgale region, the “granary” of Latvia, is called so mainly because of the fertile carbonatic (sod-calcareous soils, gley soils), suitable for obtaining high yields. In order to maintain and improve the fertility of these soils, it is important to estimate correctly their provision with plant available nutrient resources. The necessary information is provided by the data of soil analysis which enable one to set the

rates of fertilization on economic and ecological grounds. In calcareous soils particularly important is the physically-chemical and chemical immobilization and the mobilization of phosphorus. Conversion of phosphates into the plant unavailable form takes place both on the surface of carbonates and in the soil solution, where the concentration of calcium ions is comparatively high, as well as in the adsorption complex of the soil (Braschi *et al.*, 2003). The binding of the available phosphorus to plants into insoluble compounds is influenced by the soil reaction, the content of organic matter and the clay in the soil, the presence of iron compounds and moisture (Holford and Mattingly, 1975; Castro and Torrent, 1995; Carreira and Lajtha, 1997; Samadi and Gilkes, 1999; Zhou and Li, 2001). Up to now in the agrochemical research carried out in our country plant available phosphorus determination in soils was carried out by the Egner-Riehm (DL) method where calcium lactate extraction (pH 3.5 – 3.7) was used as the phosphate extracting agent. This standard method (LV ST ZM 82-97) is used for the agrochemical research of sod-podzolic and other soils. Although, as experience and research in other countries proves, this particular method is not suitable for the characterization of plant available phosphorus resources in carbonates containing soils. Carrying out the analysis by DL method carbonates and other alkaline compounds neutralize hydrochloric acid of the extracting agent by reducing its ability to dissolve potential plant available phosphates, consequently false information is obtained about the rate of phosphorus provided to plants (Zbiral, 2000). To determine the available phosphorus in carbonatic soils the most common is the Olsen method (Olsen *et al.*, 1954; Watanabe and Olsen, 1965), where a solution of 0.5 M sodium hydrogencarbonate (pH 8.5) is used as the extracting agent. The ions  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$  and  $\text{OH}^-$  of the extracting agents reduce the amount of  $\text{Ca}^{2+}$  ions in the solution by sediment of  $\text{CaCO}_3$ , as well as the ions  $\text{Al}^{3+}$  and  $\text{Fe}^{3+}$  - in the form of hydroxides, thus increasing the content of unbind phosphates in the solution. Another suitable extracting agent for such soils is ammonium hydrogencarbonate, which is the base of movable phosphorus determination in carbonatic soils according to Machigin (ГОСТ 26205 – 84). Ammonium carbonate together with DTPA (diethylenetriaminopentacetic acid) in several western states of the USA is used not only for plant available phosphorus determination, but also for the simultaneous determination of several other plants nutritive macro- and microelements (Soltanpour and Schwab, 1977). Mehlich-3 method ((Mehlich, 1984) is based on plant available compounds extraction in weak acid solutions (0.2 M  $\text{CH}_3\text{COOH}$  and 0.013 M  $\text{HNO}_3$ ), to which ammonium fluoride  $\text{NH}_4\text{F}$ , ammonium nitrate  $\text{NH}_4\text{NO}_3$  and ethylene diamine tetraacetic acid (EDTA) are added. It is known that this extraction possesses a better buffer ability, it is less neutralized by the carbonates of the soil and thus the results of this analysis better characterize the relationship with the plant available phosphorus amount of carbonatic soils (Tran *et al.*, 1990; Mallarino, 1997; Pierzynski, 2000). However the phosphorus determination results obtained by this method in the presence of carbonates frequently are considerably higher (Mallarino and Sawyer, 1999). Application possibilities of Mehlich-3 method were tested recently in many European countries including Latvia by comparing them with the existing standard methods for the determination of plant available macro- and microelements (Zbiral, 2000; Fotyma and Shepherd, 2000; Loide *et al.*, 2005; Timbare *et al.*, 2006).

Carbonatic soils comprise only a small part of the areas used for agriculture, therefore up to now when carrying out agrochemical research, they were not considered as a specific group and diverse methods of plant available phosphorus extraction was not applied to them. The aim of the research, on the basis of the Soil and Plant Research Institute crop rotation stationary archive collection of farmland soil monitoring and soil standard profile samples, was to establish the most suitable extraction method for the estimation of plant available phosphorus in carbonates-containing soils.

## Materials and Methods

There are 145 soil samples from Latvia University of Agriculture Soil and Plant Research Institute crop rotation stationary for different rate treatments of phosphorus fertilizers, the archive collection of farmland soil monitoring and soil standard profile samples with soil reaction  $\text{pH}_{\text{KCl}}$  6.05 – 8.67, carbonate content from 0.08 to 18.2 %, organic matter content from 0.7 to 95.5  $\text{g kg}^{-1}$ , with clay fraction content from 1.86 to 44.1 %, used in the research. The plant available phosphorus content of all samples was determined by the following methods: 1) Egner-Riehm method (LV ST ZM 82-97), where 0.04 M calcium lactate extraction is used as an extracting agent being acidified by hydrochloric acid up to pH 3.5 – 3.7; 2) Olsen method (LVS ISO 11263:2002) – extracting agent 0.5 M sodium hydrogencarbonate solution, its reaction with sodium hydroxide reaches the precision of pH 8.5; 3) Mehlich-3 method where solution of 0.2 M acetic acid + 0.015 M ammonium fluoride + 0.013 M nitric acid + 0.25 M ammonium

nitrate + 0.001M EDTA is used as an extracting agent. The phosphorus in soil extract is measured photometrically by the molybdate – ascorbic acid method.

Mathematical processing of obtained results from soil analysis is carried out. Statistic indices of the results of plant available phosphorus analysis are determined for the sample. With different phosphorus extraction methods for the comparison of obtained data, correlation and regression analysis is used, as well as t-test for testing phosphorus content changes.

### Results and Discussion

For the research of the methods comparison in the selected soil samples the plant available phosphorus content was different. The main statistic indices of determined phosphorus content for extracting agents by the Egner-Riehm (DL), Mehlich-3 and Olsen, as well as differences in methods by analyzing the whole sample are shown in Table 1. The average amount of phosphorus extracted from the soil by calcium lactate extraction (DL-P) was 79.6 mg kg<sup>-1</sup>, by Mehlich-3 extracting agent (M-P) - 76.5 mg kg<sup>-1</sup>, but only 30.6 mg kg<sup>-1</sup> P passed into sodium hydrogencarbonate (Ols-P). The median value by the DL-P and Ols-P results were next to average indices, but by the M-P results the median was slightly deviated (70.2 mg kg<sup>-1</sup> P) in the direction of lower value. So DL-P and M-P with quite close average content indices, the maximum value were 267.3 and 418.9 mg kg<sup>-1</sup> P respectively. Differences in the extracted phosphorus amount by mutually comparing Egner-Riehm, Mehlich-3 and Olsen extracting agents prove that, when analyzing soil samples with sharply different properties, the obtained results vary markedly both in soils with a low and high content of movable phosphorus. The boundary of three standard deviations by the correlation DL-P/Ols-P was exceeded in 3 cases, by the correlation DL/M-P – in 1 case, by the correlation M-P/Ols-P – in 3 cases. In the research when carrying out analysis of methods that affect factors, the results of these samples were not included in the analysis.

Table 1. Statistic indices of phosphorus determination results for the analyzed samples

	DL-P, mg kg <sup>-1</sup>	Ols-P, mg kg <sup>-1</sup>	M-P, mg kg <sup>-1</sup>	Difference, mg kg <sup>-1</sup>		Correlation	
				DL-P – Ols-P	DL-P – M-P	DL-P / Ols-P	DL-P / M-P
Average	79.6	30.6	76.5	48.9	3.03	2.89	1.39
Median	80.8	29.3	70.2	50.7	7.8	2.59	1.12
Minimum	0	0.81	0	-4.5	-213.7	0	0
Maximum	267.3	117.9	418.9	150.1	97.5	20.97	149.3

By carrying out results assessment of soil sample chemical analysis, the mutual relationships between phosphorus extraction methods are determined. Close linear relationship are found between phosphorus amounts measured by the Egner-Riehm and Olsen methods, as well as the Egner-Riehm and Mehlich methods (Fig. 1).

The relationship between Egner-Riehm and Olsen methods had the highest correlation coefficient (r = 0.95) there was a slightly lower relationship between Egner-Riehm and Mehlich methods (r = 0.91) and the Mehlich and Olsen methods (r = 0.89). There is little data in literature about the relationship between the Egner-Riehm and Olsen methods, but similar linear correlation between the Egner-Riehm and Mehlich-3 methods is recorded also in research in Estonia, where depending on the humus content in the soil the correlation coefficient has been found to be from r = 0.821 to 0.867 (Loide *et al.*, 2005). But in the researches carried out at the Centre of Agrochemical Researches, it is found that correlation between the phosphorus determination results obtained by the Egner-Riehm and Mehlich-3 methods is only average tight (r = 0.61 – 0.62) (Timbare *et al.*, 2006).

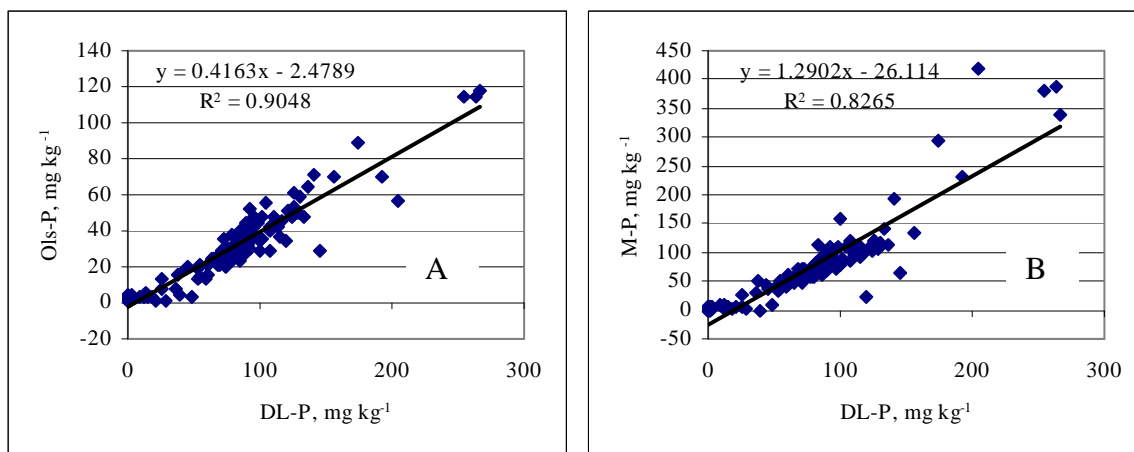


Figure 1. Mutual coherence of plant available phosphorus content determined by the Egner-Riehm and Olsen (A) and Egner-Riehm and Mehlich-3 (B) methods.

In order to find out which of the tested phosphorus determination methods mutually differ by the influence of different soil properties, the assessment of coherence changes of the determined P amount by the Egner-Riehm and Olsen was carried out for separate parts of the sample; taking into consideration agrochemical characterization of the samples. The obtained results proved that phosphorus extraction methods tested in the research show a good mutual correlation in weak acid and neutral soil reaction. The quantities of the highest correlation coefficient for the relationship between Egner-Riehm and Olsen methods ( $r = 0.96 - 0.97$ ) are determined in the exchange acidity interval  $\text{pH}_{\text{KCl}} 6.6 - 7.5$ . By the increase of soil acidity up to  $\text{pH}_{\text{KCl}} 6$ , the coherence between phosphorus amounts extracted by applying these methods, shows little tendency to reduce, which could be related to the fact, that the Olsen method can be applied for phosphorus determination in neutral and alkaline soils, but is not suitable for phosphorus determination in acid soils (Mallarino, Sawyer, 1999). Also in alkaline soils  $\text{pH}_{\text{KCl}} 7.5 - 8.6$ , the coherence between Egner-Riehm and Olsen methods becomes weaker ( $r = 0.81$ ), therefore it could be assumed, that the phosphorus amount, determined by the Egner-Riehm in calcium lactate extraction, does not adequately characterize plant available phosphorus in the soil. These relationships could get more convincing confirmation by analyzing soil sample of greater volume in a wider pH interval.

By comparing plant available phosphorus amounts determined by the Egner-Riehm and Mehlich-3, it is evident, that in the pH interval from 6 to 7.5 correlation coefficient ( $r = 0.92 - 0.94$ ) is lower than for the coherence between the Egner-Riehm and Olsen methods, which matches with the obtained coherence also for the whole soil Paired Sample analyzed. However, with soil alkalinity becoming higher, the coherence between the phosphorus amount determined by the Egner-Riehm and Mehlich-3, markedly becomes weaker ( $r = 0.50$ ). This makes us think that the Mehlich-3 method is still less suited for phosphorus determination in alkaline soils than the Egner-Riehm method. For soils with a reaction above  $\text{pH}_{\text{KCl}} 7.5$ , the coherence between Mehlich-3 and Olsen is very weak ( $r = 0.29$ ). For weak acid and neutral soils the correlative relationship between phosphorus amounts determined by these methods is tight ( $r = 0.96 - 0.98$ ). Our obtained results are confirmed also by research mentioned in the scientific literature. For example, in the Czech Republic (Zbiral, 2000) it was determined that in soils with the  $\text{pH}_{\text{KCl}}$  higher than 7.1, the amount of extracted phosphorus, measured by the Mehlich-3 method, depends on soil reaction and exchange calcium content. The plant available phosphorus amount, extracted from the soil by the Egner-Riehm, Olsen and Mehlich-3 methods, was affected also by the carbonate content. With the carbonate content not exceeding 5%, there was a close correlative coherence between the tested methods. The highest correlation coefficient ( $r = 0.95$ ) is found between the phosphorus amount which has passed into calcium lactate extraction and natrium hydrogencarbonate extraction. In soil samples, in which the carbonate content exceeded 5%, the coherence between the methods was

considerably weaker. The correlation coefficient for the coherence between the Egner-Riehm and Olsen methods was 0.44, but between the Egner-Riehm and Mehlich-3 methods – 0.49.

The correlation of phosphorus determination results, which in the Egner-Riehm and Olsen case (correlation DL-P/Ols-P) for the analyzed soil Paired Sample was 2.35 on average, but by the Egner-Riehm and Mehlich-3 methods (correlation DL-P/M-P) – 1.17, with changing soil exchange acidity, decreased. This tendency is more typical for the correlation between the phosphorus determination results ( $r = 0.53$ ) shown the by Egner-Riehm and Olsen methods, when, with the increase of soil alkalinity, in calcium lactate extraction (Egner-Riehm method) the passed phosphorus amount decreased (Fig. 2). The correlation of results by the Egner-Riehm and Mehlich-3 methods varied from 0 to 5.51 not depending on soil reaction. For soil samples with a diverse carbonate content the most essential changes are found between the results of the Egner-Riehm and Olsen methods. With the increase of carbonate content, the DL-P/Ols-P correlation decreases ( $r = 0.71$ ). This proves that in alkaline soils, rich in carbonates, a certain part of the potential plant available phosphorus amount does not pass into calcium lactate extraction.

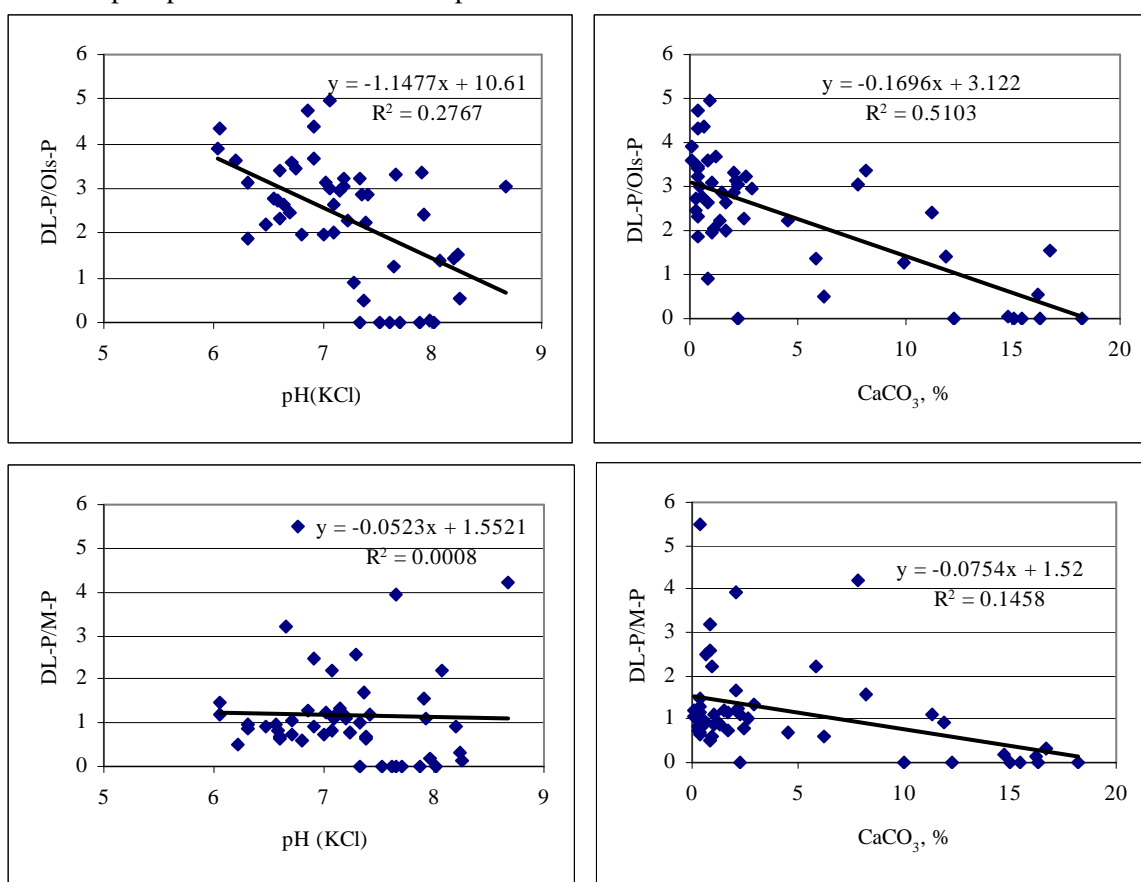


Figure 2. Influence of soil reaction and carbonate content on correlations of phosphorus determination results by the Egner-Riehm and Olsen method (DL-P/Ols-P) and the results by the Egner-Riehm and Mehlich-3 method (DL-P/M-P).

The obtained relationship prove that up to now the Egner-Riehm standard method used in agrochemical research of soils in Latvia incompletely characterises the sufficiency of plant available phosphorus in carbonatic soils with a alkaline reaction. According to the results of the research carried out and the data of literature, it is obvious that in calcareous soils the Olsen method would be more suitable. Types of phosphorus compounds, their transformations and binding mechanisms in these soils are different from soils where the dominate binding of phosphates in aluminium and iron compounds and in anion adsorption complex. Consequently, the use of one and the same plant available phosphorus determination methods in acid and neutral, as well as in alkaline carbonatic soils actually is not reasonable. The same way, depending on the soil reaction and carbonate content, sufficiency levels of soil phosphorus also should be differentiated.

## Conclusions

Close correlative coherence ( $r = 0.89 - 0.95$ ) is determined among plant available phosphorus determination results obtained from the soil sample ( $n=145$ ), analyzing these samples by the Egner-Riehm, Olsen and Mehlich-3 methods. In alkaline soils, rich in carbonates, the relationship between the results by the Egner-Riehm and Olsen, as well as between the Egner-Riehm and Mehlich-3 methods become weaker. The correlation of phosphorus content results determined by the Egner-Riehm and Olsen method with the increase of soil alkalinity essentially decreases, which proves that extraction of potential plant available phosphorus resources with calcium lactate extraction is insufficient. Similar relationship between the correlation by the Egner-Riehm and Mehlich-3 results was poorly expressed. For the determination of plant available phosphorus in alkaline carbonatic soils the most suitable is the Olsen method.

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## FOSFORA NOTEIKŠANAS METOŽU SALĪDZINĀJUMS KARBONĀTUS SATUROŠĀS AUGSNĒS

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LLU LF Augsnes un augu zinātniskajā institūtā veikti augsnes pētījumi augu sekas stacionāra atšķirīgu fosfora mēslošanas normu variantiem, lauksaimniecībā izmantojamo zemju augsnes monitoringa un augšņu etalonprofilu paraugu arhīva kolekcijai. Visiem paraugiem noteikts augiem viegli izmantojamā fosfora saturs pēc: 1) Egnera-Rīma metodē (LV ST ZM 82-97), kā ekstrāģentu lietojot 0.04 M kalcija laktāta šķīdumu, kas paskābināts ar sālsskābi līdz pH 3.5 – 3.7; 2) Olsena metodi (LVS ISO 11263:2002) – ekstrāģents 0.5 M nātrija hidroģēnkarbonāta šķīdums, kura reakcija ar nātrija hidroksīdu precizēta līdz pH 8.5; 3) Mēliha-3 metodi kā ekstrāģentu izmanto 0.2 M etiķskābes + 0.015 M amonija fluorīda + 0.013 M slāpekļskābes + 0.25 M amonija nitrāta + 0.001M EDTA šķīdumu. Fosfors augsnes izvilkumos noteikts fotometriski pēc molibdāta – askorbīnskābes metodes.

Noteikta cieša korelatīva sakarība ( $r = 0.89 - 0.95$ ) starp augiem viegli izmantojamā fosfora noteikšanas rezultātiem, kas iegūti augsnes paraugu kopai ( $n=145$ ), analizējot šos paraugus pēc Egnera-Rīma, Olsena un Mēliha-3 metodēm. Bāziskās, ar karbonātiem bagātās augsnēs, sakarība starp Egnera-Rīma un Olsena, kā arī starp Egnera-Rīma un Mēliha-3 metožu rezultātiem pavājinās. Pēc Egnera-Rīma un Olsena metodes noteiktā fosfora satura rezultātu attiecība, pieaugot augsnes bāziskumam, būtiski samazinās, kas liecina par nepilnīgu augiem potenciāli izmantojamo fosfora resursu ekstrakciju ar kalcija laktāta šķīdumu. Egnera-Rīma un Mēliha-3 rezultātu attiecībai līdzīga sakarība bija vāji izteikta. Augiem viegli izmantojamā fosfora noteikšanai bāziskās karbonātus saturošās augsnēs vairāk piemērota ir Olsena metode.

Agronomijas Vēstis. – Nr. 11.  
Latvian Journal of Agronomy No. 11.  
Jelgava, 2008  
Latvijas Lauksaimniecības universitāte  
Latvijas Lauksaimniecības un meža zinātņu akadēmija  
2008. gada 19. novembris  
Tirāža 150 eks.

Sagatavots iespiešanai Latvijas Lauksaimniecības universitātē,  
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