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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⁻¹ 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⁻¹ and the moderately susceptible clone – 51.8 t ha⁻¹. 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⁻¹. The soil type was different every year. The planting time depended on weather and varied from May 8th to 25th as well as the harvesting time – from September 2nd to 20th. 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 3rd 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.*, 2007⁻¹, 2007⁻², 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.

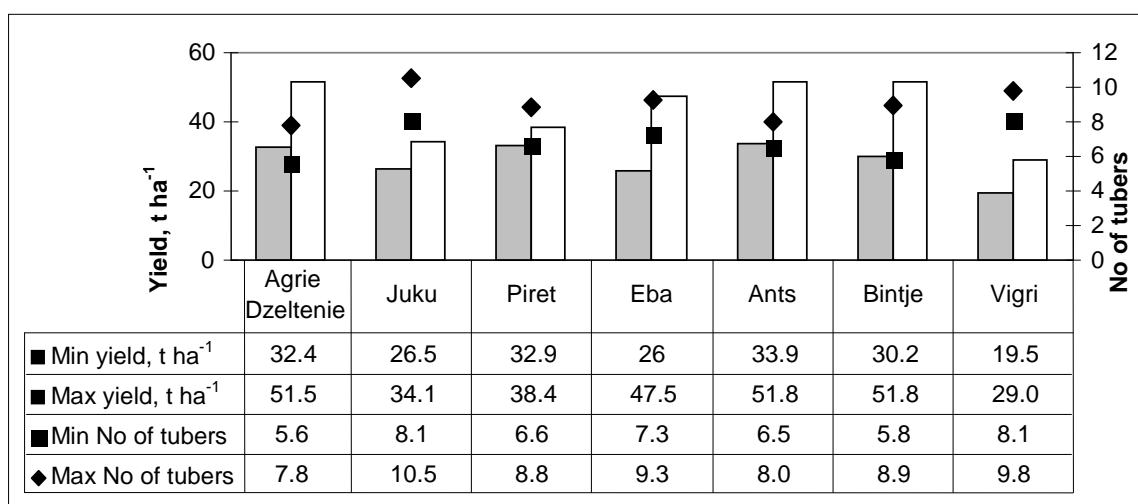


Figure 1. Variation of yield and number of tuber per plant on meristem clones* of different potato varieties

*Agrie Dzeltenie-16 clones, PD 95 % = 4.0 t ha⁻¹; PD 95 % = 0.7 tubers plant⁻¹; Juku-10 clones, PD 95 % = 3.2; PD 95 % = 0.4 tubers plant⁻¹; Piret- 10 clones, PD 95 % = 2.6; 0.8 tubers plant⁻¹; Eba- 6 clones, PD 95 % = 4.2 t ha⁻¹; PD 95 % = 0.4 tubers plant⁻¹; Ants-5 clones, PD 95 % = 4.3 t ha⁻¹; PD 95 % = 0.7 tubers plant⁻¹; Bintje-4 clones PD 95 % = 3.6 t ha⁻¹; PD 95 % = 0.8 tubers plant⁻¹; Vigri-11 clones, PD 95 % = 2.7 t ha⁻¹; PD 95 % = 0.3 tubers plant⁻¹.

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⁻¹ as an average and the moderately susceptible clone – 51.8 t ha⁻¹. 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 rd day* on <i>in vitro</i> plants, %		Injured leaf area on 26 th day* on <i>in vitro</i>		Late blight in the field, plants tubers		Yield, t ha ⁻¹ (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⁻¹. The starch content of the meristem clone No 3373 was 170 g kg⁻¹. 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.

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KARTUPEĻU MERISTĒMU KLONU MAINĪBA - JAUNI FAKTI ZINĀTNEI UN PRAKSEI

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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 Dzeltēnie' 16 meristēmu klonu trīs gadu vidējā raža variēja no 32.4 līdz 51.4 t ha⁻¹. 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⁻¹, bet vidēji izturīga klona raža bija 51.8 t ha⁻¹. 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