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 siblines 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 – $MgCl_2$, 2.5 mM $MgCl_2$, 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 94ºC for 2 min, 35 cycles of denaturation at 94 ºC for 45 sec, annealing at 51 ºC, 45 sec, elongation at 72 ºC for 45 sec, final elongation at 72 ºC 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	$(CTT)_{10}CTAC(CTT)_4$	Danin-Poleg et al 2001
CMCTT144R	AAATGGTGGGGGTTGAATAGG			
CMACC146F	CAACCACCGACTACTAAGTC	HEX	$(ACC)_9$	Danin-Poleg et al 2001
CMACC146R	CGACCAAACCCATCCGATAA			
CMGA104F	TTACTGGGTTTTGCCGATTT	NED	$(GA)_{14}AA(GA)_3$	Danin-Poleg et al 2001
CMGA104R	AATTCCGTATTCAACTCTCC			
CMAT141F	AAGCACACCACCACCCGTAA	6-FAM	$(AT)_7(GT)_6$	Danin-Poleg et al 2001
CMAT141R	GTGAATGGTATGTTATCCTTG			
CMCCA145F	GAGGGAAGGCAGAAACCAAAG	HEX	$(CCA)_5$	Danin-Poleg et al 2001
CMCCA145R	GCTACTTTTGTGGTGGTGG			
CMTC13F	TGGATGGATAAGGTGGTAAG	NED	$(TC)_{12}(CG)_5(AG)_3$	Katzir et al 1996
CMTC13R	TTCCCCTAGTCGCTCTCT			
CMGT108F	CTCCTTCAAACATTGTGTGTG	6-FAM	$(GT)_9N_{65}(CT)_7$	Danin-Poleg et al 2001
CMGT108R	GAGATAGGTATAGTATAGGGG			
CMCT134bF	GCTCCTCCTTAACTCTATAC	HEX	$(TA)_2(CT)_8(AT)_7$	Danin-Poleg et al 2001
CMCT134bR	GCATTATTACCCATGTACGAG			
CMCT44F	TCAACTGTCCATTTCTCGCTG	NED	$(CT)_{10}TGTT(CT)_3$	Danin-Poleg et al 2001
CMCT44R	CCGTAAAGACGAAAACCCTTC			
CMTC168F	ATCATTGGATGTGGGATTCTC	6-FAM	$(TC)_{14}$	Danin-Poleg et al 2001
CMTC168R	ACAGATGGATGAAACCTTAGG			
CMCT170bF	ATTGCCCAACTAAACTAAACC	HEX	$(CT)_8$	Danin-Poleg et al 2001
CMCT170bR	CACAACACAATATCATCCTTG			
CMAG59F	TTGGGTGGCAATGAGGAA	NED	$(GA)_2A(AG)_8$	Katzir et al 1996
CMAG59R	ATATGATCTTCCATTTCCA			
CMTC47F	GCATAAAAGAATTTGCAGAC	6-FAM	$(TC)_9(CT)_6$	Danin-Poleg et al 2001
CMTC47R	AGAATTGAGAAGAGATAGAG			
CMGA15F	CGGCAAGACGATTGGCAGC	HEX	$(GA)_7$	Danin-Poleg et al 2001
CMGA15R	ATCACCGTAGCGAAGCACC			
CMTA170aF	TTAAATCCCAAAGACATGGCG	NED	$(TA)_9T(TA)_3$	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 1st 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.

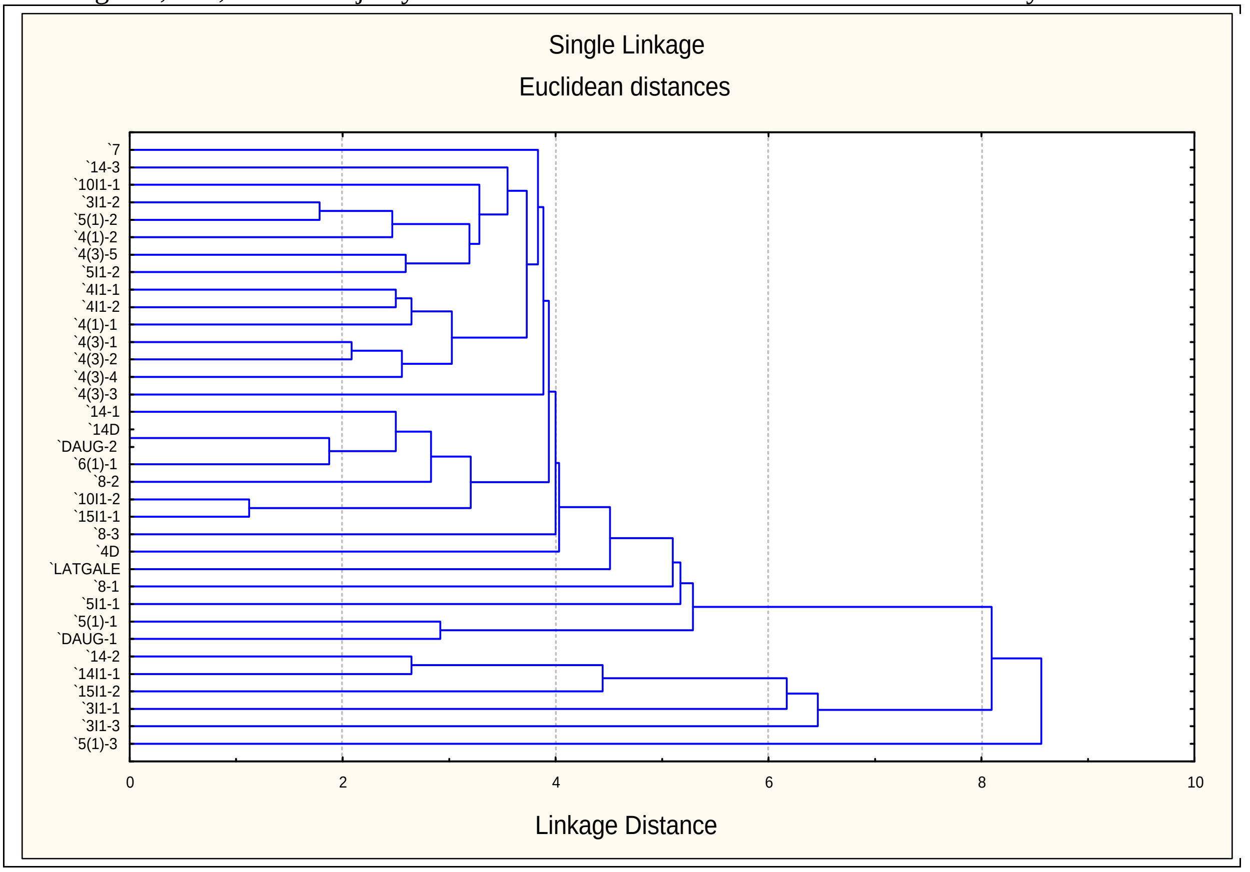

Figure 1. Relationships between lines in 2004 according to morphological traits

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.

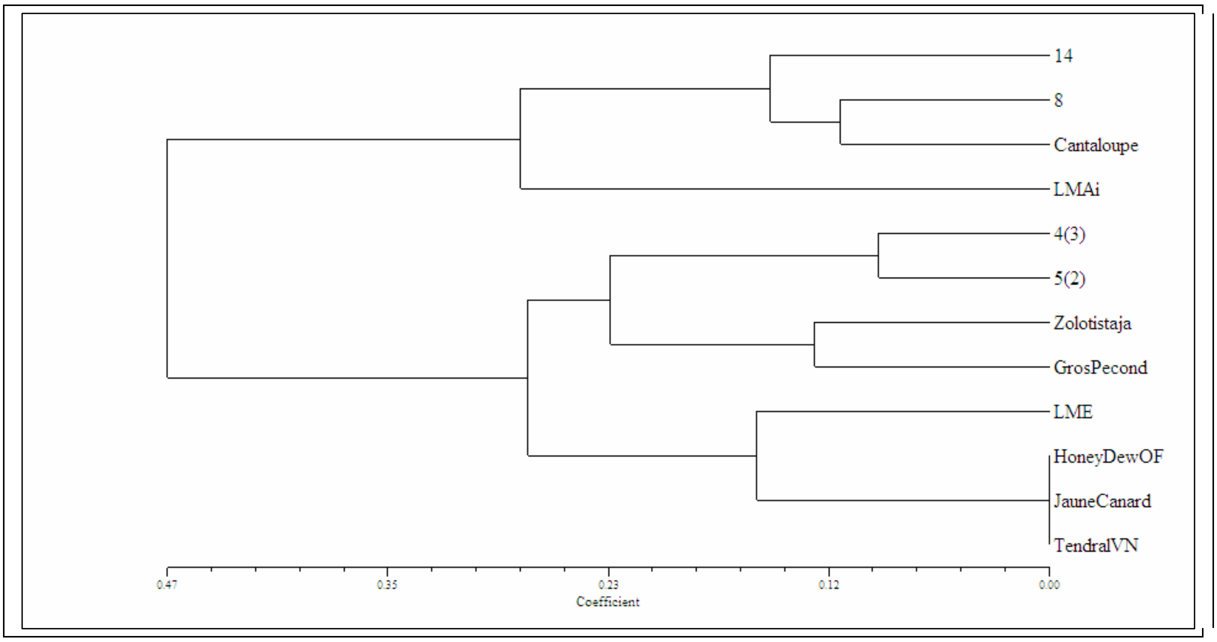

Figure 2. UPGMA dendrogram of genetic distances between Latvian melon lines and other varieties. Genetic distances were calculated using the proportion of shared alleles.

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 t broaden the genetic base of Latvian melon germplasm.

Acknowledgements:

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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