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Somaclonal variation in potato meristem culture and possibility to use this phenomenon in seed potato production and breeding

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Abstract. Regenerated plantlets obtained through virus-eradication procedure were preserved as meristem clones *in vitro*. The progeny of each meristem was the basis for meristem clone. We studied somaclonal variation of meristem clones of the new potato variety Reet created at the Jõgeva Plant Breeding Institute (PBI). The aim was to investigate the effect of thermotherapy on variability of potato meristem clones. The results showed that meristem clones differed in yield, number and weight of tubers and late blight resistance. The research provided new information about the effect of thermotherapy. For the first time we detected deviation from true-to-type in morphological characteristics of meristem clones.

Keywords: meristem clone, somaclonal variation, yield, number of tubers, weight of tuber

INTRODUCTION

Meristem method has been used as a tool for eradication of potato initial seed material from virus diseases. The method is principally the following: a slice of meristematic tissue is cut from the bud of a plant or sprout and cultivated into culture medium for the regeneration of the whole new plant. The exact method is varying according to the treatments of plants or sprouts that are used prior to the cultivation of meristems, like thermotherapy, chemotherapy, x-rays etc. The meristematic tissue can be excised from sprout or shoot; it can be lateral or apical.

In the current study we present the comparative data of agronomical traits of potato meristem clones as somaclonal variation. The number of publications of this topic is limited. Up to now the factors increasing the variability of meristem clones and possibilities to create clones with positive variations are unclear. Therefore the importance of influence of thermotherapy, growth hormones and genotype on development of variations was studied.

Despite the large number of potato varieties available and breeding of new varieties, some old varieties are still important as carriers of valuable genetic information. Meristem clones with improved traits would be helpful for potato growers – disease resistant meristem clones with uniform tuber shape and size and with higher yield capacity enable to reduce the use of chemicals and others costly inputs. Consequently the production would become environmentally friendlier.

The variability of potato genotypes was under detailed observation from the disease-resistance point of view. The resistance to bacterial and fungal diseases enables to produce potato with 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 suitable characteristics enables to improve new as well as old potato varieties. Having a close cooperation with local potato breeders, the promising new selections are brought to the EVIKA lab for eradication, meristem culture establishment and for selection of meristem clones with the best characteristics. Only then the breeding procedures with improved material will continue.

Up to present we have studied the agronomical traits of meristem clones of many varieties and our results indicated that the meristem clones differ considerable on yield and late blight resistance (Rosenberg et al., 2004, 2007, 2008).

In our earliest tests such kind of deviations in morphological characteristics between meristem clones as resulted in the present study were not evidenced. We noticed relatively low difference in such kind of morphological characteristics as the following: quicker development, stronger plants or more intensive flowering.

MATERIALS AND METHODS

The variety Reet. The new medium to late ripening potato variety Reet has been bred at the Jõgeva PBI and was included into the Estonian and European Variety List in 2007. The variety Reet is a selection from the cross between the Estonian variety Mats and the German variety Gitte. The variety Reet has resistance to nematode (Ro1) and wart disease (D1). There have been estimated a low infection with black scurf, common scab, potato virus and potato late blight. It has good cooking quality and characteristics as shallow eyes, regular shape and no enzymatic and after cooking darkening. The variety is suitable for organic farming (Tsahkna & Tähtjärv, 2008).

Cultivation of meristem clones and preparation of test material. The initial "mother" plant, from which the plants were multiplied for tests *in vitro* by micro cutting, was selected from the meristem clone 364. That meristem clone was eradicated from viruses earlier, tested and selected in field tests as the best meristem clone.

Meristem tissue, 0.2–0.3 mm in size, was cut from apical or lateral buds of green plants that passed through thermotherapy treatment 0; 16; 42 or 56 days for 16 h at 37–39^{o}C and 8 h at 33–35^{o}C. The explants were cultivated on Murashige-Skoog medium I modified by the EVIKA. The plants regenerated within 4–9 weeks. Stunted regenerates without root development, chlorotic and abnormal leaf or stem shape were eliminated from test. The first selection was made at the stage of the first meristem plants. The selected plants were propagated *in vitro* by micro cuttings. Their progenies were the basis for the meristem clone.

Field trials. The field trials were conducted in the test field of the Department of Plant Biotechnology EVIKA of the Estonian Research Institute of Agriculture in North-Estonia and at the Jõgeva Plant Breeding Institute in Mid-Estonia. In the trials the first generation tubers were used for the first year trials and in the following year the

second-generation tubers were respectively used. The trials were set in random block-placement in 4 replications, 20 tubers per plot.

Potato was fertilized with complete chlorine free mineral fertilizer (containing 50 g kg^{-1} N, 50 g kg^{-1} P and 100 g kg^{-1} K) by 500 kg ha^{-1} locally in spring. Planting time depended on weather conditions and varied from May 8th up to May 15th as well as harvesting time – from September 12th up to 30th. The chemical weed control was carried out when it was needed and potato was hilled up 2–3 times during the vegetation period. No chemical disease or pest control was used in the EVIKA test fields. At the Jõgeva Plant Breeding Institute, 4 times late blight and 3 times insect control was used.

The potato plants were evaluated during the whole growing period. The initial development, emergence, beginning and intensity of flowering, infection to late blight were evaluated. The number of tubers per plant was counted and the weight of tubers was measured at harvesting time.

Dispersion analysis was performed with unsmoothed original data by using Statistic 7.0 software (Statsoft, 2005). The results of the dispersion analysis are presented as a calculation of F statistics = MS_{model}/MS_{error} (MS – mean square), the F sub indexes are showing "model degrees of freedom" and "number observation degrees of freedom". The influence of a factor was proven at the level of significance $p < 0.05$.

RESULTS AND DISCUSSION

The main aim of our research was to study the agronomic traits and morphological characteristics of the meristem clones of the potato variety Reet. The aim of the current research was also to study the influence of thermotherapy duration to the variation of meristem clones.

In our previous tests we used for the creation of meristem clones the plants regenerated from the sprouts that passed thermotherapy. In this experiment the meristem clones were created from the meristems, operated from the plants of the meristem clone 364, which was created earlier, tested and selected in field tests as the best meristem clone. The initial plants of the meristem clone 364 were virus-free and the aim was not the virus eradication, but the study of the influence of duration of the thermotherapy on the traits of meristem clones.

In the table 1 there are presented two years yield data from the both growing locations. It is clearly indicated that the yield of meristem clones of the variety Reet depended on growing year and location. It was also evidenced that among the 20 meristem clones tested no successors from the clone 364 exceeded statistically the yield of the "mother" clone. The clone 392 had higher yield in three cases. Most of the meristem clones had lower yield compared to yield of the "mother" clone 364. For example, total yield of the meristem clone 7184 was two times lower compared to the "mother" clone. The yield difference was up to 20.2 t ha^{-1}. The generated clones were not higher yielding than the "mother" clone. We have received similar results in our earlier experiments (Rosenberg et al., 2004).

The data indicate (Fig. 1) that the yield of the meristem clones depended on the duration of thermotherapy. There are no available data about such kind of research and collected information base only on the results of our experiment.

The number of tubers per plant depended on duration of thermotherapy (Fig. 2).

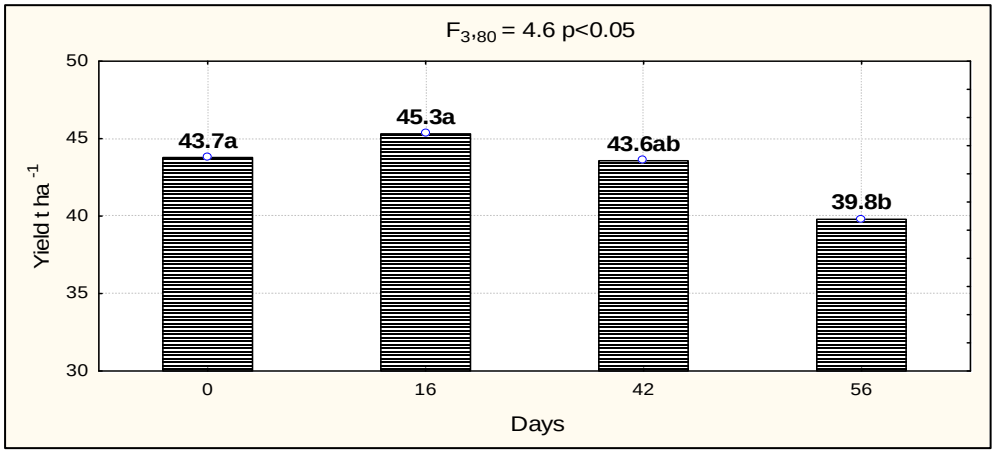

Figure1. The yield of tubers depending on days of thermotherapy.

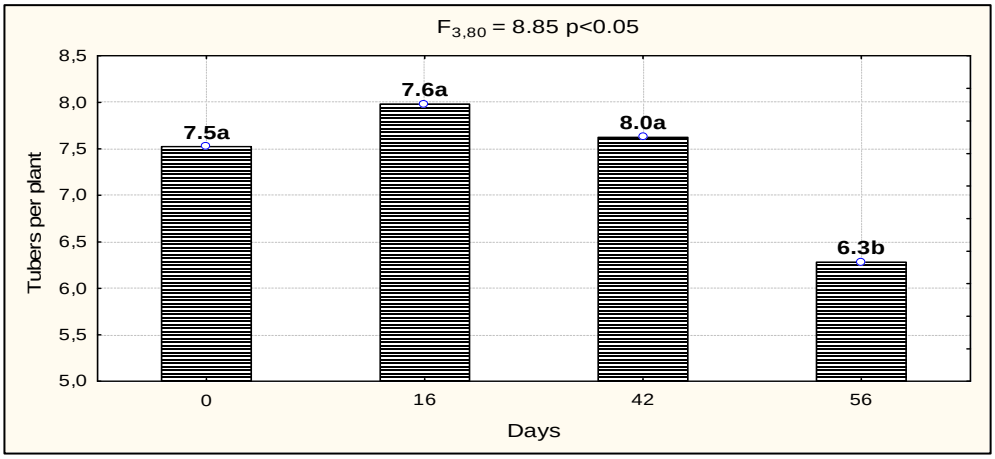

Figure 2. The number of tubers per plant depending on days of thermotherapy.

Considerable differences in the number of tubers per plant between the meristem clones were noticeable. Correlation between tubers per plant and yield was found. The number of tubers per plant was higher after 16 days and the lowest after 56 days of thermotherapy. The data indicate that the weight of tubers was influenced by duration of thermotherapy (Fig. 3).

The longer period of thermotherapy resulted in higher tuber weight. In the case without thermotherapy and thermotherapy of 42 days, the average weight of tubers was similar By visual evaluation, different appearance of the plants of the clones from 7181 to 7189 occurred, in both years and both locations. The plants were higher, the stems were finer and pigmented leaves were narrow and flowering period started later. Those

meristem clones were more late blight resistant and had longer growing period. The tubers were bigger, but fewer per plant and the stolons were longer comparing to the tubers of other meristem clones. At same time the shape and colour of tubers were true-to-type.

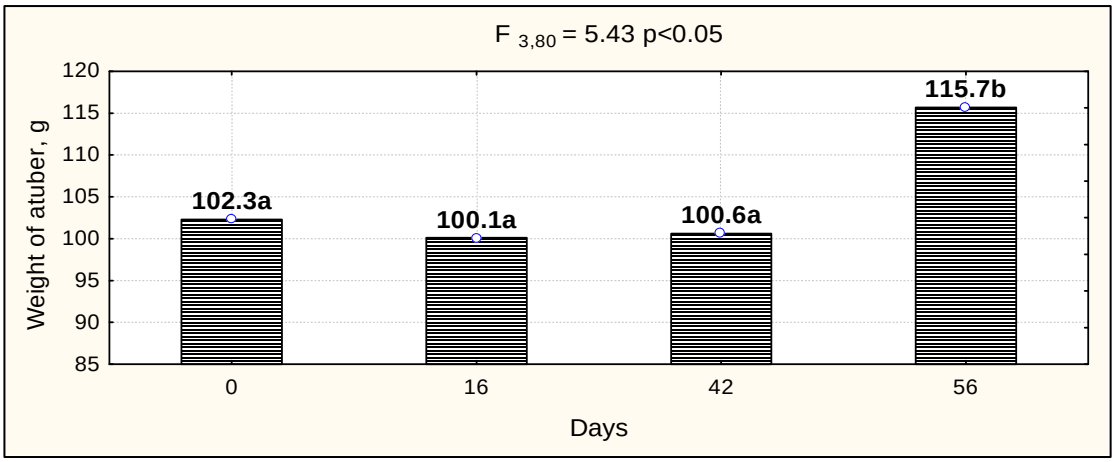

Figure 3. The average weight of tubers depending on days of thermotherapy.

The yield of the most meristem clones with abnormal appearances was lower, except the meristem clone 7185. The yield of the clone 7185 was similar to "mother" plant in EVIKA and even higher at the Jõgeva PBI in 2008. There was severe infection of late blight in 2008. The clones of 7185 occurred to be more resistant to the infection.

In 2008, the plants of meristem clones true-to-type flowered fully on July the 15th and the stems were completely destroyed up to middle of September. The plants of atypical meristem clones started to flower just at the end of July and were green up to the end of September until the first night frosts. The clone 7185 had the highest late blight resistance and higher yield capacity.

We have to conclude, that two years results are not enough to make final conclusions about the traits of the meristem clones. But the results confirmed our earlier findings that there were differences between meristem clones and they could be both – positive or negative. The new fact was that there existed variation of clones in tuber size influenced by the length of thermotherapy. The somaclonal variation induced by meristem culture is not thoroughly studied yet. However, there are data about variations induced in callus culture and the possibilities to use the technique in plant breeding (Sebastiani et al., 1994). Different genotypes were received from the callus culture generated by growing hormones from leaf segments (Tikan et al., 2008). The authors concluded that the obtained results were promising on creating the late blight and virus resistant potato varieties.

The beneficial use of variations induced by callus culture on potato breeding and improving of varieties, has been considered in many research investigations. By the results of many years trials was found that 17 donor varieties and more than 13,000 variations induced by callus culture differed from the donor variety in number of tubers per plant, tuber size, starch content, deepness of eyes and others traits. The authors have expected to use this phenomenon in breeding process (Thieme & Griess, 2005).

Table 1. Yield of tubers t ha^{-1} and relative yield at the EVIKA and the Jõgeva PBI trial fields in 2007 and 2008.

Clone No	Yield 2007*	Relative yield 2007*	Yield 2008*	Relative yield 2008*	Yield 2007**	Relative yield 2007**	Yield 2008**	Relative yield 2008**
364	47.2	1.0b	40.1	1.0b	42.8	1.0b	64.0	1.0b
68	46.9	1.0a	41.1	1.0a	42.9	1.0a	60.6	0.9a
73	36.7	0.8a	35.2	0.9a	37.2	0.9a	56.0	0.9a
74	36.6	0.8a	34.4	0.9a	42.9	1.0a	54.9	0.9a
108	48.9	1.0a	38.1	1.0a	34.3	0.8a	59.5	0.9a
111	49.1	1.0a	34.0	0.8a	42.8	1.0a	41.7	0.7a
122	53.6	1.1a	36.4	0.9a	37.2	0.9a	65.7	1.0a
130	45.0	1.0a	39.3	1.0a	40.0	0.9a	59.5	0.9a
392	46.5	1.0ab	40.3	1.0ab	45.7	1.1ab	46.3	0.7ab
417	38.7	0.8ab	36.3	0.9ab	31.4	0.7ab	63.4	1.0ab
705	38.3	0.8b	36.1	0.9b	37.1	0.9b	61.7	1.0b
714	44.7	0.9b	29.6	0.7b	42.9	1.0b	59.5	0.9b
7181	32.0	0.7b	32.8	0.8b	31.4	0.7b	58.8	0.9b
7182	46.1	1.0b	42.1	1.0b	34.3	0.8b	59.4	0.9b
7183	28.2	0.6b	30.9	0.8b	31.4	0.7b	57.7	0.9b
7184	27.0	0.6b	20.5	0.5b	31.4	0.7b	52.6	0.8b
7185	39.9	0.8b	25.4	0.6b	31.4	0.7b	66.3	1.0b
7187	32.0	0.7b	21.6	0.5b	37.2	0.9b	49.2	0.8b
7188	29.3	0.6b	21.7	0.5b	31.4	0.7b	54.8	0.9b
7189	37.1	0.8b	19.1	0.5b	31.4	0.7b	60.7	0.9b
794	38.7	0.8b	26.8	0.7b	42.9	1.0b	52.5	0.8b

N[1]=84

SE[2]=0,015

CL[3]=0,063

N[1] = number of samples; SE[2] = standard error; CL[3] = confidence limits at $p = 0.05$
*EVIKA; **Jõgeva PBI

Use of different growth hormones and ultraviolet light can increase the development of variations and mutations of callus culture. The authors concluded that in such way is possible to increase salt and drought tolerance (Ehsanpour et al., 2007). The callus of the variety Bintje was obtained from stem internodal tissue, subterminal tuber tissue culture and mesophyll protoplasts of *in vitro* grown plants. Nine somaclones were analysed, all of them were morphological and cytological chimeras (Jelenik et al., 2001). The previous studies showed that the somaclones obtained by use of callus culture could differ from mother or donor variety in morphological or cytological characteristics.

Meristem culture and its variability are supposed to be in between intact plant culture and callus or cell culture. There are no lethal chromosomal or gene mutations in meristem culture as a rule, but the frequency of point-mutations can be higher than has intact plant culture. It is assumed that meristem culture itself produces only a little amount of somaclonal variations but the variability can be increased by the other factors such as higher concentration of growth hormones in nutrient medium. Our studies with many varieties have evidenced that meristem clones may differ in their agronomic traits and disease resistance. More than 600 meristem clones of 40 varieties have been studied for a long time period. The results showed that meristem clones differed: in yield, tuber weight, number of tubers per plant, starch content and disease resistance (Rosenberg, 1995; 2004; Rosenberg et al., 2008; 2007).

Meristem clones as the basic material for somaclonal variation and its application possibilities were studied together with the Jõgeva Plant Breeding Institute. The varieties Anti and Juku and breed J1488-88 (Reet) were included in the test. The variety Juku demonstrated the highest variation in agronomic traits. The breed J1488-88 was characterized by the highest variation in resistance to *Phytophthora infestans* (Koppel et al., 1999; Koppel & Rosenberg, 1998). The intensity of virus biosynthesis and susceptibility to the viruses in 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 the same plant, were studied. The intensity of virus biosynthesis and susceptibility to the viruses of PVX in 17 meristem clones of the varieties Premiere, Eba and Kondor and PVM in 27 meristem clones of varieties Vigri, Eba, Kondor and Premiere were studied. The dependence of virus resistance of the meristem clones on the location of meristem in the plants was obtained (Agur & Rosenberg, 1999).

Our experiences have shown that the variability of meristem clones serve as excellent tool in improving yield and other agronomic traits of varieties in seed production and breeding. In the future the detailed studies about variations of meristem clones, their genetic background and the possibilities to improve the resistance to viruses, bacterial and fungal diseases will be continued.

CONCLUSIONS

Our experiences have shown that the variability of meristem clones can serve as a valuable tool in improving yield and other properties of potato varieties and breeds. For the first time not true to-type morphological characteristics of clones were detected. We have reason to presume that such kind of deviation was affected by longer period of thermotherapy and higher concentration of growth hormone in medium.

We can conclude that before supplying the seed production with initial disease-free material it is necessary to test meristem clones in field trials. Good quality of initial material can be obtained and preserved long term *in vitro* and be multiplied for seed production by using rapid methods. The studies of resistance to viruses and *Phytophthora infestans* provided a lot of useful data but needs more detailed investigations to use the somaclonal variation in plant breeding.

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REFERENCES

Agur, M., & Rosenberg, V. 1999. Biosynthesis of viruses (PVX, PVM) in meristem clones of potato. In: Aaviksaar, A.: *Taimekoekultuurid, uurimine ja rakendamine*. EPMÜ, EBI, Harku, pp. 194–202 (in Estonian).

Ehsanpour, A.A., Madani, S. & Hoseini, M. 2007. Detection of somaclonal variation in potato callus induced by UV-C radiation using RAPT – PCR. Gen. Appl. *Plant Phys*. **33**, 3–11.

Jelenic, S., Bežljak, J., Papeš, D. & Jelanska, S. 2001. Mixoploidy and Chimeric Structures in Somaclones of Potato (*Solanum tuberosum L.*) cv Bintje. *Food technol. Biotechn*. **39** (1), 13–17.

Koppel, M. & Rosenberg, V. 1998. Possibilities of use of somaclonal variation caused by meristem cultures in potato breeding. *Biotechnology in Plant Breeding*. Kaunas, pp. 25–28.

Koppel, M., Tsahkna, A. & Rosenberg, V. 1999. Study and use of somaclonal variation in potato breeding. In: Aaviksaar, A. (Ed.). *Taimekoekultuurid, uurimine ja rakendamine*. EPMÜ, EBI, Harku, pp. 178–184 (in Estonian).

Lebecka, R., Sobkowiak, S. & Zimnoch-Guzowska, E. 2006. Resistance of Potato Tubers to Higly Aggressive Isolate of *Phytophtora infestans* in Relation to Tuber age. *Pot. Res*. **49** (2), 99–107.

Pasco, C., Bozek, M., Ellisseche, D. & Andrivon, D. 2006. Resistence Behaviour of Potato Cultivars and Advanced Breeding Clones to Tuber Soft Rot caused by *Pectobacterium atrosebticum*. *Pot. Res*. **49** (2), 91–98.

Rosenberg, V., 1995. Results, showing possibilities of meristem method for improving some characteristics of potato varieties. *Current Issues in Plant Molecular and Cellular Biology*. Kluwer Academic, Netherlands, pp. 423–426.

Rosenberg, V., Kotkas, K., Särekanno, M. & Talvoja, P. 2004. Research of potato meristem clones. *Proceedings of EAPR Agronomy section meeting*, Brasov, 2004, pp. 109–119.

Rosenberg, V., Kotkas, K., Särekanno, M., Ojarand, A. & Vasar, V. 2008. Variation of potato meristem clones – new facts for science and practice. *Agr. Ves.*, **11**, 129–135.

Rosenberg.V., Särekanno, M., Kotkas, K., Vasar, V. & Ojarand, A. 2007. Variation of agronomic traits of potato somaclones produced by meristem culture. *S. Afr. J. Plant Soil* 2007, **24** (2), 95–99.

Sebastiani, L., Lenzi, A., Pugliesi, C. & Fambrini, M. 1994. Somaclonal variation for resistance to *Verticillum dahliae* in potato (*Solanum tuberosum* L.) plants regenerated from callus. Euphytica, **80**, 1–2, pp. 5–11.

STATSOFT, 2005. Statistica 7.0. Copyright 1984–2005. Tulka, OK, USA, 716 pp.

Thieme, R. & Griess, H. 2005. Somaclonal variation in tuber traits of potato. *Pot. Res*. **48**, 153–156.

Tikan, A., Campeanu, G., Chiru, N. & Ivanovici, D. 2008. Using of unconventional methods for obtaining somaclonal variations, having as goal making of new potato varieties with resistance to diseases and pests. *Roum. Biotec. Let.* **13** (4), 3791–3798.

Tsahkna, A. & Tähtjärv, T. 2008. The new potato variety Reet. *Agr. ves.*, **11**, 159–164.