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THE DYNAMICS OF CELL DIVISION IN SOME LOCAL POTATO VARIETIES CULTIVATED *IN VITRO* ON MICROPROPAGATION MEDIUM

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ABSTRACT. This study was performed to reveal the changes in cell division, as a result of the prolonged period of subculture on micropropagation medium, of five local varieties of *Solanum tuberosum* L. maintained on *in vitro* collection at Suceava Genebank, Romania. For this purpose it was used the Murashige-Skoog medium (MS-1962) with addition of 40 g/l sucrose, and 6 mg/l daminozide. The effect of prolonged period of subculture up to two and 12 months was expressed as mitotic index and frequency of cells with abnormal division. Mitotic index ranged from 20.1 to 22.1% after 12 days, between 15.5 - 17.7% after two months and between 17.7 - 19.2% after 12 months of subculture. The results obtained showed that the frequency of aberrant cells increased with the preservation time on the *in vitro* cultures and their accumulation rate depended on the genotype. Were identified interphases with micronuclei, metaphases with retarded chromosomes, ana-telophases with chromosomal bridges, retarded chromosomes and chromosomal fragments, but their percentage was low in all the genotypes.

Key words: *Solanum tuberosum* L.; Local varieties; Mitotic index; *in vitro.*

REZUMAT. Dinamica diviziunii celulare la unele varietăţi de cartof, cultivate *in vitro* pr mediul de propagare. Acest studiu a fost efectuat pentru a evidenţia modificările din diviziunea celulară, rezultate ca urmare a prelungirii perioadei de subcultură pe un mediu de micropropagare, la cinci varietăţi locale de *Solanum tuberosum* L., menţinute în colecţia *in vitro* a Băncii de Gene Suceava. În acest scop a fost folosit mediul de micropropagare Murashige-Skoog (MS-1962) cu adaos de 40 g/l zaharoză şi 6 mg/l daminozidă. Efectul prelungirii perioadei de subcultură până la două şi, respectiv, 12 luni a fost exprimat prin indicele mitotic şi prin frecvenţa celulelor cu anomalii ale procesului de diviziune. Indicele mitotic a

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variat între 20,1 şi 22,1 % după 12 zile, între 15,5 şi 17,7 % după două luni şi între 17,7 şi 19,2% după 12 luni de subcultură. Rezultatele obținute au arătat că frecvența celulelor aberante a crescut odată cu prelungirea perioadei de menținere a culturilor *in vitro*, iar rata acumulării acestora a depins de genotip. Au fost identificate interfaze cu micronuclei, metafaze cu cromozomi restanțieri şi anatelofaze cu punți cromozomale, cromozomi restanțieri şi fragmente cromozomale, dar procentul lor a fost mic la toate genotipurile.

Cuvinte cheie: *Solanum tuberosum* L.; varietăți locale; indice mitotic; *in vitro.*

INTRODUCTION

In order to reduce the frequency of subculture, the growth rate of potato plantlets *in vitro* may be restricted by various methods including manipulation of nutrients in the culture medium, incubation in a lower temperature, and light intensity and a shorter photoperiod (Westcott, 1981a,b; Estrada *et al.,* 1983; Ishige, 1995; Siddiqui *et al.,* 1996; Lopez-Delgado *et al.,* 1998; Gopal *et al.,* 2005).

In any program of germplasm conservation, which is using tissue culture methods, the stored genotypes must remain sufficiently stable, knowing that sometimes undesirable aspects such as genetic instability and loss of morphogenetic potential could appear (Denton *et al.,* 1977; Ghiorghiță *et al.,* 2005).

A parameter used successfully in cytogenetic studies is the mitotic index. It is reflecting the genotype response to the action of various factors and it is an important criterion in the cell division rate assessment (Ghiorghiță, 1999; Cîmpeanu *et al.,* 2002; Ciobanu *et al.,* 2011).

MATERIALS AND METHODS

The research has been initiated using minicuttings prelevated from local populations of potato SVGB 14376, SVGB 15079, SVGB 15102, SVGB 15140 and SVGB 15446, maintained as plantlets on *in vitro* collection of Suceava Genebank by *slow growth*.

The experiments were conducted on micromultiplication medium (M_{14}) based on Murashige-Skoog (MS-1962) recipe, supplemented with 1 mg / l K, 0.02 mg/l NAA, 40 g/l sucrose and 6 mg/l daminozide. The high quantity of kinetin added to the medium allowed a better survival of the inoculums in the storage conditions during the experiment, knowing the positive effect of this hormone to increase tolerance in seedlings at low temperatures and long periods of subculture (Kotkas, 2004). The five local varieties of potato have been investigated in terms of cytogenetic after three periods of cultivation *in vitro* on the micropropagation medium, respectively: 12 days, 2 months and 12 months. There were three different experimental versions and the results obtained at 12 days after inoculation was considered the control.

For the study of mitosis, after 12 days, were used the root meristems obtained by placing the minicuttings on solid micropropagation medium and the culture flasks were placed at a temperature of 20-22^{0}C, photoperiod 16/24 hours, with a light intensity of 2500 lx. The biological material used to study the mitosis at 2 and 12 months was collected from root of plantlets

maintained under micropropagation medium at temperatures between 6-12^{0}C, photoperiod 10/24 hours, with a light intensity of 1000 lx .

After 12 days, 2 and 12 months, respectively, after inoculation, root tips of 10-12 mm were collected and fixed in Carnoy solution for 48 hours at 5^{0}C. It was followed by hot hydrolysis with HCl 1N at 60^{0}C for 25 minutes and the material was colored with Carr solution. It was used the fresh squash type method to examine the specimens under the optical microscope Olympus CX 41. The images were done with the camera included in the microscope.

RESULTS AND DISCUSSION

The mitotic index. The effect of the cultivation period and genotype, on the dynamics of cell division in five varieties of *Solanum tuberosum* L. studied, is presented in *Table 1* and *Fig. 1*.

The data inserted in *Table 1* present the dynamics of mitotic index which is decreasing, compared to the 12 days control, in the plantlets cultivated for an extended period on micropropagation medium. The bigger reduction was registered from 12 days to 2 months. At the end of this subculture period it was noted the variety SVGB 15140 with a maximum of mitotic index, the difference from the control (2.9%) is less than in case of the other varieties analyzed.

The changes in mitotic activity of root meristems of the samples grown for 12 months on M_{14} medium, regardless of variety, reflect the start of adaptation to the preservation conditions, the number of cells in division was positively influenced, and therefore, the mitotic index values are higher than those of 2 months. An explanation of this development could be that cells in the roots began to divide to produce secondary roots, leading to a relatively large number of cells in mitosis.

The results were validated by the statistical relationship between the subculture period and the number of cells in division (*Fig. 1*). The analyses of limit differences are reflecting distinct or very significant decreases statistically assured in the samples at 2 and 12 months; it was an exception for the genotype SVGB 15079 with smaller differences statistically insignificant. The variety SVGB 15079 was noted at 12 months because it was the only genotype having the number of cells in division higher compared to the control, but the difference is also insignificant.

It has to be emphasized, however, that in four from five of the analyzed varieties the average values of this parameter, after 12 months, was lower than control.

Table 1. The effect of genotype and period of subculture on mitotic index in five varieties of *Solanum tuberosum* L. grown on micropropagation medium M_{14}

Genotype	Mitotic index / Subculture period		
	12 days	2 months	12 months
SVGB 14376	22,1%	16,4%	19,2%
SVGB 15079	20,1%	17,0%	17,7%
SVGB 15102	21,9%	16,1%	18,8%
SVGB 15140	20,6%	17,7%	19,1%
SVGB 15446	20,3%	15,5%	18,4%

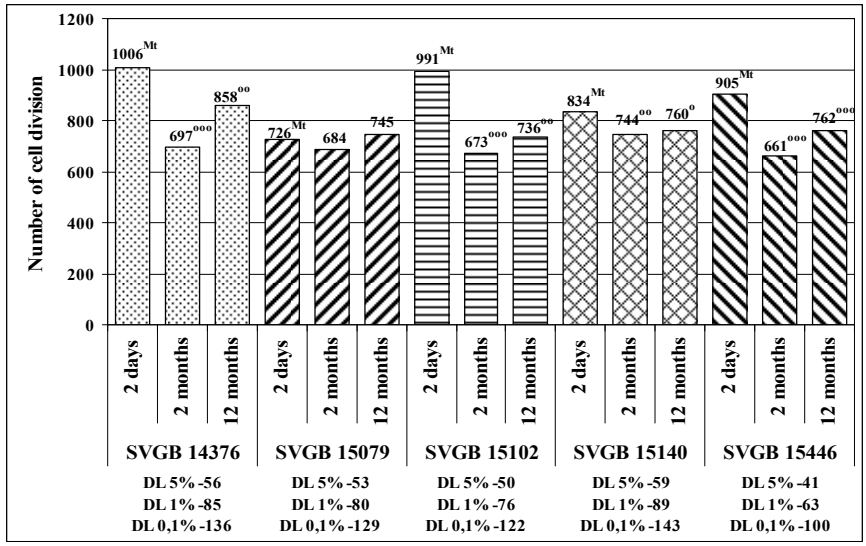

Figure 1 - The influence of genotype and cultivation period on the cell number in division in root meristems of the five local varieties of *Solanum tuberosum* L. inoculated on medium M_{14}

The frequency of cells with chromosomal aberrations. The relationship between the cultivation period of the explants on medium M_{14} and average frequency of cells with chromosome aberrations in root meristems of studied potato varieties is shown in *Fig. 2*.

Compared to the control, the biggest increases were recorded after 12 months of culture in varieties SVGB 14376 (with 200%) and SVGB 15102 (with 75%), the differences having distinct significations for the first genotype, and without statistical signification for the second. In case of variety SVGB 15140 the aberrant cells appeared in identical numbers after 2 and 12 months of subculture on M_{14} medium (0.39%).

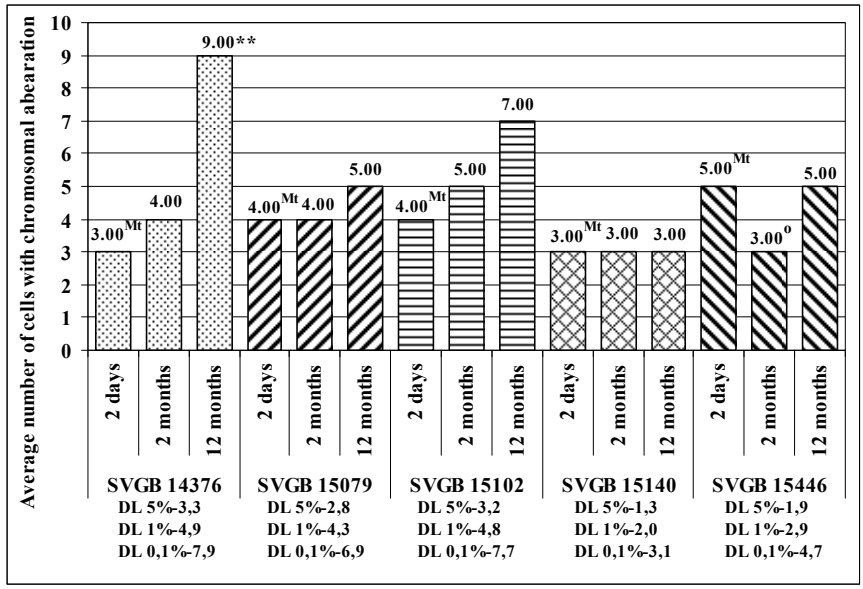

Figure 2 - The number of cells with chromosomal aberrations in root meristems of five local varieties of *Solanum tuberosum* L. inoculated on medium M_{14}

A special behavior was registered in the variety SVGB 15446 because the average frequency of cells with chromosome aberrations after 2 months of subculture was lower than in control version with 40%, the difference being statistically significant. In other cases the analyzed, differences from control were small and statistically uninsured.

The microscopic analysis revealed the following types of chromosome aberrations: interphase with micronuclei, metaphases with retarded chromosome, ana-telophases with chromosomal bridges, retarded chromosomes and chromosomal fragments (*Table 2*).

The micronuclei were present in cell interphases of control cultures in very small percentage (0.10 to 0.13%) to four of the five local varieties and in potato varieties SVGB 15079 and SVGB 15102 after 2 and 12 months, respectively, after the inoculation on the medium.

Retarded chromosomes were found in all the experimental variants, including control, where they recorded the lowest frequency (between 0.10 to 0.29%).

Some aspects of chromosomal aberrations recorded after different periods of subculture on micropropagation medium M_{14} are presented in *Fig. 3÷8.*

Also, chromosome bridges were registered in all experimental variants, but in subunit proportions; an exception was the variety SVGB

15079, at 2 months of subculture, to which this type of aberrations was not met. Most ana-telophases with bridges were registered in the variety SVGB 14376 after 12 months after inoculation.

Ana-telophases with fragments were not present in the control variant, being present only in genotypes SVGB 14376 and SVGB 15102 after 12 months of cultivation on the culture medium M_{14}.

Table 2 - The frequency of chromosomal aberrations types in root meristems of five local varieties of *Solanum tuberosum* L. inoculated on M_{14} medium, after different *in vitro* culture periods

Genotype	Subculture period	Cells in division	Aberrations types					
			Metaphases with retarded chromosome	A-T with simple bridges	A-T with double bridges	A-T with multiple bridges	A-T with retarded chromosomes and chromosomal fragments	Micronuclei
SVGB 14376	12 days	1006	1	0	0	2	0	0
	2 months	697	2	0	0	2	0	0
	12 months	858	4	1	1	1	2	0
SVGB 15079	12 days	726	2	0	0	1	0	1
	2 months	684	3	0	0	0	0	1
	12 months	772	3	0	0	2	0	0
SVGB 15102	12 days	991	1	1	0	1	0	1
	2 months	673	3	0	0	2	0	0
	12 months	736	3	0	0	1	1	2
SVGB 15140	12 days	834	1	0	1	0	0	1
	2 months	744	1	1	0	1	0	0
	12 months	760	2	0	0	1	0	0
SVGB 15446	12 days	905	2	1	0	1	0	1
	2 months	661	3	0	0	0	0	0
	12 months	762	3	1	1	0	0	0

A-T = ana-telophase

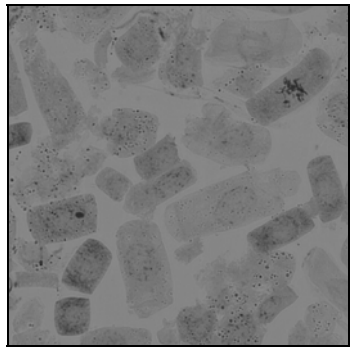

Figure 3 – Metaphase with retarded chromosomes (top) and interphase with micronucleus (left) to the genotype SVGB 15102, after 12 days of subculture (*original*)

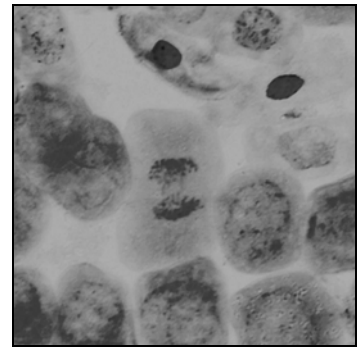

Figure 4 – Ana-telophase with double bridges to the genotype SVGB 15140, after 12 days of subculture (*original*)

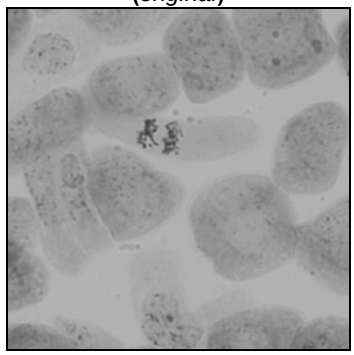

Figure 5 – Ana-telophase with fragments and retarded chromosomes to the genotype SVGB 14376 after 12 months of subculture (*original*)

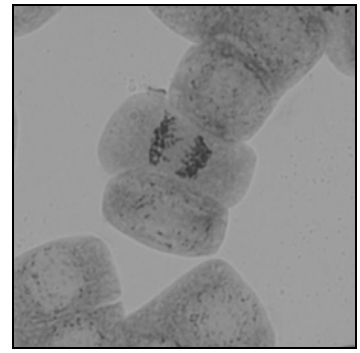

Figure 6 Ana-telophase with retarded chromosomes to the genotype SVGB 15446, after 2 months of subculture (*original*)

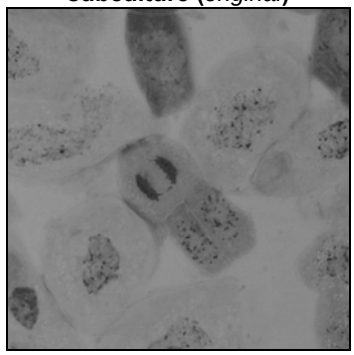

Figure 7 – Ana-telophase with fragment and retarded chromosomes to the genotype SVGB 15102, after 12 months of subculture (*original*)

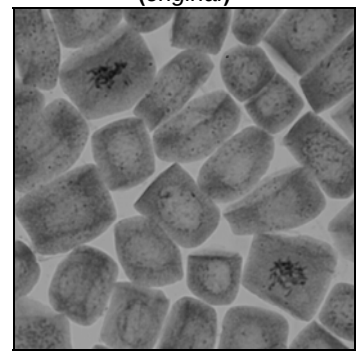

Figure 8 – Metaphases with retarded chromosomes to the genotype SVGB 15079, after 12 months of subculture (*original*)

CONCLUSIONS

The two storage period influenced the mitotic activity in root meristems of the five varieties of *Solanum tuberosum* L. Cell division rate decreased from the control variant after both cultivation periods, but the most pronounced reduction of the mitotic index was found after 2 months of subculture.

The results obtained showed the presence of chromosomal aberrations which ranged from 0.29 to 0.55% at 12 days, between 0.39 - 0.74% at 2 months and between 0.39 - 1.04% at 12 months of subculture.

The extension of subculture up to 12 months increased the recorded values with 52.6%, compared to the control variant.

The increasing of culture period on a micropropagation medium and in slightly restrictive conditions of storage room, act in many ways, affecting cell growth and the process of cell division.

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