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RESEARCH ON THE DYNAMICS OF CELL DIVISION IN SOME LOCAL POPULATIONS OF *SOLANUM TUBEROSUM* L. FROM *IN VITRO* COLLECTION OF SUCEAVA GENEBANK

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Abstract: This study was conducted to analyze the cytogenetic effects induced by some growth inhibitors (daminozide, mannitol, and sorbitol) on cell division on five local varieties of potato from in vitro collection maintained by the Gene Bank of Suceava. We used four different culture media: medium of micromultiplication (control medium) - M_{14}, medium with 30 mg/l daminozide - C_{22}, the medium with 40 g/l mannitol - C_{23}, the medium with 40 g/l sorbitol - C_{24}. The effect of three substances on root meristems of genotypes analyzed was expressed as mitotic index, which ranged from 16,46% limits, the plantlets inoculated on medium with mannitol and 24,74% for the seedlings inoculated with daminozide medium. We observed a decrease in mitotic index in all five genotypes on mannitol and sorbitol medium and increase its on medium with daminozide. The studies showed that in most cells, regardless of culture medium used were normal mitosis. Were also found abnormal cell division, which included delayed, expelled and picnotic chromosomes, bridges and chromosomal fragments and micronuclei, but their percentage was lower in all genotypes, in all medium variations experienced.

Keywords: potato, the mitotic index, daminozide, mannitol, sorbitol

Introduction

Cell division represents the fundamental biological mechanism that ensures both the individual and the species conservation and differentiation and evolution. Mitosis ensures the continuity of hereditary in every organism and preserves the genotype in a very exact type [8].

The intensity of cell division proportionality is kept constant during the different ontogenetic stages of plant development of and therefore, the determination of mitotic activity in root meristems contribute to reveal the features cell division [2].

The determination of plant cytogenetic aspects play a major role in the understanding of base sustaining principles, and how the specific conditions of *in vitro* cultivation affect the processes of growth and development.

Because the mitosis duration can be influenced by the culture medium components, (in sense of stimulation or inhibition), we proposed to quantify the effects of three substances (daminozide, mannitol and sorbitol) on cell division by using the main cell indices, in five local potato populations preserved *in vitro*.

Materials and methods

The research was initiated by using the microcuttings prelevated from five local potato populations: SVGB 14376 (genotype 1), SVGB 15079 (genotype 2), SVGB 15102 (genotype

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3), SVGB 15140 (genotype 4) and SVGB 15446 (genotype 5) derived from plantlets maintained to *in vitro* collection of Suceava Genebank through slow growth techniques.

The experiments were conducted on four culture media: a micromultiplication medium (M_{14}), as control, and three variants of conservation media (C_{22}, C_{23}, C_{24}) with growth inhibitors. The control sample was inoculated on the Murashige-Skoog (1962) medium [7], having the normal concentration of macro and microelements, supplemented with 40 g/l sucrose and 6 mg/l daminozide. The experimental media were based on MS nutrient formula, in a dilution of ½, with low concentrations of growth regulators, sucrose 2-3%, and the addition of 30 mg/l daminozide, 40 g/l mannitol and 40 g/l sorbitol (Tab. I).

Table I. Variants of cultured media used for *in vitro* growth of the roots to local potato varieties

Variant of culture medium	Basal culture medium and hormonal balance	Other components
M_{14} (control)	MS [1] + 1 mg/l K [2] + 0,02 mg/l ANA [3] + 6 mg/l daminozide	40 g/l sucrose
C_{22}	MS ½ + 0,02 mg/l K, 0,02 mg/l BA [4] + 0,02 mg/l ANA	30 g/l sucrose + 30 mg/l daminozide
C_{23}	MS ½ + 0,02 mg/l K, 0,02 mg/l BA + 0,02 mg/l ANA	20 g/l sucrose + 40 g/l mannitol
C_{24}	MS ½ + 0,02 mg/l K, 0,02 mg/l BA + 0,02 mg/l ANA	20 g/l sucrose + 40 g/l sorbitol

[1] Murashige-Skoog; [2]Kinetin; [3] α naphthyl acetic acid; [4] Benzyladenine

To establish the effect of the three substances on the mitosis process it were used roots meristems from the plantlets developed from the microcuttings on solid media, in glass jars, in a temperature of about 20-22^{0}C, photoperiod 16/24 hours, with a light intensity of 2500 lx. (Figs. 1-3).

After 12 days of microcuttings development on the media, the primary roots, of about 10-12 mm have been collected and fixed in Carnoy solution for 48 hours at 4^{0}C. It was followed by a hot hydrolysis with HCl 1N, to 60^{0}C, for 25 minutes and then the staining of the material with Carr solution [3]. The fresh microscopic preparations were of squash type, and have been surveyed using the optic microscope Olympus CX 41. The microphotographies were done with the camera from the microscope endowment.

Results and discussions

The plants of *Solanum tuberosum* L. are tetraploide, having a small amount of DNA assigned to the total chromosome number (2n = 4x = 48). They are very small (near punctual), but could be stained well enough with the Carr solution (Figs. 4, 5).

Mitotic index and the frequency of the mitotic division phases

The microscopic observations carried in the five potato genotypes, obtained on the four culture media analyzed, revealed the inhibitory effect of the mannitol and sorbitol, expressed by the lower percentage of the cells in division compared to control samples, and a slight stimulating effect the risogenesis produced by the daminozide (Figs. 6-10). The largest decrease of the percentage of cells in division was recorded in the genotype 2

(16.46%), followed by the genotype 5 (16.60%) on C_{23} medium, which shows that cell division occurs at a lower in the presence of mannitol. The highest values were recorded in the samples of genotypes 3 (24.74%) and 4 (23.39%) on medium with 30 mg/l daminozide (C_{22}).

Among the phases of mitotic division, most examined cells have been in the prophase both to the control samples and on the other three medium variants, followed to long distance of cells in metaphase, anaphase and telophase (Tab. II).

Table II. The frequency of the phases of mitotic division in radicular apex of plantlets by 12 days to *Solanum tuberosum* L., depending on the experimentation variant

Variant to experiment		Total analyzed cells	Total cells in interphase		The distribution of the mitotic division phases							
					Prophase		Metaphase		Anaphase		Telophase	
Medium of culture	Genotype	Nr.	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
M_{14} control	**G1**	4544	3538	77,86	897	19,74	50	1,10	37	0,81	22	0,48
	G2	3614	2888	79,91	654	18,02	39	1,07	18	0,49	15	0,41
	G3	4532	3441	75,92	898	19,81	48	1,05	31	0,68	14	0,30
	G4	4045	3211	79,38	769	19,01	32	0,79	19	0,46	14	0,34
	G5	4466	3561	79,73	830	18,58	47	1,05	16	0,35	12	0,26
C_{22}	**G1**	4475	3477	77,69	905	20,22	51	1,13	23	0,51	19	0,42
	G2	3777	2969	78,60	728	19,27	42	1,11	20	0,52	18	0,47
	G3	4166	3135	75,25	897	21,53	64	1,53	41	0,98	29	0,69
	G4	4479	3431	76,60	952	21,25	55	1,22	26	0,58	15	0,33
	G5	4393	3492	79,49	814	18,52	50	1,13	19	0,43	18	0,40
C_{23}	**G1**	4230	3445	81,44	723	17,09	36	0,85	15	0,35	11	0,26
	G2	3686	3079	83,53	568	15,40	24	0,65	8	0,21	9	0,24
	G3	4122	3381	82,02	688	16,69	38	0,92	10	0,24	5	0,12
	G4	4007	3282	81,90	676	16,87	25	0,62	14	0,34	10	0,24
	G5	3807	3175	83,39	587	15,41	28	0,73	11	0,28	6	0,15
C_{24}	**G1**	4534	3603	79,46	847	18,68	48	1,05	23	0,50	13	0,28
	G2	3831	3164	82,58	617	16,10	27	0,70	14	0,36	9	0,23
	G3	4455	3628	81,43	752	16,87	43	0,96	18	0,40	14	0,31
	G4	3994	3226	80,77	702	17,57	33	0,82	18	0,45	15	0,37
	G5	4281	3551	82,94	664	15,51	36	0,84	22	0,51	8	0,18

Frequency of the cells with chromosome aberrations

Microscopic analysis of biological material revealed the presence of various types of chromosome aberrations relative to the five local potato varieties studied, distributed randomly depending on genotype and culture medium variant. It was observed interphase with a micronucleus, cells with delayed, expelled or picnotic chromosomes and cells with chromosomal bridges and fragments in ana-telophase, due either poor separation of chromosomes or the welding chromosome terminal who have suffered terminal deletions (Tab. III).

The highest percentage of chromosomal aberrations was registered in the ana-telophase. The maximum proportion of the aberrant cells in ana–telophase was induced by variant of medium with 30 mg/l daminozide (C_{22}) to the variety SVGB 15446 (0.77%). In all variants, except for the variant SVGB 15102 on medium C_{24}, were registered prophase and metaphase with delayed, expelled or picnotic chromosomes.

The most aberrant cells were found on the C_{22} medium, with 30 mg/l daminozide (23) and the less on the C_{24} medium, with 40 g/l sorbitol (13).

The research revealed the specificity of each genotype in the occurrence of chromosomal aberrations: most aberrations were registered in the genotype 4 and the fewest in the genotype 1.

Different aspects of chromosomal aberrations recorded on the four media analyzed are presented in Figs. 11-18.

Table III. Frequency and types of the cells with chromosome aberrations in the mitosis of local varieties of potato radicular meristems inoculated on four culture media

Genotype	Medium of culture	Cells in the division	Types of chromosomal aberrations			Total	
			Delayed, expelled, picnotic chromosomes	Bridges and fragments in the A-T	Micronuclei	Nr.	%
G1	**M_{14}**	1006	1	2	0	3	0,29
	C_{22}	998	1	2	1	4	0,40
	C_{23}	785	1	1	0	2	0,25
	C_{24}	931	2	1	0	3	0,32
G2	**M_{14}**	726	2	1	1	4	0,55
	C_{22}	808	1	1	1	3	0,37
	C_{23}	607	1	2	0	3	0,49
	C_{24}	667	2	1	0	3	0,44
G3	**M_{14}**	991	1	2	1	4	0,40
	C_{22}	1031	1	1	2	4	0,38
	C_{23}	741	1	2	2	5	0,67
	C_{24}	827	0	0	1	1	0,12
G4	**M_{14}**	834	1	1	1	3	0,35
	C_{22}	1048	2	1	2	5	0,47
	C_{23}	725	1	2	2	5	0,68
	C_{24}	768	1	2	1	4	0,52
G5	**M_{14}**	905	2	2	1	5	0,55
	C_{22}	901	2	**4**	1	**7**	**0,77**
	C_{23}	632	1	0	1	2	0,31
	C_{24}	730	1	1	0	2	0,27

Conclusions

The culture media C_{22}, C_{23} and C_{24} have influenced the mitosis process and the three substances tested had different influence on the mitotic division.

The cytogenetic tests show a decrease in mitotic index compared with the control sample at inoculums placed on the media with mannitol (C_{23}) and sorbitol (C_{24}), leading decrease, probably, an inhibition of the growth of plantlets when they are maintained for a longer time on these media. The rizogenesis was stimulated on the medium variant with 30 mg/l daminozide (C_{22}), which recorded a larger number of cells in division than on the control variant. The microscopic observations are also supported by the findings of macroscopic, respectively reduction of the number and size of roots grown on the C_{23} and C_{24} media and increased values of both characteristics when using C_{22} medium.

The mitosis analysis revealed a low number of interphase and chromosomal aberrations which percent ranged from 0,29 to 1.68% on the control medium (M_{14}), from 0,37 to 0,77% on medium C_{22}, 0,25 – 1,68% in medium C_{23} and 0,12 – 0,52% on the medium C_{24}.

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Explanation of the plates

Plate I

Figure 1. Roots development on the culture medium with daminozide to the genotype 1
Figure 2. Roots development on the culture medium with manitol to the genotype 1
Figure 3. Roots development on the culture medium with sorbitol to the genotype 1
Figure 4. Chromosomes of *Solanum tuberosum* L. (X 100) to the genotype 3 (SVGB 15102) cultivated *in vitro*
Figure 5. Aspect of a microscopic field to the genotype 2 (SVGB-15079) (x 40)
Figure 6. Graphical representation of the mitotic index to the genotype 1 cultivated on the four variants of medium used in the experiment
Figure 7. Graphical representation of the mitotic index to the genotype 2 cultivated on the four variants of medium used in the experiment
Figure 8. Graphical representation of the mitotic index to the genotype 3 cultivated on the four variants of medium used in the experiment
Figure 9. Graphical representation of the mitotic index to the genotype 4 cultivated on the four variants of medium used in the experiment

Plate II

Figure 10. Graphical representation of the mitotic index to the genotype 5 cultivated on the four variants of medium used in the experiment
Figure 11. Ana-telophase with ragged bridges and chromosomal fragments (x 100) to the genotype 5 from medium C_{22} (zoom 3)
Figure 12. Delayed chromosomes (x 100) to the genotype 2 from medium M_{14}
Figure 13. Interphase with micronucleus (x 100) to the genotype 4 from medium C_{23}
Figure 14. Ana-telophase with bridges (x 100) to the genotype 3 from medium C_{23}
Figure 15. Delayed chromosomes (x 100) to the genotype 4 from medium $C_{24,}$
Figure 16. Ana-telophase with bridges (x 100) to the genotype 2 from medium $C_{22,}$
Figure 17. Ana-telophase with ragged bridges (x 100) to the genotype 1 from medium $C_{22,}$
Figure 18. Ana-telophase with multiple bridges (x 100) to the genotype 2 from medium M_{14}

Figure 1

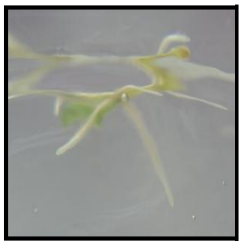
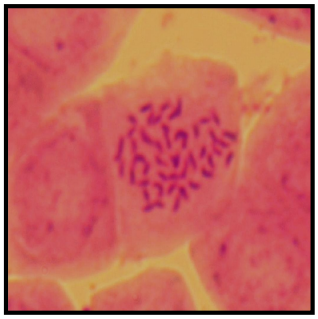


Figure 2

Figure 3



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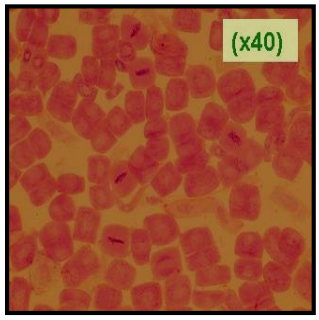

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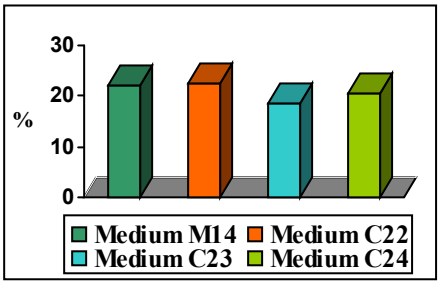

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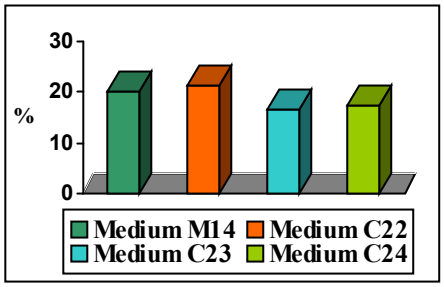

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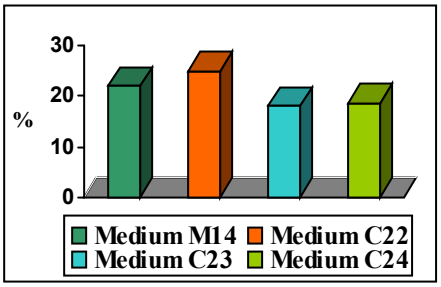

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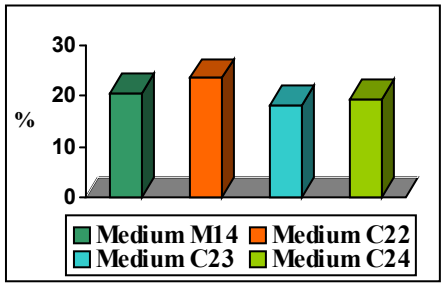

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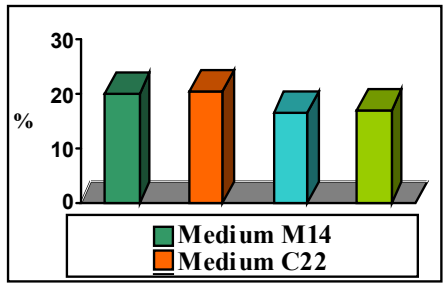
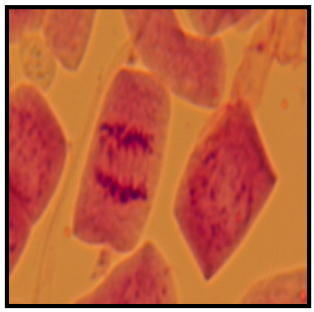


Figure 10



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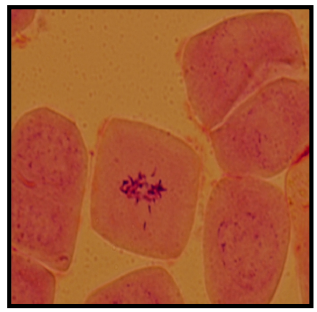

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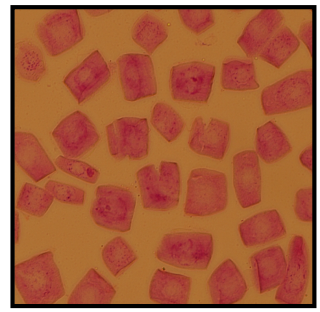

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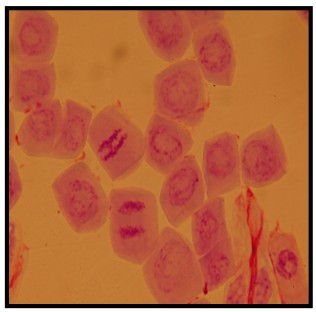
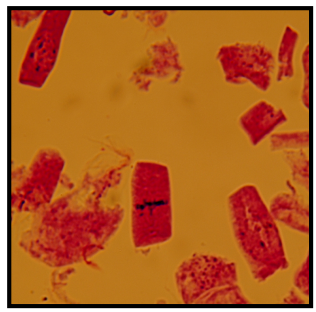


Figure 14



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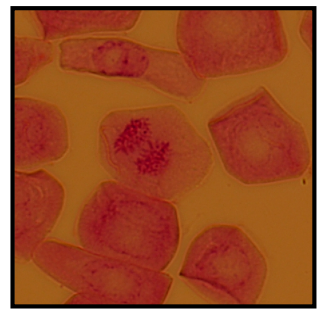

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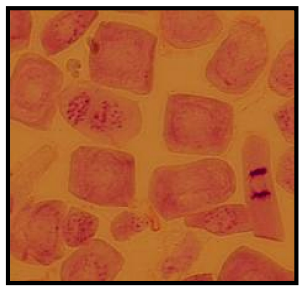

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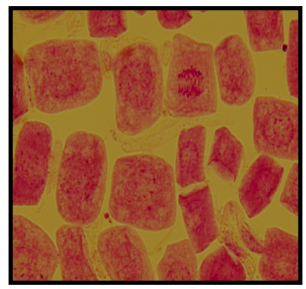

Figure 18