

THE EFFECT OF SORBITOL AND CONSERVATION PERIOD ON THE *IN VITRO* EVOLUTION OF *SOLANUM TUBEROSUM* L. PLANTLETS

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ABSTRACT. Local potato varieties are kept at Suceava Genebank, Romania, in the experimental field collection and *in vitro* by *slow growth* method which allows limited plantlets growth, thus prolonging the time between two subcultures. The paper presents some results concerning the influence of a growth inhibitor, namely sorbitol (as osmotic agent), in the development of plantlets belonging to five local varieties of potato (*Solanum tuberosum* L.), after different periods of *in vitro* storage, while reducing temperature and light intensity. During the experiment were evaluated several morphological aspects: number, length and branching of shoots, roots, number of viable nodes and number of viable shoots. The observations revealed that there has been registered a growth retardation of inoculums, while maintaining their viability and vitality. Although there were differences in growth patterns of these five potato varieties, as a general rule, the main effects of growing conditions has been a reduced plantlets height (2-4 cm) by the shortening of internodes and the contraction of leaflets size, to 1.5-2 mm. The results obtained

during *in vitro* conservation for 17 months, of five local potato varieties on MS medium with 40 g/l sorbitol (C₂₄ culture media), recommend this solution for a wider use in the preservation of potato collection from the Suceava Genebank.

Key words: Potato; Local varieties; Sorbitol; Slow growth.

REZUMAT. Efectul sorbitolului și al perioadei de conservare asupra evoluției *in vitro* a plantulelor unor varietăți locale de *Solanum tuberosum* L. La Banca de Gene Suceava, varietățile locale de cartof sunt menținute în câmpul experimental și în colecția *in vitro* prin metoda *creșterii lente*, care limitează creșterea plantulelor, permițând, astfel, prelungirea duratei dintre două subculturi. Lucrarea prezintă o parte din rezultatele referitoare la influența unui inhibitor de creștere, respectiv sorbitolul (ca agent osmotic), asupra evoluției plantulelor a cinci varietăți locale de cartof (*Solanum tuberosum* L.), după perioade diferite de conservare *in vitro*, concomitent cu reducerea temperaturii și a intensității luminoase. În timpul experimentului au fost

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evaluate o serie de aspecte morfologice: numărul, lungimea și ramificarea lăstarilor, înrădăcinarea, numărul de noduri viabile și numărul de lăstari viabili. Observațiile efectuate au relevat faptul că s-a obținut o încetinire a creșterii inoculilor, concomitent cu păstrarea viabilității și a vitalității lor. Deși s-au înregistrat diferențe în tipul de creștere a celor cinci varietăți de cartof, efectul principal al condițiilor de cultivare a fost reducerea înălțimii plantulelor (2-4 cm) prin scurtarea internodiilor, precum și reducerea dimensiunii frunzulițelor, care au devenit solziforme (1,5 – 2 mm). Rezultatele obținute în cursul conservării *in vitro*, timp de 17 luni, a celor cinci varietăți locale de cartof pe mediul MS, cu 40 g/l sorbitol (mediul C₂₄), recomandă această soluție pentru o folosire la scară mai largă în prezervarea colecției de cartof de la Banca de Gene Suceava.

Cuvinte cheie: cartof; varietăți locale; sorbitol; creștere lentă.

INTRODUCTION

In Romania, after cereals, potato (*Solanum tuberosum* L.) is the second important culture and a base foodstuff in the diet of the people. The importance of research on potato local varieties derives from the fact that, with their high heterogeneity, they have the advantage of being much better adapted to biotic and abiotic stress conditions (diseases, pests, drought, low nutrient content etc.). Unfortunately, many of these varieties are lost because their replacement with new varieties, genetically uniform but more productive.

For this reason this part of biodiversity conservation must be ensured through germplasm collections in gene banks where the

native genetic material has to be protected and maintained for further use. An appropriate method of potato germplasm storage is the material conservation in *slow growth* conditions (Cachiță - Cosma, 1987; Engelmann and Drew, 1998; Sarkar and Naik, 1999).

The principle of slow growth storage allows a safe use of *in vitro* culture without the disadvantages of frequent subcultivation. The cultures can be observed while they grow and can be returned to normal multiplication subculture (Withers, 1991).

The combination of osmotic active substances with low temperatures and reduced light intensity is the most effective way to extend the period of subculture (Golmirzaie and Toledo, 1998) and particularly useful for local varieties of potato (Kotkas, 2004; Ciobanu *et al.*, 2011).

MATERIALS AND METHOD

The experiments were conducted at the Suceava Genebank on five local potato varieties grown *in vitro* and the aim was to get information about the evolution of minicuttings on conservation medium C₂₄, for a period of 2, 7, 12 and 17 months to assess the influence of sorbitol (as osmotic agent) for the medium-term conservation of this species.

The culture medium used was based on Murashige-Skoog formulation (MS-1962) in ½ dilution with low concentration of growth regulators, sucrose 2% and 40 g/l sorbitol (*Table 1*).

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Table 1 - The main components of the culture medium used for *in vitro* conservation of local potato genotypes

Variant	Basal culture medium & hormonal balance	Other components
C ₂₄	MS ½ ¹ + 0,02 mg/l K ² , 0,02 mg/l BA ³ +0,02 mg/l NAA ⁴	20 g/l zaharoză + 40 g/l sorbitol

¹Murashige-Skoog; ²Kinetin; ³Benzyladenine; ⁴Naphthaleneacetic acid;

The researches were initiated by using minicuttings with 1-2 nodes prelevated from potato local populations SVGB 14376, SVGB 15079, SVGB 15102, SVGB 15140 and SVGB 15446, maintained in the *in vitro* collection of Suceava Genebank.

The pH of the culture medium was adjusted to 5.7 with NaOH 0.1 N, before the agar addition, and medium was solidified with 0.75% agar-agar. Sterilization of media was done by autoclaving at 121°C for 20 minutes.

After inoculation and flask closing, they were transferred into growth chamber, where they were placed under the light of 2000-2500 lx with a photoperiod of 16/24 hours, at a temperature of 20-22°C and an ambient humidity of about 60 -70%.

After four weeks, the samples were transferred to storage room, where cultures were maintained at temperatures between 6-12°C, in white fluorescent light with an intensity of 1000 lx. and photoperiod 10/24 hours, for experimental periods of 2, 7, 12 and 17 months, respectively.

Because after 2 months of subculture the plantlets regenerated on the medium C₂₄, had a vigorous growth and a good evolution for all five local potato varieties analyzed, and the survival rate of the shoots was 100%, this period was taken as a reference (control) in the further *in vitro* development of the plantlets.

RESULTS AND DISCUSSION

The reaction of the explants taken from the five local potato varieties, inoculated and grown on medium C₂₄ was assessed during storage in order to understand, especially, how the plantlets adapt to the conditions and the genotype interaction with the culture medium.

Analyzing the effects of extending the storage period on the number of viable shoots/plantlet it was found that at seven months after the start of the experiment, the number of viable shoots/plantlet increased compared to the control. The differences are statistically significant in variety SVGB 14376 and very significant in case of varieties SVGB 15102 and 15140 SVGB. The increases recorded for other two varieties analyzed, SVGB 15079 and 15446 SVGB were without statistical signification (*Table 2*).

When the preservation period was extended to 12 months the most substantial increase, compared to the control, was observed on the variety SVGB 14376, the average value of this character was about 5.4 times higher, while for the variety SVGB 15079 the value was only 1.1 times higher, corresponding to a statistically insignificant increase.

Table 2 - The influence of genotype and storage period on the number of viable shoots/plantlet from five potato varieties grown in C₂₄ culture medium

Specification		Number of viable shoots (shoots and branches) / plantlet			
Potato genotype	Storage period	\bar{x}	%	Difference	Signification
SVGB 14376	2 months	5.7	100.0	0.0	cv
	7 months	12.0	210.5	6.3	*
	12 months	31.0	543.8	25.3	***
	17 months	26.2	459.6	20.5	***
		LSD 5% = 5.4	LSD 1% = 7.3	LSD 0.1% = 9.8	
SVGB 15079	2 months	6.1	100.0	0.0	cv
	7 months	6.6	108.2	0.5	ns
	12 months	7.0	114.7	0.9	ns
	17 months	7.5	122.9	1.4	ns
		LSD 5% = 2.1	LSD 1% = 2.8	LSD 0.1% = 3.7	
SVGB 15102	2 months	7.2	100.0	0.0	cv
	7 months	16.1	233.6	8.9	***
	12 months	18.6	258.3	11.4	***
	17 months	13.9	193.0	6.7	***
		LSD 5% = 3.4	LSD 1% = 4.6	LSD 0.1% = 6.1	
SVGB 15140	2 months	4.8	100.0	0.0	cv
	7 months	12.3	256.3	7.5	***
	12 months	17.1	356.2	12.3	***
	17 months	11.5	239.5	6.7	***
		LSD 5% = 3.0	LSD 1% = 4.0	LSD 0.1% = 5.3	
SVGB 15446	2 months	4.5	100.0	0.0	cv
	7 months	5.2	115.5	0.7	ns
	12 months	10.9	242.2	6.4	***
	17 months	19.4	431.1	14.9	***
cv – control variant		LSD 5% = 1.9	LSD 1% = 2.6	LSD 0.1% = 3.5	

Also, after 17 months of keeping plantlets in storage conditions, the differences between the average number of viable shoots/plantlet and the control values registered at 2 months were statistically very significant, except for the variety SVGB 15079, with statistically insignificant differences.

The evolution of this character, observed in the variety SVGB 15079, which values, compared to control, have no statistically significant differences, regardless the storage period on medium with sorbitol, reveals more clearly the influence of genotype on the adaptation way to relatively restrictive growth conditions.

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From the data presented the *Table 3* could be observed that the average shoot length/plantlet in the control variants ranged from 2.8 cm/plantlet in the variety SVGB 15102 to 7.8 cm/plantlet for the variety SVGB 14376, while the average number of nodes/shoot varied only from 5.9 to 7.4. These close average values in case of nodes number/shoot shows that the influence of sorbitol was quite constant on this character.

By extending the storage period from 2 months to 7 months a quite similar evolution of the five varieties of potato is relieved. In terms of shoots length growth the mean values ranged between 4.1 to 4.9 cm/plantlet, but the signification of the differences compared to the control varied from very negative to very positive. Thus, if on the variety SVGB 14376 the increase of conservation period to 7 months had a very significant inhibitory effect on the average of shoots length for other three varieties (SVGB 15102, SVGB 15140 and SVGB 15446) the effect was to stimulate shoot elongation, differences related to the control ranged from significant to very significant.

This evolution of the average of shoot length was accompanied by a very significant increase in the average number of nodes/shoot, regardless the variety, for 7 months of conservation. Compared to the control the differences for the average number of nodes/shoot show that the extending of conservation period, in

the conditions of this experiment, induced an accentuated shortening of internodes.

By increasing the storage period to 12 months the value of mean nodes number/shoot were higher compared to the control, except the variety SVGB 15079 where it was registered a decrease of about 15.9%, and the difference has a distinct significance.

When the storage period was extended to 17 months it was noted an accentuated reduction in the average of shoot length, which was accompanied by the decrease in the number of nodes/shoot. There was not a correlation statistically significant between the two characters. At the end of experimental period the differences from the control were all negative, but statistical significance was higher for shoot length.

If the evolution the average number of roots/plantlet it is analyzed it is clear that with the extension of the conservation period the average value of this character is increasing, and the differences from the control are at very significant level after 12 and 17 months, respectively, of storage, regardless of genotype (*Fig. 1*).

Could be noted however, that in case of variety SVGB 14376 the extension the storage period to 17 months resulted in a mean value of the character that exceeded the control about 2.6 times; at the opposite was the variety SVGB 15446, with an increase of about 1.8 times. The survival rate of plantlets after different conservation periods on the culture medium supplemented with 40 g/l sorbitol is presented in the *Fig. 2*.

Table 3 - The influence of genotype and storage period on shoot length and number of nodes, on five local potato varieties of grown on medium C₂₄

Specification		Shoot length / plantlet (cm)				Number of nodes / shoot			
Potato genotype	Storage period	\bar{x}	%	Difference	Signification	\bar{x}	%	Difference	Signification
		SVGB 14376	2 months	7.8	100.0	0.0	cv	6.5	100.0
7 months	4.4		56.4	-3.4	ooo	11.2	172.3	4.7	***
12 months	3.4		43.5	-4.4	ooo	8.4	129.2	1.9	**
17 months	1.3		16.6	-6.5	ooo	5.3	81.5	-1.2	o
		LSD 5% = 0.9 LSD 1% = 1.2 LSD 0.1% = 1.6				LSD 5% = 1.1 LSD 1% = 1.5 LSD 0.1% = 2.0			
SVGB 15079	2 months	4.8	100.0	0.0	cv	6.3	100.0	0.0	cv
	7 months	4.1	85.4	-0.7	ns	7.7	122.2	1.4	***
	12 months	2.8	58.3	-2.0	ooo	5.3	84.1	-1.0	oo
	17 months	1.9	39.5	-2.9	ooo	5.1	80.9	-1.2	oo
		LSD 5% = 0.9 LSD 1% = 1.3 LSD 0.1% = 1.6				LSD 5% = 0.7 LSD 1% = 1.0 LSD 0.1% = 1.3			
SVGB 15102	2 months	2.8	100.0	0.0	cv	5.9	100.0	0.0	cv
	7 months	4.5	160.7	1.7	***	10.6	179.6	4.7	***
	12 months	5.2	185.7	2.4	***	8.9	150.8	3.0	***
	17 months	2.4	85.7	-0.4	ns	5.6	94.9	-0.3	ns
		LSD 5% = 0.9 LSD 1% = 1.2 LSD 0.1% = 1.6				LSD 5% = 1.0 LSD 1% = 1.4 LSD 0.1% = 1.8			
SVGB 15140	2 months	4.2	100.0	0.0	cv	7.4	100.0	0.0	cv
	7 months	4.9	116.6	0.7	*	11.8	159.4	4.4	***
	12 months	4.0	95.2	-0.2	ns	7.8	105.4	0.4	ns
	17 months	2.8	66.6	-1.4	ooo	6.7	90.5	-0.7	ns
		LSD 5% = 0.5 LSD 1% = 0.7 LSD 0.1% = 1.0				LSD 5% = 0.9 LSD 1% = 1.2 LSD 0.1% = 1.6			
SVGB 15446	2 months	3.6	100.0	0.0	cv	6.8	100.0	0.0	cv
	7 months	4.7	130.5	1.1	**	12.1	177.9	5.3	***
	12 months	4.1	113.8	0.5	ns	10.5	154.4	3.7	***
	17 months	2.0	55.5	-1.6	ooo	6.4	94.1	-0.4	ns
cv – control variant		LSD 5% = 0.7 LSD 1% = 1.0 LSD 0.1% = 1.3				LSD 5% = 0.9 LSD 1% = 1.3 LSD 0.1% = 1.7			

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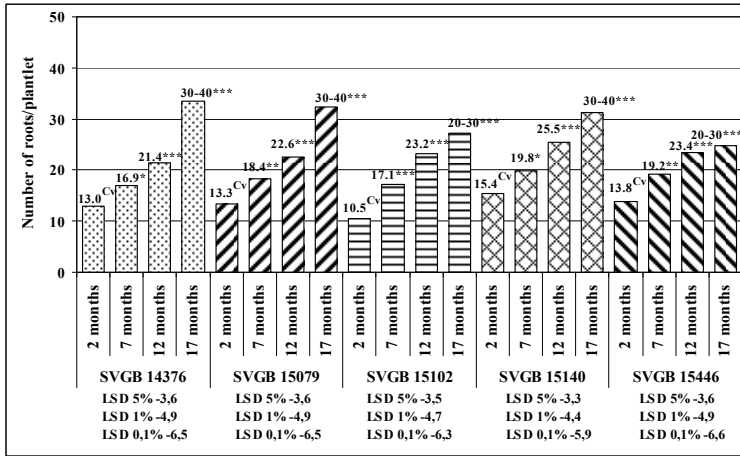


Figure 1 - The influence of genotype and conservation period on the average number of roots/plantlet from the five potato varieties maintained on the medium C₂₄

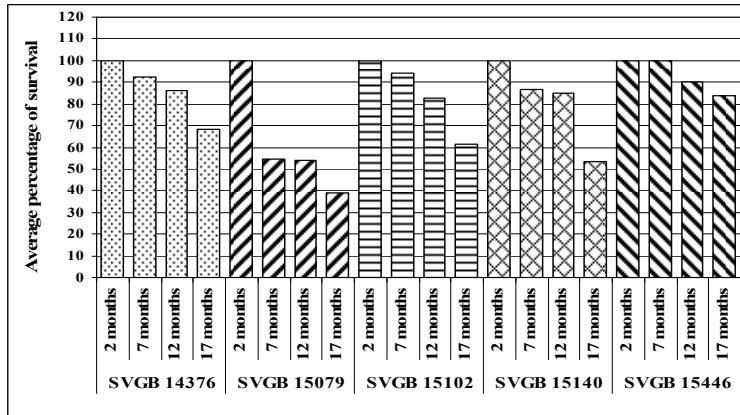


Figure 2 - The influence of genotype and subculture period on the average rate of shoot survival on conservation medium C₂₄

The increase of plantlets storage period on the medium with sorbitol, in the conditions of conservation room, induced a decrease in the number of shoots survival with an average of 14.5% after 7 months, 20.5% after 12 months and 38.9%, respectively, after 17 months of conservation.

It was also found that, if the extension of conservation until 7 months had no negative effect on the variety SVGB 15446, the survival rate of shoots remaining at 100%, instead caused a serious decline, with 45.5% of it on the variety SVGB 15079. On the other hand, the increase the storage period to 12 months caused a

minor variation of this character on the varieties SVGB 15079 (0.7%) and SVGB 15140 (1.6%).

After 17 months of preservation it was noted the variety SVGB 15446, which had the maximum survival rate (83.8%), associated with a relatively good state of plantlet vitality, thus having a high potential for regeneration in the subculture.

CONCLUSIONS

The studies have concluded that the sorbitol and physical conditions from the conservation room influenced the morphological characteristics of potato plantlets belonging to the five genotypes.

The sorbitol, added to the amount of 40 g/l in the culture medium, inhibited progressively the growth of plantlets, producing compact shoots with short internodes, and positively influenced the rooting process. At the same time the sorbitol had a beneficial effect on the plantlets during the extended storage period.

In terms of *in vitro* conservation on medium-term of the five local varieties under study, the culture medium C₂₄ proved to be quite effective, reporting a medium survival rate of 85.5% shoots after 7 months, 79.5% after 12 months and 61.1% after 17 months of conservation.

From the five local potatoes varieties included in the experiment, after the four periods of maintenance *in vitro* on *slow growth* storage conditions, on the tested medium, the best development of shoots was registered on the variety SVGB

15446, with 93.4% average survival of sprouts, and the lowest development was recorded in variety SVGB 15079, with a shoot survival of about 61.8%.

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