IN VITRO CONSERVATION OF *SOLANUM TUBEROSUM VAR. GERSA*

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Abstract

In this paper we describe a slow growth technique (Living Collection) for micropropagation optimization of Solanum tuberosum var. Gersa. We used 2 systems: double layer system (using silicone oil, paraffin oil and castor oil) and for single layer (manithol, sorbithol, AgNO3, B9). For the double layer system the vitroplantlets were inoculated on simple MS media without growth regulators, after 2 weeks – when the plantlet manifests a caulo- and rizogenetic process, we applied the second layer – the oil; for the single layer system – the growth inhibitors were integrated in MS nutritive media. This is on going experiment, until this moment the plantlets were vitroconservated 24 weeks in Living Collection, the control lot being subcultived already 2 times: after 12 and 24 weeks of vitroculture. In 24 weeks of Living Collection the vitroplantlets from the slow growth system did not reach the growth values which control lot achieved in 12 weeks of vitroculture – this showing the efficiency of the presented system.

Key words: potato, *Solanum tuberosum*, slow growth, Living Collection, double layer system

INTRODUCTION

According to the latest revision, the world population is projected to grow by 34% from over 7 billion today to 9,1 billion in 2050. According to *FAO's baseline projections*, it should be possible to meet the future food and feed demand of the projected world population in 2050 within realistic rates for land and water use expansion and yield development. However, achieving this will not be automatic and several significant challenges will have to be met (Bogdan et al., 2010; Pop and Cachiţă, 2011; Blidar et al., 2011, 2012; FAO, 2012). For the future, the current economy must be replaced with an new concept of bio-economy, based on ecology and environment protection, adapted to the rural area based on Agrifood Biodiversity, and scientists have a major contribution in this regard (Bogdan et al., 2010, 2011; Petruş and Cachiţă, 2011; Pop et al., 2011; Purcărea et al., 2010).

To achieve these objects using the modern biotechnology, both in animals (Ipate et al., 2011) and the plant (Petruş and Cachiţă, 2010; Petruş, 2011a; 2011b), is a future solution, and using with maximum efficiency of

biotechnological methods which provide scientific data and certainty procedures used. Plant biotechnology will be the domain that, in the future, will provide much needed human nutrition, in addition to traditional methods *In vitro* cultures are used for many plant species, which enables us to obtain healthy plants in a short time (Antofie et al., 2010 a and b; Petruș et al., 2011). These plants are maintained in gene banks and periodically subcultured (Cachiță and Constantinovici, 2008; Ciobanu at. al, 2012), potato (*Solanum tuberosum*) is a major crop worldwide, providing at least 12 essential vitamins, minerals, proteins, carbohydrates and iron (Ciobanu et al., 2011; Cachiță and Sand, 2011). In this paper we describe a slow growth technique (*Living Collection*) for micropropagation optimization and efficiency of *Solanum tuberosum var*. *Gersa*, ensuring the virus-free plants production using non invasive, ecosanogenic and bio economic methods - by reducing the frequency of subcultures, can be reduced the working staff, the propagation costs and the energy consumption.

MATHERIAL AND METHODS

In this experiment we used local variety of potato from Suceava Gene Bank: *Solanum tuberosum var*. *Gersa* (14381 – blue potato), cultivated on MS media without growth regulators. For creating the Living Collection we used 2 systems: double layer system (using silicone oil, paraffin oil or castor oil) and for single layer (manithol, sorbithol, AgNO3, B9) table 1. For the double layer system the vitroplantlets were inoculated on simple MS media without growth regulators – lick the control group (the MS nutritive media is Murashige-Skoog (1962), modified by us, without fitohormons and glycine, with vitamins (thiamine HCl, pyridoxine HCl, nicotinic acid, each 1 mg/l), meso-inositol 100 mg/l, sucrose 30 g/l and agar 7 g/l), after 2 weeks – when the plantlet manifests a caulo- and rizogenetic process, we applied the second layer – the oil; for the single layer system – the growth inhibitors were integrated in MS nutritive media.

Table 1.

Used variants.

Type of system	Cod	1st layer	2end layer
Control group	V0	MS single layer without growth inhibitors	
Double layer system	V1	MS without growth inhibitors	3 cmc silicone oil
	V2	MS without growth inhibitors	3 cmc paraffin oil
	V3	MS without growth inhibitors	3 cmc castor oil
Single layer system	V4	MS with 50 mg/l manithol	-
	V5	MS with 50 mg/l sorbithol	-
	V6	MS with 10 mg/l AgNO3	-
	V7	MS with 50 mg/l alar (B9)	-

This is on going experiment, until this moment the plantlets were vitroconservated 24 weeks in Living Collection, after each 4 weeks the vitroplantlets were measured (the vitroplantlet´s length, average number of nods on the main stem, average number if ramification and leafs, and average number of roots, percentage of survival).

DISCUSSIONS

As it may be observed in figure 1–2, the potato vitroplantlets manifested a good response to the slow growth techniques, so in 24 weeks of vitroconservation in **double layer system** can be observed the fact that vitroplantlets have a very slow growth in compare with control lot. The inocula from V1 and V2 had the same length during the period of 24 weeks of slow glow system: 2,6 cm (V1 – being with 391,31% inferior in compare with control lot) and 2,8 cm (V2 – being by 382,61% inferior to control lot), instead a necrosis at the apical area, leafs and shoots has occurred at the V3 variant, these vitroplantlets have lost 30,43% of the stem length (fig. 1).

Instead, in the **single layer system** was observed a moderate positive evolution, so analyzing the inocula the lowest value of the stem length was remarked at V7 lot: 3,2 cm (the increase growth in 24 weeks of vitroculture was by 52,17%) – being lower than control lot by 365,22%, close values was observed at V4: 3,6 cm and V5: 3,5 cm, but the highest stem length was determined at V6 lot: 4,5 cm – being by 308,7% lower comparing to control lot witch at 12 weeks reached 11,6 cm (fig. 1). As can be observed, the vitroplantlets from the Living Collection even after 24 weeks on slow growth could not reach the control lot length – this aspect being more accentuated in double layers system – witch demonstrate the efficiency of the slow growth methods.

The control lot at 12 weeks of vitroculture had 11 nods/inocula, 8,9 shoots on the main stem and 22,19 leafs/inocula. In double time – 24 weeks of vitroconservation, the plantlets form double layer from V1 and V2 lot had only 4 nods, 3 (V1) and 2,9 (V2) shoots and both variants had 11 leafs/vitroplantlet, instead, because of the necrosis observed at the V3 variant, these plantlets had only 3 nods which caped the green color, 1 shoot which manifested a beginning of necrosis and only 4 leafs (fig. 1-2).

The biggest values - from the **single layer system** - was observed at V6 lot: 6,9 nods, 4 shoots on the main stem and a total average number of 15 leafs/inocula; more close values was registered at V4 – V5 and V7 variants: 5,31 (V4), 5,29 (V5) and 5,9 (V7) nods an the main stem, 2,9 (V4), 3 (V5) and only 2 (V7) shoots on the main stem and a total average number of leafs: 11,31 (V4), 12,85 (V5) and respectively 12 (V7) leafs/inocula – fig. 1-2.

The control lot of *Gersa* variety at 12 weeks of vitroculture had 18,24 roots – before the first subculture, but even in double time – in 24 weeks – the vitroplantlets from Living Collection did not reach that result, so, the biggest number of roots from double layer system was observed at V2 plantlets: 10,15 roots/ inocula, 8,24 roots at V1 lot, and the smallest number of this system at V2 variant: 6,1 roots/plantlet. In the single layer system the weakest results were observed at V6: 6,36 roots and V7: 8,1 roots/inocula, higher number of roots being counted at V4: 10,24 and V5: 11,17 roots/vitroplantlet, also even after 24 weeks these plantlets did not reach the performance of the control lot, which is the demonstration of the efficiency of the slow growth techniques.

The survival percent of *Gersa* variety was 95% and 90% at V1 and respectively V2 variants, a much lower percent: only 60% of vitroplantlets survived an V3 lot – even that remained plantlets presented a small degree of necrosis at apical area, at some shoot and leafs area, instead in single layer system that survival percent was 100% in all variants, excepting V7: only 80% of vitroplantlets stayed viable (fig. 2).

CONCLUSION

Studying the evolution in Living Collection 2 *Solanum tuberosum var. Gersa*, after 24 weeks of slow growth systems, we can recommend the double layer system, using V1 and V2 variants for efficient inhibition of growth and a good plant survival percent, but also, the V4 and V5 variant in case we need a rapid propagation of the germplasm, these variant being capable of generating a lot of nods on the main stem and a lot of new shoots.

ACKNOWLEDGEMENT

This work was co financed from the European Social Fund through Sectorial Operational Programme Human Resources Development 2007-2013, project number POSDRU/89/1.5/S/63258 ”Postdoctoral school for zootechnical biodiversity and food biotehnology based on the eco-economy and the bio-economy required by eco-sanogenesys”, coordinated by the National Institute of Economic Research ”Costin C. Kirițescu”, Romania, in collaboration with ”Lucian Blaga” University of Sibiu, ”Vasile Goldiş” West University Arad, S.C. GNIR Holding S.A. and S.C. SIAT S.A. The content of this material does not necessarily represent the official position of the European Commission or Romanian Government.

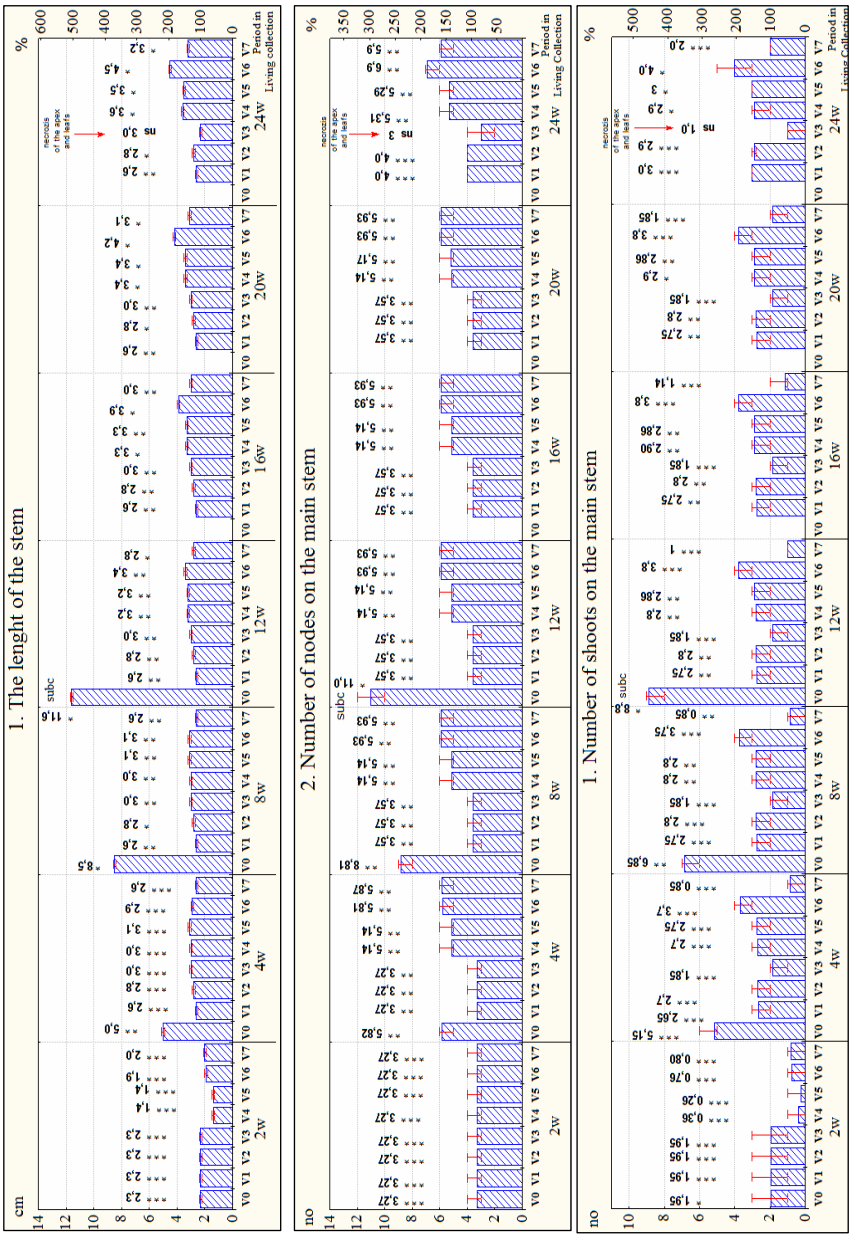

Figure 1. *Solanum tuberosum var*. *Gersa* vitroplantulets length, nodes number and number of shoots on the main stem and total number of leafs/inocula in the period of 2 - 24 weeks in Living Collection, where: V0-control: monolayer MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil, V2-MS with 3 ccm of paraffin oil, V3-MS with 3 ccm of castor oil, single layer system: V4-MS with 50 mg / l manitol, V5-MS with 50 mg / l sorbitol, V6-MS with 10 mg / l AgNO3, V7-MS with 50 mg / l alar (B9). P = statistical significans: p>0,05 = ns non significant; p<0,05 * significant; p<0,01=** distinctly significant; p<0,001=*** very significant. Subc. = first subculture of the control variant.

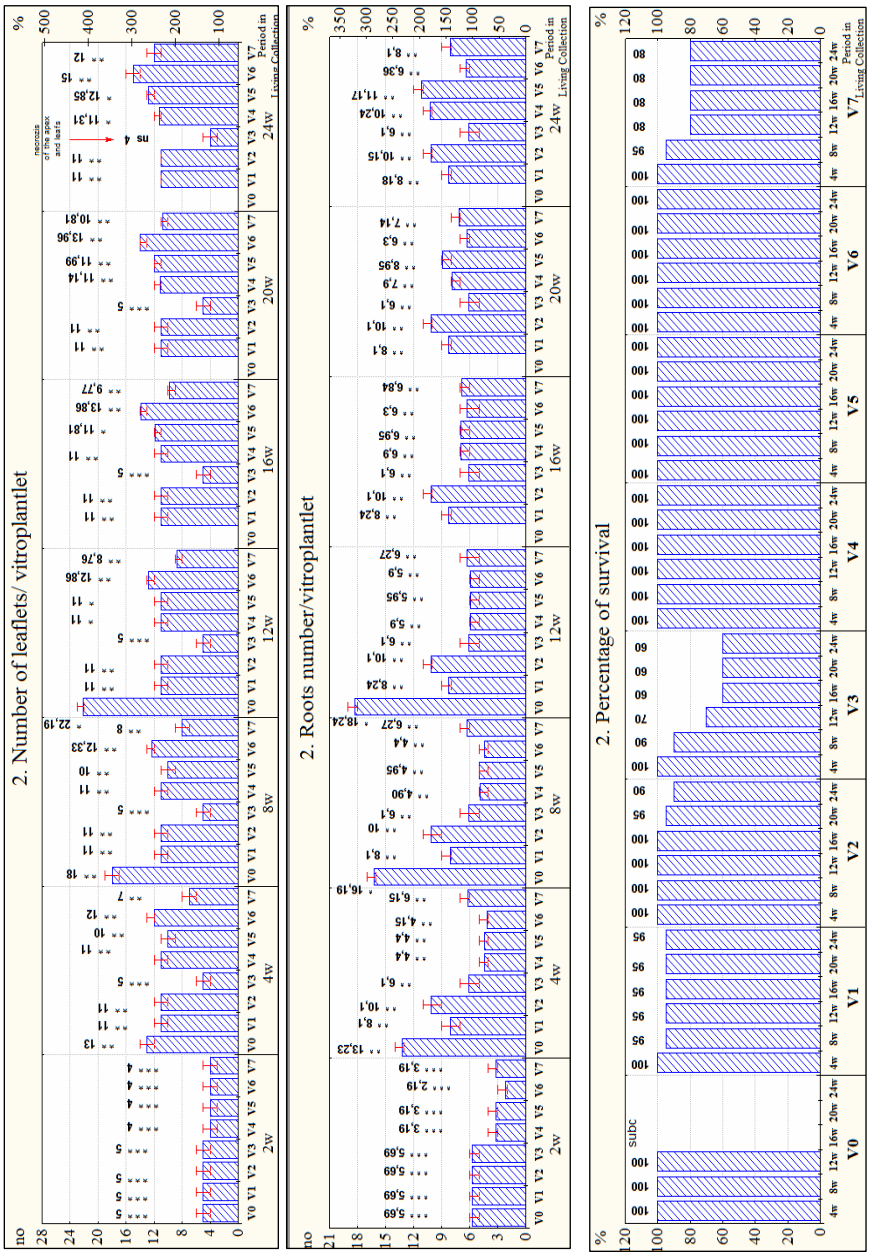

Figure 2. *Solanum tuberosum var. Gersa* vitroplantulets number of leaflets, number of roots and percentage of survival in the period of 2 - 24 weeks in Living Collection, where: V0-control: monolayer MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil, V2-MS with 3 ccm of paraffin oil, V3-MS with 3 ccm of castor oil, single layer system: V4-MS with 50 mg / l manitol, V5-MS with 50 mg / l sorbitol, V6-MS with 10 mg / l AgNO3, V7-MS with 50 mg / l alar (B9). P = statistical significans: p>0,05 = ns non significant; p<0,05 * significant; p<0,01=** distinctly significant; p<0,001=*** very significant. Subc. = first subculture of the control variant.

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