TESTING THE REGENERATIVE CAPACITY OF *SOLANUM TUBEROSUM* VAR. *GERSA* EXPLANTS AFTER 24 WEEKS STORAGE IN LIVING COLLECTION

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Abstract

In this paper analyse the regenerative capacity of Solanum tuberosum var. Gersa resulting in subculture from a slow growth system (Living Collection) applied for a period of 24 weeks. The storage system was formed from double layer system (using silicone oil, paraffin oil and castor oil) and for single layer (manithol, sorbithol, AgNO3, B9). After 12 and 24 weeks of vitroconservation we subcultured binodal minicutings on new nutritive media without growth inhibitors for testing the regenerative capacity. For vitroconservation in Living Collection of potato vitroplantlets we recommend silicone oil in double layer system – in subculture the inocula keep the regenerative capacity. Also, for a rapid regeneration of a big number of explants from the single layer storage system we recommend manitol or sorbitol as growth inhibitor, the vitroplantlets resulted in subculture from this variants being capable to generate large number of nods an ramifications/inocula.

Key words: potato, *Solanum tuberosum*, slow growth, Living Collection, regenerative capacity

INTRODUCTION

According to the latest literature, which is particularly rich in this area, it was proved that conventional methods of preserving plant material in gene banks are: conserving seeds, pollen or vegetative propagation organs or plants collections maintained in the field (which, unfortunately, does not provide certainty regarding maintaining the variety of the vegetation, an other issue is the variability, and extreme weather conditions), or as vitrocultures (Cachiţă şi Sand, 2011).

The methods of storage and preservation of plant material in the form of *tissue culture and cells culture*, in total aseptic conditions, are part of modern procedures to conserve valuable genotypes. Currently, biotechnology is focused on developing solutions for storage and then rapid cloning in vitro after stage period (Withers, 1990, Yoshimatsu şi colab., 2000; Cachi□ă and Constantinovici, 2008; Radove□ and Cachi□ă, 2012), which is very important aspect in micropropagation (Ciobanu şi colab., 2011).

Over the years various methods were used for slowing the growth rate of fitoinocula, they increase the range of subculture and improve the yield of micropropagation, in the following we describe some of the main procedures used for this purpose:

- using a minimal quantity of culture medium for slow growth, or introduction into the culture media some growth regulators or using sucrose-free culture media (Jones, 1974), or introducing abscissic acid in the culture media (Henshaw et al., 1978), or adding silver nitrate (Turhan, 2004), or diaminozid, mannitol or sorbitol (Ciobanu et al., 2011b) etc.
- using mineral oil, as second layer, especially on callus tissue cultures of potato plantlets for delaying the growth rate (Caplin, 1959, Augereau et al., 1986), conservation of *Lilium martagon* and *Lilium candidum* vitroplantlets under paraffin oil (Bolba, 2004), or paraffin oil and castor oil (Petrus et al., 2006), or storage of *Coleus* vitroplantlets under silicone oil (Radoveț et al., 2008; 2012);
- storage for one year of dehidtared *Daucus carota* callus, or somatic (Gray, 1987; Nitzsche , 2004; Baciu et al., 2007);
- Low pressure / low amount of oxygen in the atmosphere of fitoinocula (Bridgen and Staby, 1981);
- using positive low temperature, between 2-8° – to prevent freezing of fitoinocula (Aitken and Singh, 1987) or 10°C (Hassan, 2004);
- for long-term conservation is practiced the cryopreservation method by bringing to zero the cell metabolism, by freezing the cultures in liquid nitrogen (-196°C) - (Bajaj, 1990; Halmagyi et al, 2003; Baciu et al., 2007; Cachi□ă and Sand, 2011).

MATHERIAL AND METHODS

In this experiment we used the *Solanum tuberosum var. Gersa* vitroplantlets vitroconserved for 24 weeks in Living Collection (Radove□ and Cachi□ă, 2012). The Living Collection contains 2 systems: double layer system (using silicone oil, paraffin oil or castor oil) and for single layer (manithol, sorbithol, AgNO3, B9) table 1. For testing the regenerative capacity, after 12 and 24 weeks of vitroconservation, we subcultured binodal minicutings on new MS nutritive media without growth inhibitors: 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 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).

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)	-

DISCUSSIONS

In this experiment we used local variety of potato from Suceava Gene Bank: *Solanum tuberosum var*. *Gersa*, 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 (Radove□ and Cachi□ă, 2012). After 12 (Subculture 1) and 24 weeks (Subculture 2 – fig. 1) of Living Collection storage we performed a subculture for testing the regenerative capacity of the vitroplantlets.

The evaluation of the potato vitroplantlets growth resulted in subculture after 12 weeks of storage in Living Collection showed a normal growth, without any inhibition effects. But after 24 weeks of storage, the resulted vitroplantlets in subculture had a some signals of slowed growth even being subcultived on MS media without growth regulators. So, even from 4 weeks of subculture all vitroplantlets showed a positive reaction, but the influence of the type of the growth inhibitor from the Living Collection was visible during all 12 weeks of subculture period. The vitroplantlets resulted in subculture from the V1 variant (Silicone oil) at the end of 12 weeks of subculture have the stem length of 6 cm (being by 250% lower them control lot, witch marked 11 cm), the inocula resulted from V2 variant (paraffin oil) have a stem length on only 5 cm (this value being lower by 300% comparative to control lot) and the lowest reaction of the vitroplatlets in subculture was observed at V3 variant (resulted from double layer system using castor oil) – at 4 weeks no change was remarked, at 8 weeks first signs of necrosis was observed, and after 12 weeks all inocula was senescent (fig. 2).

Instead, the vitroplantlets resulted in subculture from single layer system survived in proportion of 100%, after 12 weeks of subculture the

inocula resulted from the V4 and V5 variants have the same stem length of 10 cm (being lower then control lot by 1 cm=50%), 9 cm have vitroplantlets resulted from V6 lot (being by 2 cm = 100% lower then control lot), the lowest value of this parameter in the single layer system being the length of the vitroplantlets coming from the V7 variant only 6 cm – being lower by 250% comparative with control lot (B9 had the strongest inhibitor effect even in subculture) – fig. 2.

Regarding the number of nods and the number of ramifications on the main stem on the vitroplantlets resulting from double layer system, the heist values was observed at the inocula resulting from V1 variant (siliconic oil), at the end of 12 weeks of subculture they presented 5 nods (being by 91,74% lower then control lot) and 3 ramifications (3 times lower comparative with control lot). The lowest values of this parameter was registered at the vitroplantlets resulting from V3 (castor oil), starting from 8 weeks of subculture at the inocula was identified the necrosis process, the neoformation of nods or ramifications was not observed.

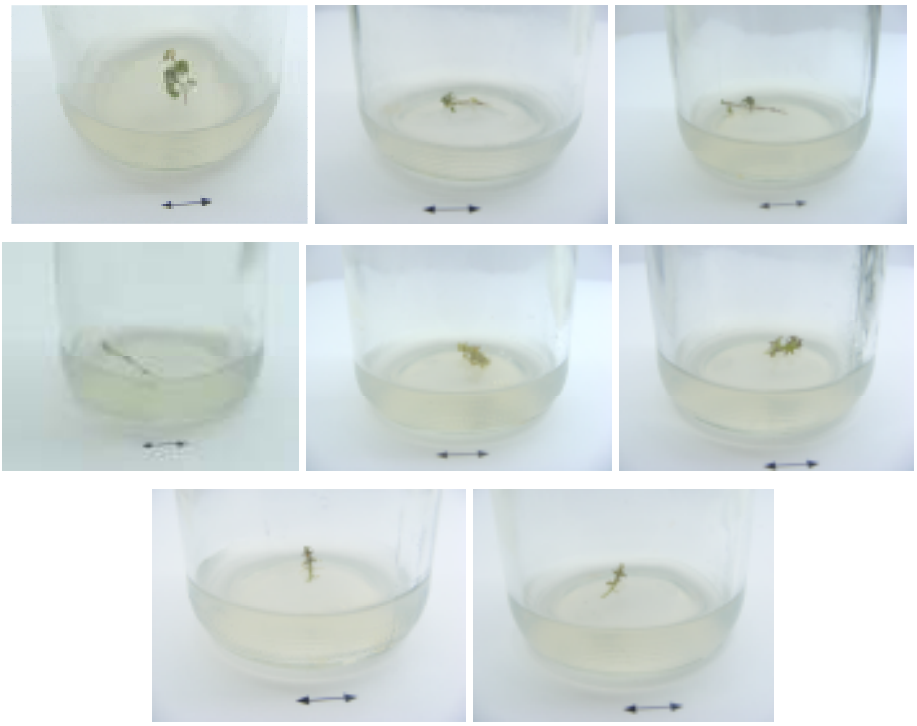

Figure 1. The evolution of *Solanum tuberosum* var. *Gersa* vitroplantlets after 4 weeks of subculture, resulted from slow growth system in Living Collection - 24 weeks, where: V0-control: monolayer MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil, V2-MS with 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).

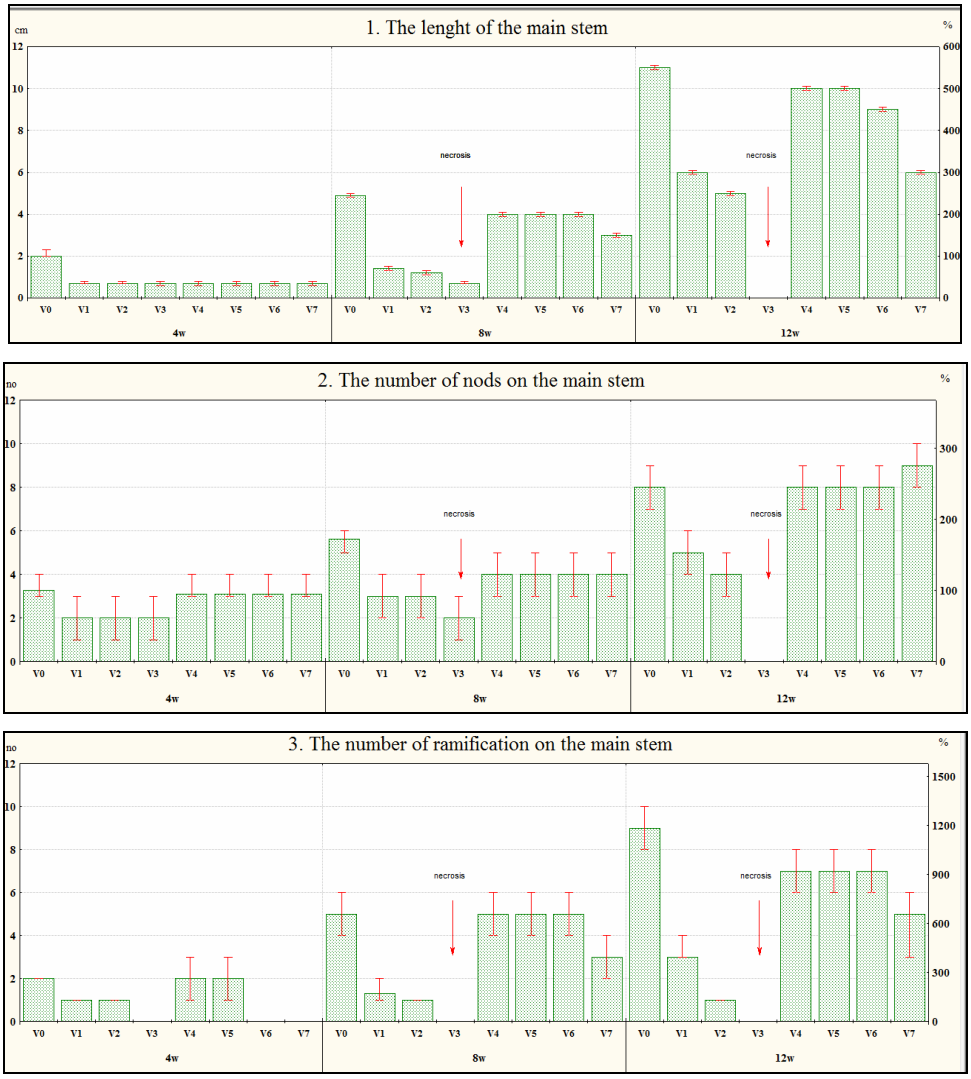

Figure 2. The evolution of *Solanum tuberosum* var. *Gersa* (blue potato), in 12 weeks of subculture from Living Collection storage, where: V0-control: monolayer MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil, V2-MS with 3 ccm of paraffin oil, V3-MS with 3 ccm 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).

Instead, the vitroplantlets resulted in subculture from the single layer system marked a positive reaction, the vitroplantlets resulted from the V4-

V6 variants after 12 weeks of subculture having 8 nods on the main stem – the same value lick control lot and 7 ramification (lower them control lot by 100%=2 ramification), an interesting situation was observed at the inocula resulting in subculture from V7 lot: 9 nods/vitroplantlet (same value as control lot) and only 5 ramifications (lower by 200% comparative with control lot) - the stem had a general compressed look, having very small internodal spaces (fig. 2).

CONCLUSIONS

The regenerative capacity of *Solanum tuberosum var. Gersa* vitroplantlets is depending on the type of the vitroconservation system: double or single layer and the storage period. The prolonging of the storage period affect more or lass the regenerative capacity depending on the growth ingibitor. So, for vitroconservation in Living Collection of potato vitroplantlets we recommend silicone oil in double layer system – in subculture the inocula keep the regenerative capacity. Also, for a rapid regeneration of a big number of explants from the single layer storage system we recommend manitol or sorbitol as growth inhibitor, the vitroplantlets resulted in subculture from this variants being capable to generate large number of nods an ramifications/inocula.

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