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THE MICTROPROPAGATION EFICIENSY BY SLOWING THE GROWTH RATE OF *SOLANUM TUBEROSUM VAR. GERSA* VITROPLANTLETS

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ABSTRACT. In plant "living collection" for micropropagation optimization of potato local variety we used a slow growth technique, we used 2 systems: the double layer system (using a basal agarized medium and as supernatant a second layer with silicone oil or paraffin oil, or castor oil), and a normal agarized medium - by adding in culture media: manithol, sorbithol, AgNO3 or B9. The plantlets were vitroconservated 48 weeks in slow growth system; the control lot - during the 48 weeks - was subcultived 4 times: after 12, 24, 36 and 48 weeks of vitroculture. In 48 weeks of vitroplantlets slow growth did not reach the growth values which control lot achieved in 12 weeks of vitroculture – this showing the efficiency of the presented system. Silicone oil - as supernatant - is the best variant of slowing the growth in double layer system of *Solanum tuberosum var. Gersa*, B9 (diaminozide) had the highest inhibitory effect in normal agarized media.

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

INTRODUCTION

Food security, according to FAO, exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and preferences for an active and healthy life. With 30 percent of the earth's land used for growing crops and pastureland, another 30 percent covered by forests, and a full 70 percent of abstracted fresh water used by agriculture, there is no question that agriculture must be at the centre of any discussion of natural resource management and global environmental objectives. 726 million tons of roots and tubers were collected from world soils in 2010! Given agroclimatic constraints, potato is the main root crop cultivated in temperate zones, while in the tropics, a broad array is cultivated but cassava is the major root crop (FAO, 2012). According to FAO′s statistics, the average potato production in Romania starting from 1992 to 2010 was 3.604.030 tones (FAO, 2012 – www1). Around the world, as well as in Romania, exists a lot of potato varieties in culture, some of them having a great value witch worth to be preserved. In the latest decades started a idea of germplasm conservation in gene banks, which assure the preservation of the genetic vegetal resources during long period of time 10 to 20 years, having the necessary condition to prevent the genetic or physic deterioration. Generally in gene banks are preserved some particularly parts of the plats lick: seeds, pollen, organs witch assure the vegetative multiplication of the plants (bulbs, rhizomes, tubers) (Cachiță and Sand, 2011). Conventional methods of preserving the vegetative material in gene banks as seeds, pollen, organs or clone collection in the field do not assure the preservation of the entire variety of the vegetal material. This methods come with high costs, big preserving spaces, the field collections – at a high density – exist the risk to be destroyed by pathogen agents or unfavorable climate factors, in conclusion al field culture can be destroyed overnight (Baciu et al., 2007a, 2007b). The impossibility to solve these disadvantages of the conventional methods of preservation and the strong need to preserve vegetal resources lied to elaboration of new non-conventional storage methods in gene banks; lack the preservation of the tissue and cell culture in aseptic condition (Cachiță and Sand, 2011). There are a lot of methods of vitroconservation applied to fitoinocula:

- using a minimal quantity of culture medium for slow growth (Jones, 1974), using different growth inhibitors lick diaminozid, manithol or sorbithol (Ciobanu at al., 2011; Motallebi – Azar and Kazemianni, 2011); manipulation of the chemical components in the nutritive media (Rahman et al., 2011; Sultan et al., 2011);
- inducing hypoxia using mineral oil (Augereau et al., 1986), paraffin oil (Bolba, 2004), or paraffin oil and castor oil (Novakova et al., 2010), silicone oil (Radoveț and Cachiță, 2012a, 2012b);
- dehydrated organs or callus (Gray, 1987; Nitzsche et al., 2004; Baciu et al., 2007b);
- manipulation of the temperature (Aitken-Christie and Singh, 1987; Augustin Moh et al., 2011; Hassan, 2004);
- cryopreservation method (-196°C) (Bajaj, 1990; Halmagyi et al, 2003; Baciu et al., 2007b; Cachiță and Sand, 2011);
- using magneto-fluidic nanocomposites (Baciu et al., 2007a; Baciu, 2008b).

In plant breeding programs, the old varieties frequently represent an important and valuable gene source, these being characterized by homeostasis, many valuable particularities but most of them still insufficient known. The most characteristics of the old varieties relate to tolerance, various biotic, abiotic

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stress factors and also to quality (Baciu, 2008b; Novakova et al., 2010; Ticona Benavente et al., 2011). For this study we used a local old variety of potato (*Solanum tuberosum var. Gersa* – blue potato) from Gene Bank of Suceava, by slowing the growth, our goal is to rise the efficiency of the microprogation and vitroconservation of one of the most important root crop cultivated all other the Word.

MATERIALS AND METHODS

In this experiment we used a local variety of potato from Suceava Gene Bank: *Solanum tuberosum var. Gersa* (14381 – blue potato), cultivated on MS medium (Murashige-Skoog, 1962) modified by us, without fitohormons and glicine, with vitamins (thiamine HC, pyridoxine HCl, nicotinic acid, each 1 mg/l), meso - inositol 100 mg/l, sucrose 20 mg/l (30 g/l in the original recipe) and agar-agar 7 g/l. For creating the slow growth system in „living collection„ we used 2 systems: the double layer system (using a basal agarized medium and as supernatant a second layer with silicone oil or paraffin oil, or castor oil), and a normal agarized medium - single layer – by adding in the agarized medium: manithol, sorbithol, AgNO3 or B9 (alar). For the double layer system the vitroplantlets were inoculated on simple MS media, without growth regulators, and after 2 weeks – when the plantlet manifests a caulo- and rizogenetic process, we covered the basal agarized medium with a second layer – the oil; for the normal medium – the growth inhibitors were integrated in aseptic MS nutritive medium (table 1). The nutritive medium was distributed in glass test tubes by 1.6 cm diameter and 16 cm height and sterilized by autoclavation. The inoculation was performed in aseptic room; the culture was incubated at 22 - 23ºC, at 1700 lux light intensity, generated by white fluorescent lamps, with 16/24 light photoperiod. The plantlets were vitroconservated 48 weeks in slow growth system 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 of ramification and leafs, and average number of roots, percentage of survival). All data was data were processed and statistically analyzed in STATISTICA 6.0.

Table 1.

Used variants for experiments

Type of system	Cod	normal agarized medium	2end layer (as supernatant)
Control group	V0	MS single layer without growth inhibitors	-
	V1	MS without growth inhibitors	3 ccm silicone oil
Double layer system	V2	MS without growth inhibitors	3 ccm paraffin oil
	V3	MS without growth inhibitors	3 ccm castor oil
	V4	MS with 50 mg/l manithol	-
Normal agarized mediu	V5	MS with 50 mg/l sorbithol	-
Single layer system	V6	MS with 10 mg/l $AgNO_3$	-
	V7	MS with 50 mg/l alar (B9)	-

Note: ccm = cubic centimeters.

RESULTS AND DISCUSSIONS

At 12 weeks form the initiation of the experiment, the control lot reached the size of 10.8 cm (the growth increase from the first evaluation was 440%), occupying the entire available space of the container needed to be subcultived, so from the 12th week the control lot no longer appears in the graphics - over 48 weeks (the entire period of the experiment) - were subcultived 4 times (at 12, 24, 36, and 48 weeks after initiation of the experiment).

During the first 24 weeks of vitroconservation in Living Collection by two systems: double layer (covering the vitroplantlets with silicone oil, paraffin or castor oil) and normal agarized medium single layer system (by adding in the culture medium the growth retardants: manithol, sorbithol, nitrate silver or B9) we achieved to maintain these inocula in vitroculture without the need to perform subcultures, while the control group (MS without growth inhibitors) was subcultivated already 2 times. The vitroplantlets from the double layer system did not exceed the oil layer until the age of 48 weeks in slow growth system. The vitroplantlets grown in Living maintained the viability, as evidenced by slow progress, but positive one: at all levels as caulo- and rizogenetic aspects (according to the masurements and statistical processing of data), their regenerative capacity was evaluated by conducting regular subcultures on new growth medium without growth inhibitors.

After 48 weeks of slow growth in double layer system the growth slowing effect was highly emphasized in terms of size of the vitroplantlets, so, the **stem size** of the V1 group was 2.6 cm (with a total increase of 13.04% over the 48 weeks of vitroconservation), V2: 2.8 cm (with a total increase of 21.74%) and V3: 2.3 cm (with a total increase of growth by 30.43% up to 24 weeks of vitroconservation - when necrosis phenomenon was observed at the apex level of the vitroplantlets, losing 30.34% of the stem length, the remaining part of the inocula remaining green) - fig. 1 and 2. In contrast, in the normal agarized monolayer system, was observed a slow growth - but steady, so the highest value of this parameter was remarked at V5 lot: 7.27 cm (the stem of these vitroplantlets increased 3.63 times in the 48 weeks of vitroconservation), the size of the stem of the V6 lot was 5.09 cm (their size increased 2.67 times during 48 weeks of micropropagation), lot V4 had the stem

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length of 4.45 cm (their size increased 3.17 times during the entire experiment), instead the vitroplantlets from V7 lot had the shorter stem length: only 3.2 cm, their size increased only 1.6 times during the 48 weeks of vitroconservation.

The stagnation of growth evolution of the cormophytoinocula was observed in the **average number of knots** on the main stem, so at the end of 48 weeks of vitroconservation, the vitroplantlets from double layer system, showed the same number of 4 knots/inocula (the variants V1 and V2 – the total growth was only 22.23% increase over the entire period of vitroconservation during the 48 weeks) and 3 knots at the stem on V3 lot – at this variant was observed an accentuated partial necrosis phenomenon (up to 20 weeks of vitroconservation this variant was observed a positive trend of growth, reaching 3.61 knots/inocula, necrosis occurred after that time decreased all growth parameters) - fig. 1 and 2. A positive development - at the end of 48 weeks - have accomplished vitroplantlets from the normal agarized single layer system: the lowest value of this parameter was marked by lot V7: 6 knots/inocula (their size increased 1.83 times during the 48 weeks of vitroconservation), followed in ascending order values marked by lot V6: 8 knots/vitroplantlet (their knots number increased 2.44 times during the entire period of vitroconservation); 9 knots/inocula at lot V5 (their number increased 2.75 times during the entire period of the experiment) - fig. 2.

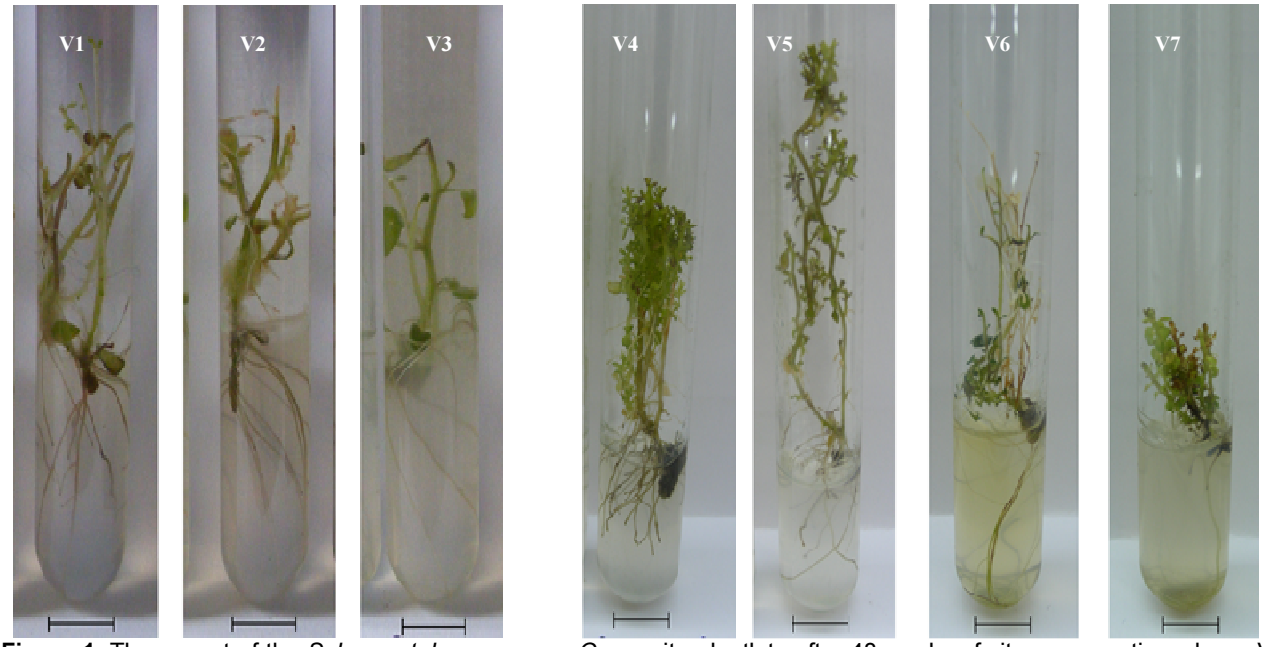
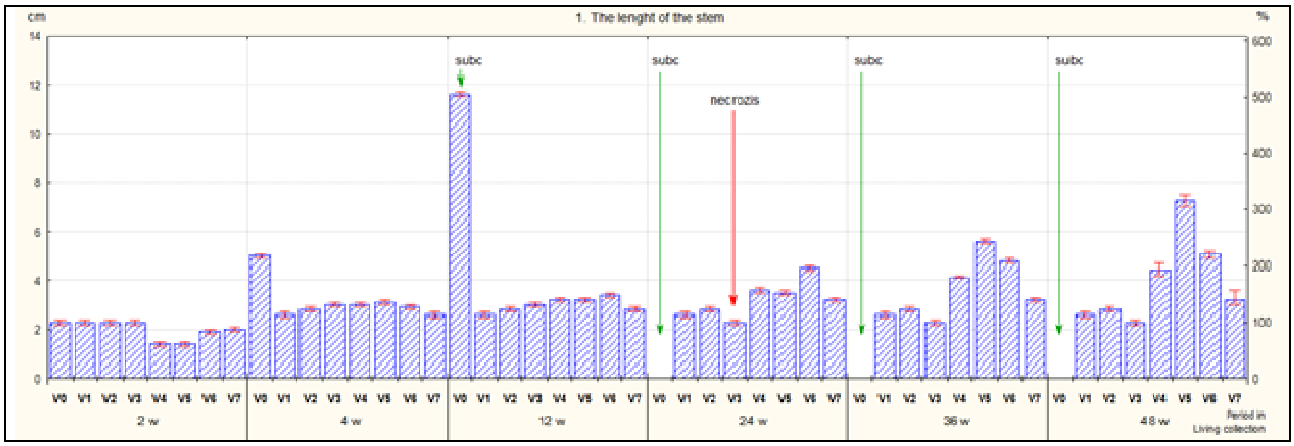


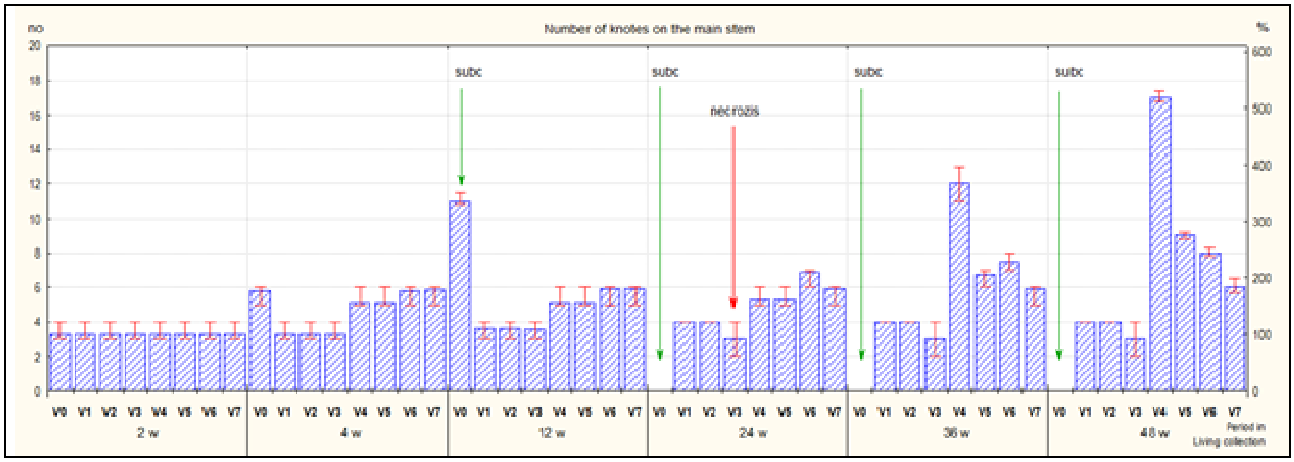
Figure 1. The aspect of the *Solanum tuberosum var. Gersa* vitroplantlets after 48 weeks of vitroconservation where: V0 control lot, MS normal agarized medium without growth inhibitors, the double layer system: V1-MS with 3 ccm of silicone oil, V2-MS with 3 ccm of paraffin oil, V3-MS with 3 ccm of castor oil , normal agarized medium (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).

Regarding the average number of ramifications, during the 48 weeks of vitroconservation in double layer system, at the plantlets level was observed a slow steady growth, at the end of this period was observed 3 ramifications at V1 lot (with a total increase of 53.85%), 2.9 ramifications in the V2 lot (with an increase of only 48.72%), the lowest value of this parameter - taking into account the necrosis throughout the entire period of vitroconservation (marking a loss of 49% of total number of ramification) - was observed in variant V3 - 1 each branch only viable / vitroplantlet. Regarding the development of the vitrocultures from normal agarized single layer system, they have a positive slow development, the lowest value of this parameter after 48 weeks from the initiation of the experiments was atV7 lot: only 7 branches/inocula - the most poor result - and the most effective for inducing the slowing of the growth regarding this parameter, the number of ramifications increased 8.75 times during the 48 weeks of vitroculture fig. 1 and 3. In ascending order was the values of lot V5 and V6 which marked: 9 branches / inocula (number of ramifications in V5 group increased 34.61 times, while the V6 group: 11.86 times during the 48 weeks vitroconservation), the highest value of this parameter was observed in the group V4: 20 ramifications (number of branches in this group increased 55.55 times throughout the experiment) - fig. 1 and 3.

A steady increase in the average number of leaves / inocula was observed during the 48 weeks in most variants of the normal agarized medium – single layer system, compared with the number of leaves from the double layer system where the found values at the age of 4 weeks remained constant up to 48 weeks in groups

V1 and V2: each having 11 leafs/vitroplantlet (with a total growth by 120% during the entire experiment), the lowest value of this parameter was observed at V3 variant, where a positive development was observed until the age of 20 weeks - marking the value of 7 leafs/vitroplantlet, while at the age of 24 weeks - when necrosis was observed - there was a loss of 60% from the leafs effective, so by the end of the experiment remained viable only 4 leafs/inocula - fig. 1 and 3. In contrast, the vitroplantlets from the normal agarized medium – single layer system was registered a positive slow positive trend of growth, taking into account the positive evolution in the average number of knots and ramifications/cormofitoinocula, the lowest value of this parameter was recorded at V6 lot: only 44 leaves/inocula (the number of leaves - throughout the experiment - increased 8.8 times), 56 specimens in group V7 (number of leaves increased 11.2 times), 70 copies at V5 group (number of leaves increased 14 times), and the highest value of this parameter was recorded in the group V4: 140 leaves/fitoinocula - their number increased 28 times - fig. 3.





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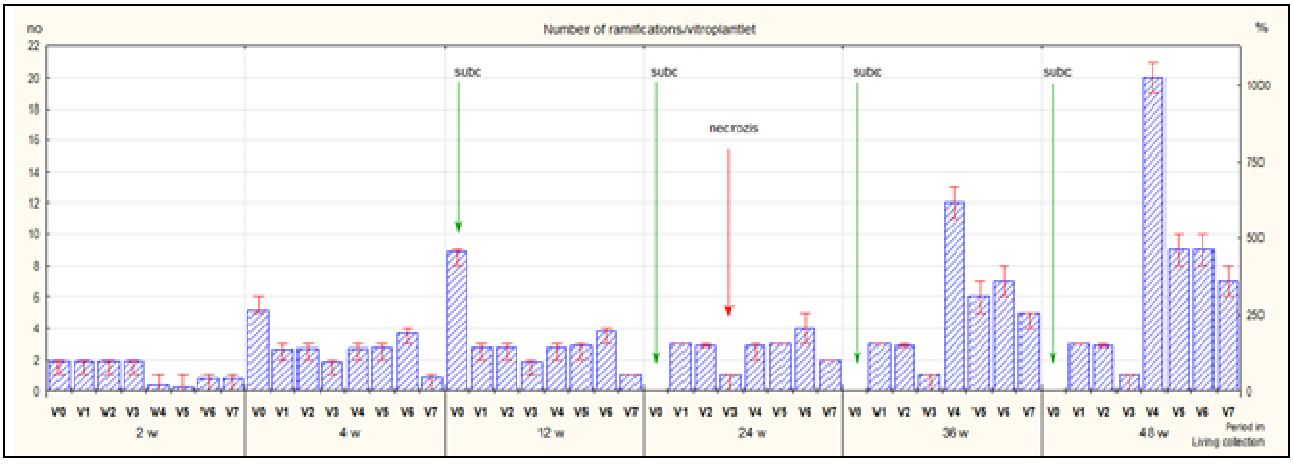
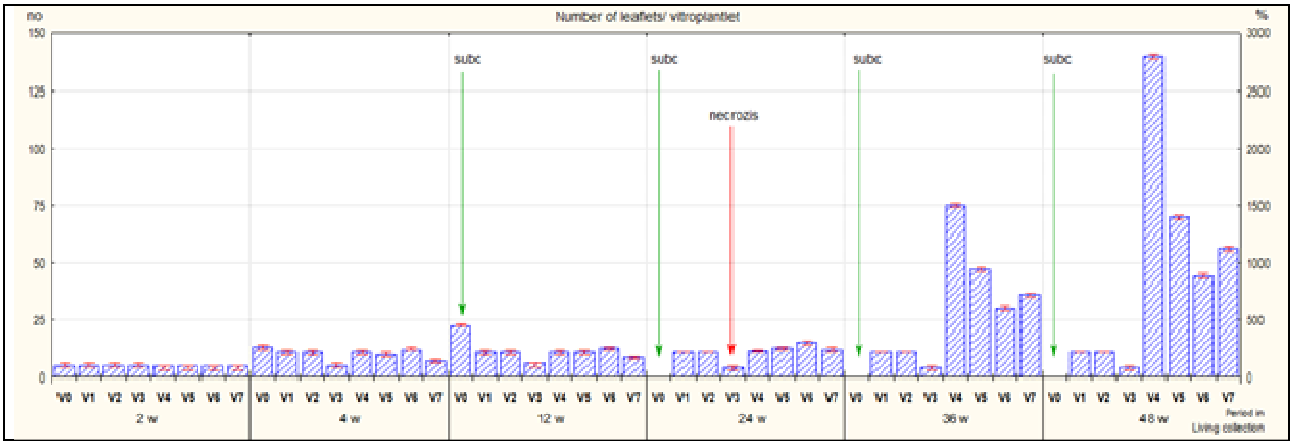
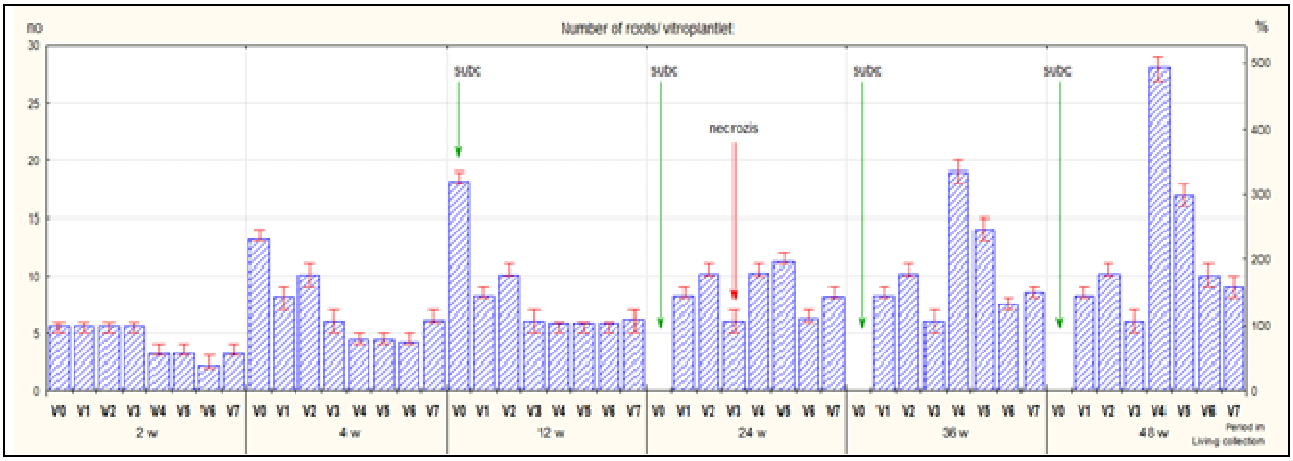


Figure 2. *Solanum tuberosum var. Gersa* vitroplantlets length, knotes and ramification number in the period of 2 - 48 weeks in slow growth system in „Living collection", where: V0-control: normal medium - MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil as supernatant, V2-MS with 3 ccm of paraffin oil as supernatant, V3-MS with 3 ccm of castor oil as supernatant normal medium 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). ccm = cubic centimeters, MS = Murashige-Skoog medium, w = weeks (orig.).





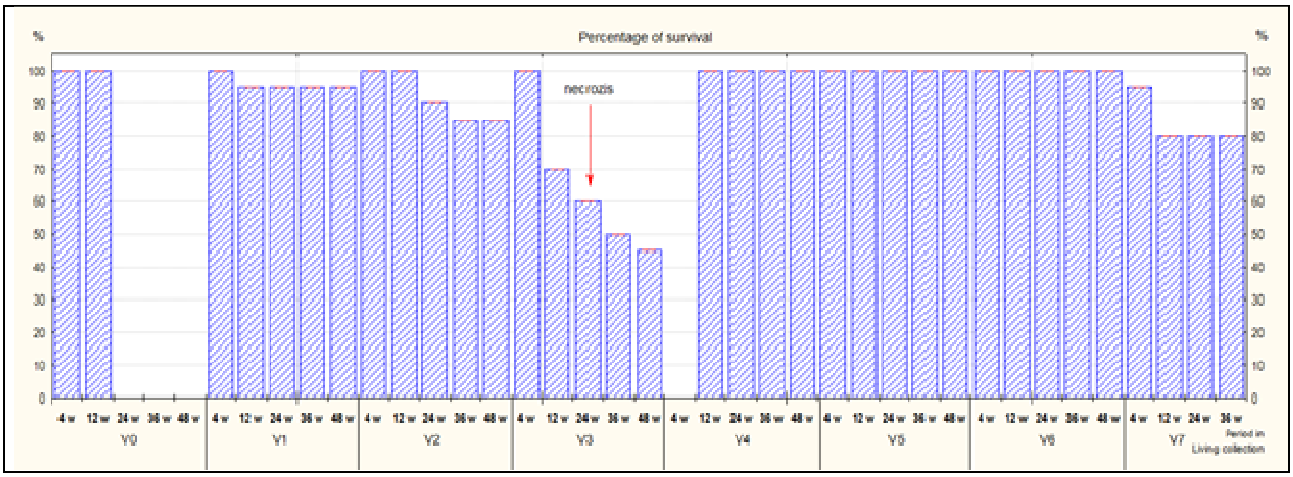


Figure 3. *Solanum tuberosum var. Gersa* vitroplantlets number of leafs, roots and percentage of survival in the period of 4- 48 weeks in slow growth system in „living collection", where: V0-control: normal medium - MS media free of growth inhibitors; double layer system: V1-MS with 3 ccm of silicone oil as supernatant, V2-MS with 3 ccm of paraffin oil as supernatant, V3-MS with 3 ccm of castor oil as supernatant normal medium 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), w = weeks, ccm = cubic centimeters, MS = Murashige-Skoog medium (orig.).

At the end of 48 weeks of vitroconservation in slow growth system, the vitroplantlets from the double layer system have maintained the same **average number of roots** from the period of 24 weeks - by the end of the experiment, so at V1 lot was noted a number of 8.24 roots (total growth marked by this group is 44.82%), V2 lot marked value of 10.15 copies/inocula (with a total increase of 78.38%), the lowest value this parameter was observed in group V3: 6.1 copies – a total increase by 7.21% - fig. 1 and 3. The vitroplantlets from the double layer system still showed a positive development, even if it was very small, shows that inoculas have maintained the viability during vitroconservation - this fact was tested by periodically subcultures from these vitroplantlets, also proved the efficiency of the double layer system to slowdown the growth rate of the vitroplantlets. Regarding the average number of roots within the normal agarized medium – single layer system, the lowest value of this parameter was recorded in group V6: 9 copies (number of roots increased 2.8 times during the entire experiment), 10 roots/inocula marked the cormofitoinocula in group V6 (number of roots increased 4.56 times during those 48 weeks), 17 root market V5 lot (their number increased by 5.32 times), the highest value this parameter was evaluated in V4 lot: 28 roots (number of roots increased 8.77 times during the 48 weeks of slow growth system) - fig. 1 and 3.

After 48 weeks of vitroconservation **the survivor percent** of the vitroplantlets was: 95% at V1 lot (siliconic oil), 85% at V2 lot (paraffin oil) and the smallest value of this parameter was at lot V3: only 45% because of the necrosis observed starting from 24 weeks of vitroconservation. Instead, la vitroplantlets from normal agarized media from V4-V6 had survived during the 28 weeks of experiment, the single exceptions being V7 lot witch lost 25% of vitroplantlets during the experimental period, the final surviving percent being only 75% - fig. 3.

As it can be seen from those mentioned above, the vitroplantlets from the double layer system did not exceed the oil layer over the 48 weeks of vitroconservation - we achieved the goal to slow the growth rate of the inocula, although there were significant losses of the effectives in vitroplants submerged in castor oil, which suggests that the high density of this oil determines a too hard hypoxia regime for cormofitoinocula, affecting their viability during the vitroconservation. It was also found that the most favorable/positive reaction of the vitroplantlets was by submersing them under silicone oil, which we can lead to the conclusion that this oil is suitable for forming a slow growth system – a double layer system.

From the normal agarized media (single layer system) all vitroplantlets showed a positive development - they have not reached the foil witch close the culture container - consequently we have achieved the goal of slowing the growth rate of the vitroplantlets - avoiding their subcultivation over 48 weeks - while the witness lot was already subcultived 4 times. The aim - to slow the growth - was best revealed by using Alar (B9) - as growth inhibitor - even if Gresa variety proved to be more sensitive – by a loss of 15% of vitroplantlets.

CONCLUSIONS

Silicone oil - as supernatant - is the best variant of slowing the growth in double layer system of *Solanum tuberosum var. Gersa*, the inocula kept the viability during the 48 weeks of the vitroconservation in Living Collection, and the subcultures, resulted after 12, 24, 36 or 48 weeks of vitroconservation manifested the best regenerative capacity from the double layer system

and the highest survivor percent, resulting - in vitroculture - vigorous vitroplantlets capable of caulo- and rizogenetic process.

B9 (Diaminozide) had the highest inhibitory effect in normal agarized media (being included in the agarized media), the resulted inocula – in subculture on fresh nutritive media without growth regulators – being capable of regenerative process as caulo- and rhyzogenesis.

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