SIMPLE METHOD OF *Solanum tuberosum* L. cv. Gersa TUBERIZATION STIMULATION

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Abstract. This paper presents the results of a study regarding the increasing *in vitro* tuberization of *Solanum tuberosum* L. cv. Gersa (potato), valuable from the eco-sanogenous point of view, due to the high anthocyanin content, by adding sucrose (30, 60, 90 g / l) in the solid Murashige-Skoog culture medium, without growth regulators. At 90 days of culture, the addition of 90 g / l sucrose was found to be the optimal stimulation *in vitro* tuberization process. In terms of morphological and anatomical feature, the newly-formed tubers showed normal aspects specific to this type of vegetative organ. Stimulation method has proven to be a simple, no extra additives ingredient in the base medium, cheap, namely bio-economic.

Keywords: tuberization; potato; vitrotuber; sucrose; morphology; anatomy.

INTRODUCTION

With the requirements of humanity beyond Earth's natural resources and environmental damage significantly affect food production; there is a need to identify new ways to avoid looming economic collapse [6-8].

Potato is an important food to economy, ranked in the fifth place among worldwide cultivated species, the fourth global harvest and production of proteins. The tubers are rich in vitamins, minerals, proteins, carbohydrates and iron [12, 14].

The overall needs of seed materials increase from year to year, and the best method to produce it, both in financial and the quality terms, is by using *in vitro* culture [10, 11, 28- 30]. Modern methods of plant biotechnology have to facilitate increasing the productivity of agricultural crops, and agricultural quality product [24]. In Romania, *in vitro* production of potato for seed started in 1988, to the National Institute of Research and Development for Potato and sugar Beet Brasov [13].

Moreover, biotechnology focused on research related to the bio-economy is one of the ways to eradicate hunger. Other cheap and convenient method for the increasing formation of microtubers *in vitro* was identified by replacement the sucrose, from the culture medium, with honey [23], or adding nanoparticles into the medium [1-3, 9], or by illuminating the culture with colored lights emitted by LEDs (Light Emitting Diodes) [25]. Ziv and Shemesh [36] have developed tuber from buds in subculture derived from the tuber inducing medium with 23.2 µM kinetin, 5.19 µM ancymidol and 8.6% sucrose. The number and size of the tubers per group were higher on solid medium, which was added over a second layer of liquid medium. Also tuberization was increased at lots which containers had maintained on intense agitation and decreased at bioreactor cultures. The authors emphasized the possibility of using cultures in bioreactors, bud proliferation and stimulating the development of tubers in liquid medium, in double layer culture.

Another bio-economic method [34] involved the replacement agar-agar with Phytagel™ 2%, allowing *in vitro* faster and higher tubers *Solanum tuberosum* L. cv. Baraka, compared with Difco Bacto-agar. The gelling agent also resulted in the stimulation of tuberization in the dark. The authors reported that the moisture did not influence growth and tubers differences.

Other authors [27] tested the effects of jasmonic acid (JA) on *in vitro* tuberization from nodal explants of Sangre and Russet Burbank potato cultivars in the liquid and solid medium with 0, 8-16 hours photoperiod. The explants were obtained from plants grown material medium supplemented with 2.5 µM JA. The most obvious benefits of pre-treatment with JA were observed at 16 h photoperiod conditions that are known to inhibit the tubers.

The nature of the carbon source contained in the culture medium and its concentration were studied over time by researchers in the field [4, 5].

An explanation of potato *in vitro* tuberization was given by studying the effect of potato *in vitro* sucrose metabolism (cv. Kennebec) [33]. The plants were grown in three different media: Murashige and Skoog basal medium containing high concentrations of nitrogen with 0 or 20 g l^{-1} sucrose; or a modified medium containing small amounts of nitrogen and 20 g l^{-1} sucrose. Researchers show that the sucrose has reduced quantities of the organic acid; both in leaves and in roots when plants were given a large amount of N [33]. Sucrose sustain phosphoenolpyruvate carboxylase activity, increased protein synthesis. Sucrose metabolism is involved in restoration of the tricarboxylic acid cycle, with carbon skeletons required to sustain phosphoenolpyruvate utilization during high nitrate assimilation.

The aim of this study was to develop a biological and economical method to stimulate *in vitro* potato tuberization.

MATERIALS AND METHODS

Inoculs, provided from subculture, consisted in one nod minicuttings of *Solanum tuberosum* L. cv. Gersa -

Table 1 Experimental protocol

Culture medium		Culture conditions	Measurements time	Measurements type
V_0	MS + 30 g/l sucrose (control)	- fluorescent white of 1700 lux - 16h light/24 h photoperiod; - 21°C ± 2°C temperature	90 days	Stem no. Stem L. (cm) Ramification no. Tubers no. Callus
V_1	MS + 60 g/l sucrose			
V_2	MS+ 90 g/l sucrose			

Note: MS –Murashige-Skoog basal solid culture medium [22], without growth regulators; L. - length; no - number.

14381, a genotype received from Suceava Plant Genetic Resource Bank (Romania), and the experimental protocol is shown in Table 1.

Culture vessel consisted by colorless jars with 11 cm height and 2 cm diameter, containing 5 ml of solid medium.

Sections of fresh plant material were created manually using shaving blades in a transversal plan. These were immediately analysed using an optical microscope (Leitz brand, Webster M) and the most representative images were taken with the adapted Cannon digital camera. The macroscopic photos were made with a Nikon 3100 digital camera.

RESULTS

At 90 days after inoculation, growth was similar in all of the three tested variants, growth indices of *in vitro* plants from culture media with the supplementary sucrose, with differences either positive or negative, compared to the control, but not statistically significant (Table 2). The single and most imported difference, comparing the control lot, was the significantly increasing of the tuberization, by statistical point of view (Table 2). In the lot with the 60 g / l sucrose addition was reported the callus presence (Table 2).

Table 2. Statistical processing of the data measured in the *in vitro* plants of potato (*Solanum tuberosum* L. cv. Gersa), in a 90 days on the following culture media: V0 - MS culture medium with sucrose 30 g / l, V1 - MS culture medium, sucrose 60 g / l, V2 - MS culture medium with sucrose 90g/l.

V_0-30 g/l sucrose – *control lot*

Statistical data	X ± Sx
Stem no.	1.23±0.43
Stem L. (cm)	10.25±4.84
Ramification no.	4.69±2.26
Tubers no.	0.03±0.12

V_1-60 g/l sucrose

Statistical data	X ± Sx	±d	P
Stem no.	1.45±0.51	0.22	Ns
Stem L. (cm)	11.00±1.98	0.75	Ns
Ramification no.	4.32±1.64	-0.37	Ns
Tubers no.	0.82±0.59	0.79	***
Callus	++		

V_2-90 g/l sucrose

Statistical data	X ± Sx	±d	P
Stem no.	1.44±0.73	0.21	Ns
Stem L. (cm)	11.00±1.32	0.75	Ns
Ramification no.	3.56±1.67	-1.14	Ns
Tubers no.	1.11±0.60	1.08	***

Notă: X ± Sx [average (cm) ± standard deviation], ±d [difference to control lot (cm)], p (significance of difference to control lot): ns – no significance difference, *** - very significant difference.

From the ***morphological*** point of view, during the *in vitro* period, have been regenerated stems, each having one or more ramification (Fig. 1). At each node was formed one leaflet with whole edge of foliar limb, arranged alternate on the stem. From axillary buds was formed stolons, which gradually thickened at distal end, developing tubers. The tuberization which appeared at 30 days at lot with 60 g / l sucrose determined slowing plantlets growth, especially aerial organs. At 90 days after inoculation, tuberization was identified in all experimental lots with more intensively to the addition of 90 g/l sucrose (suppose that the sucrose reserve, at the lot with the addition of 60 g/l, was exhausted after 60 day of culture). From the *in vitro* regenerated tuber were both regenerated adventitious roots and stems, which in turn showed adventitious roots (Fig. 1). Stems and tubers were covered with multicelular, uniseriate (unbranched), tector trichomes (Fig. 2 and 3A).

The ***anatomy***, *in vitro* new formed potato tubers showed the characteristic structure of this vegetative organ (Fig. 3B). Thus, from the outside to the inside, we have identified phellem, a suberized layer, a secondary tissue with protective role, as periderm

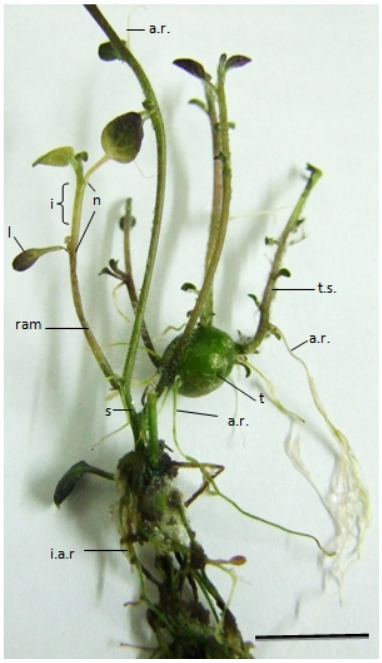

Figure 1. Aspects of potato (*Solanum tuberosum* L. cv. Gersa) tuber *in vitro* regenerated on V_2 lot (a.r. –adventive roots; i – internode; i.a.r. –adventive roots, new formed at inocul base; l – leaflets; n – node; ram – ramification; t – tuber, with adventive roots; s – stem new formed from inocul, with adventive roots; t.s. – stem newly formed from tuber, with adventive roots) (ruler mark 1 cm)

Figure 2. Morphological detail of potato (*Solanum tuberosum* L. cv. Gersa) tuber *in vitro* new formed on V_2 lot, with tector hairs and stem newly developed from tuber (s- stem; t.h. – tector hairs) (ruler mark 0.5 cm)

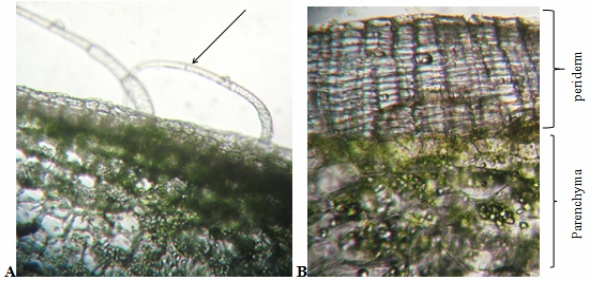

Figure 3. Tector hairs on potato (*Solanum tuberosum* L. cv. Gersa) tuber *in vitro* new formed on V_1 lot (A - 200X) and tuber structure (B - 400X)

component. This tissue was composed from flattened and death cells, radial arranged without intercellular spaces, being the soft elastic tissue, less permeable. Next layer, under periderm, was a cortical parenchyma, as storage tissue, rich in starch grains (Fig. 3B), with scarce and dissipated vascular tissue.

DISCUSSIONS

By increasing the sucrose content in the culture medium, but supplemented with kinetin and chlorocholine chloride (CCC), has been reported [26] in tuber formation to *in vitro* cultured node explants of *Solanum tuberosum* cv. Superior. The authors noted, however, that the addition of growth regulators was not essential for the formation of tubers, although smaller tubers were formed in the free kinetin and CCC. Also that chlorocholine chloride 200 mg/l or 90g/l sucrose in the medium resulted in maximum tuber induction [20].

However, paradoxically, in high concentrations, K^+ is not usefully to the development of *in vitro* tubers once the induction took place [32]. The results were discussed in the context of the possible effects of high K^+ in preventing sucrose intake and metabolism.

According to other authors [16], some mutant potatoes have complex change of development and phenotypic characteristics that are typical for strong induced tubers formation. These changes are likely to be related to photosynthesis and carbohydrate metabolism rather than impaired gibberellin transduction inhibitor. Moreover any axillary buds on stems or tuber cuttings may form a tuber, provided that there is tuberization inducing conditions. Only cuttings take from induced plant develop directly tuber, regardless of the concentration of sucrose in the medium [18].

Tubers was found to be under various types of hormones control, including gibberellins, cytokinins, abscisic acid, jasmonic acid and others, although, evidences for the involvement of hormones remains controversial [31]. In contrast, exogenous application of gibberellins produced stolons elongation and tuber formation was inhibited, while a decrease in the level of gibberellin preceding first peaks visible signs of stolon swelling [35]. *In vitro* potato tuberization increased in hormone-free systems on solidified medium, including the effects of environmental factors, composition of medium, genotype and explants [15].

Romanian researchers have improved the method of potato multiplication and tuberization stimulation by adding phytohormones in the culture medium, especially 2 mg/l or 2.5 mg/l BA and 0.05 mg/l NAA, as well the addition of 60 g/l sucrose [37].

Other simple method for increased tuberization by 60 g/l sucrose addition was reported for ‘Hunde’ and ‘Ararsa’ potato varieties [17] and sucrose 60 g/l with 15 g/l BAP showed maximum number of tuber [21]. Culture medium Murashige Skoog with 6% sucrose and 4 mg/L KIN combination of treatment was best for *in vitro* tuberization of potato [19].

In conclusion, in our experiment addition of 90 g/l sucrose in the culture medium without growth regulators, stimulated *in vitro* tuberization without morpho-anatomical changes of newly formed tubers. The method is simple, cheap and convenient for the user.

REFERENCES

[1] Baciu, A., (2009): The answer of some old varieties of *Solanum tuberosum* L. for *in vitro* cultivation. Analele universităţii din Oradea, Fascicula Biologie, University of Oradea Press, TOM XVI(1): 18-23.

[2] Baciu, A., Petruş-Vancea, A., Zehan, R., (2007): The evolution of the *Solanum tuberosum* l. neoplantles cultivated *in vitro* on cuture media supplimented with bioactive magneto-fluidic nanocomposites. Analele Universităţii din Oradea, Fascicula Biologie, TOM XI: 19-22.

[3] Baciu, A., Danci, O., Petruş-Vancea, A., Sărac, I., Mike, L., (2009): Results regarding potato (*Solanum tuberosum* L.) cultivars reaction to *in vitro* culture conditions. Journal of Horticulture, Forestry and Biotechnology, 13: 174-178.

[4] Blidar, C.F., Cachiţă, C.D., Petruş-Vancea, A., Drăghici, F., (2004): Vitroculturile de protocormi de *Cymbidium* pe medii cu amidon de porumb ca înlocuitor parţial sau total al zaharozei, pp. 203-212. In: Cachiţă, C.D., Ardelean, A., Fati, V. (eds): Lucrările celui de al XII-lea Simpozion Naţional de Culturi de ţesuturi şi Celule Vegetale „Fitopatologia celulei vegetale în regim de vitrocultură. Editura Daya, Satu-Mare.

[5] Blidar, C.F., Cachiţă, C.D., Petruş-Vancea, A., Pop, L., Marian, M., (2007): The *Cymbidium* protocorms reaction, cultivated „in vitro” on a bistratified medium, using a fructose solution as supernatant, pp. 419-423. In: Proceeding of the International Conference „Agricultura land food sciences, process and technologies”. Editura „Lucian Blaga” University, Sibiu.

[6] Brown, C.R., (1994): Potato. Encyclopedia of Agricultural Sciences 3. Academic Press Inc., pp. 5-10.

[7] Brown, L.R., (2005): Depăşind Resursele Planetei - probleme globale ale omenirii. Editura Tehnica Bucuresti, pp. 100-130.
[8] Brown, L.R., (2011): The Great FoodCrisis of 2011. Foreign Policy, 10: 1-5.
[9] Butnaru, G., Baciu, A., (2005): Capacitatea de tuberizare *in vitro* la unele cultivare de *Solanum tuberosum* L, pp. 131-137. In: Lucrările celui de al XIV-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale. Editura Alma Mater-Sibiu.
[10] Cachită, C.D., Ardelean, A., (2009): Tratat de biotehnologie vegetală, Vol. II. Editura Dacia Cluj-Napoca, pp. 7-50.
[11] Cachiță, C.D., Constantinovici, D., (2008): Conservarea resurselor vegetale în Băncile de Gene sub formă de fitoinoculi, pp. 152-161. In: Lucrările celui de al XVII-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale, Bucureşti.
[12] Cachiță, C.D., Sand, C., (2011): Biodiversitatea şi băncile de gene. "Vasile Goldiş" University Press, pp. 10 -100.
[13] Chiru, N., (1999): Înmulțirea *in vitro* a cartofului (*Solanum tuberosum* L.) prin culturi de țesuturi. Universitatea din Oradea.
[14] Ciobanu I.B., Constantinovici, D., Ceţu, I., (2011): Influence of genotype and cultivation conditions on vitroplantlets evolution of *Solanum tuberosum* L. local varieties. Analele Ştiințifice ale Universității „Al. I. Cuza" Iaşi, Tomul LVII(2), s. II a. Biologie vegetală: 13-20.
[15] Dobránszki, J., Magyar-Tábori, K., Hudák, I., (2008): In vitro tuberization in hormone-free systems on solidified medium and dormancy of potato microtubers, pp. 83-94. In: Fruit, vegetable and cereal science and biotechnology. Global Science Books.
[16] Fischer, L., Lipavska, H., Housman, J.F., Opatrny, Z., (2008): Morphological and molecular characterization of a spontaneously tuberizing potato mutant: an insight into the regulatory mechanism of tuber induction. BioMed Central Plant Biology, 8: 117-121.
[17] Fufa, M., Diro, M., (2013): The effects of sucrose on *in vitro* tuberization of potato cultivars. Advances in Crop Science and Technolgy 1:114.
[18] Gregory, L.E., (1956): Some factors for tuberization in the potato plant. American Journal of Botany, 43: 281-288.
[19] Hoque, E.M., (2010): In vitro tuberization in potato (*Solanum tuberosum* L.). Plant Omics Journal, 3(1):7-11.
[20] Iqbal H., Chaudhry, Z., Muhammad, A., Asghar, R., Saqlan Naqvi, S.M., Rashid, H., (2006): Effect of chlorocholine chloride, sucrose and bap on *in vitro* tuberization in potato (*Solanum tuberosum* L. cv. Cardinal). Pakistan Journal of Botany, 38(2): 275-282.
[21] Imani, A.A., Qhrmanzadeh, R., Azimi, J., Janpoor, J., (2010): The effect of various concentrations of 6-benzylaminopurine (BAP) and sucrose on in vitro potato (*Solanum tuberosum* L.) microtuber induction. American-Eurasian Journal of Agricultural & Environmental Sciences, 8 (4): 457-459.
[22] Murashige, T., Skoog, F., (1962): A revised medium for rapid growth and bioassays with tobacco tissues cultures. Physiologia Plantarum, 15: 155-159.
[23] Pătru, D., Cachiță, C.D., (2005): Micropropagarea la cartof (*Solanum tuberosum* L.) pe medii de cultură în care zaharoza a fost substituită cu miere de albine sau cu fructoză, pp. 207-216. In: Lucrările celui de al XIV-lea Simpozion Național de Culturi de Țesuturi şi Celule Vegetale. Editura Alma Mater-Sibiu.
[24] Petruş-Vancea, A., Cachița, C.D., Purcarea, C., (2013): Proceduri bioeconomice, ecoeconomice de optimizare a conservării si multiplicării in vitro a biodiversității vegetale. "Vasile Goldis" University Press, pp. 6-50.
[25] Pop, L., Cachiță, C.D., (2011): Contribution to Solanum tuberosum l. tubergenesis, vitrocultivated under ultrabright color L.E.D.. Analele Universității din Oradea - Fascicula Biologie, TOM XVIII(2): 124-127.
[26] Protacio, C.M., Flores, H.E., (1992): The role of polyamines in potato tuber formation. In Vitro Cellular & Developmental Biology - Plant, 28: 81-86.
[27] Pruski, K., Astatkie, T., Nowak, J., (2002): Jasmonate effects on in vitro tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum* L.) under different media and photoperiod conditions. In Vitro Cellular & Developmental Biology - Plant, 38: 203-209.
[28] Radoveț-Salinschi, D., Cachiță, C.D., (2012): Testing the regenerative capacity of *Solanum tuberosum* var. Gersa explants after 24 weeks storage in living collection. Analele Universității din Oradea, Fascicula: Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, XII/B: 423-430.
[29] Radoveț-Salinschi, D., Cachiță, C.D., (2012): *In vitro* conservation of *Solanum tuberosum* var. Gersa. Analele Universității din Oradea, Fascicula Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară, XII/B: 431-438.
[30] Radoveț-Salinschi, D., Cachiță, C.D., (2013): The mictropropagation eficiensy by slowing the growthrate of *Solanum tuberosum* var. Gersa vitroplantlets. Studia Universitatis "Vasile Goldiş", Seria Ştiințele Vieții, 23(1): 49-56.
[31] Sarkar, D., Pandey, S.K., Sharma, S., (2006): Cytokinins antagonize the jasmonates action on the regularization of potato (*Solanum tuberosum*) tuber formation *in vitro*. Plant Cell, Tissue and Organ Culture, 87: 285-295.
[32] Sarkar, D., Pandey, S.K., Sharma, S., (2010): High K^+ does not affect potato (*Solanum tuberosum* L.) tuber induction, but represses its development *in vitro*. In Vitro Cellular & Developmental Biology - Plant, 46: 569-577.
[33] Sima, B.D., Desjardins, Y., Quy, L.V., (2001): Sucrose enhances phosphoenolpyruvate carboxylase activity of *in vitro Solanum tuberosum* L. under non-limiting nitrogen conditions. In Vitro Cellular & Developmental Biology - Plant, 37(4): 480-489.
[34] Veramendi, J., Villafranca, M.J., Sota, V., Mingo-Castel, A.M., (1997): Gelrite as an alternative to agar for micropropagation and microtuberization of *Solanum tuberosum* L. cv. Baraka. In Vitro Cellular & Developmental Biology - Plant, 33: 195-199.
[35] Xu, X., van Lammeren, A.A.M., Vermeer, E., Vreugdenhil, D., (1998): The role of gibberellin, abscisic acid, and sucrose in the regulation of potato tuber formation *in vitro*. Plant Physiology, 117: 575-584.
[36] Ziv, M., Shemesh, D., (1996): Propagation and tuberization of potato bud clusters from bioreactor culture. In Vitro Cellular & Developmental Biology - Plant, 32: 31-36.
[37] ***, (2012): Raport ştiințific şi tehnic, http://www.potato.ro/_graphx/Raport%205%20MICROCAR.pdf, available in 10september 2014.

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