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MICROPROPAGATION OF POTATO SEED TUBERS (*Solanum tuberosum* L.) UNDER *IN VITRO* CONDITIONS

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Abstract

In this paper the results of influence of phytohormone gibberellic acid GA_3 on sprout formation in *in vivo* conditions and *in vitro* microtuberization of the potato varieties Agria, Agata, Sunshine, Ultra and Marabel are presented. The tubers from all varieties utilized in the experiment were certified potato seed tuber material.

The experiment in *in vitro* conditions was established on sprout explants and nodal segment explants on the MS medium (Murashige & Skoog, 1962) with addition of different combination and concentration of auxins and cytokines. Microtuberization was stimulated by rising the concentration of sucrose in MS medium from 40g/l to 60 g/l and 80g/l, respectively.

The *in vivo* tuber treatment with 30mg/l GA_3 was the most effective treatment for all potato varieties in proliferation of sprouts. All tubers that were treated with GA_3 resulted in *de novo* sprouting of tubers.

The variety Agata resulted with 100% of microtuberization from nodal segment explants on MS medium supplied with 40g/l and 80g/l sucrose. Microtuberization of the variety Sunshine was stimulated with addition of 80 g/l sucrose in MS medium.

The developed microtubers were detached from the nodal segments and subcultured on new MS medium supplied with BAP 4mg/l, KIN 4mg/l and 8% of sucrose to increase their weight.

Key words: *phytohormones, microtuberization, gibberellic acid, sucrose, variety of seed potatoes*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is widely grown worldwide because of its rich nutrition, easiness of cultivation and high yield performance (Wang et al., 2020). Nowadays it is the fourth most important food crop in the world, after wheat, rice and maize, cultivated on 19.3 million hectares with yield of 388.2 million tons of potato tubers (Waqas et al., 2021). Potato (*Solanum tuberosum* L.) is grown in more than 100 countries and feeds more than a billion people worldwide (Islam et al., 2020).

The formation of the tubers is a very complex process, but it can be stimulated in *in vitro* conditions, process known as microtuberization (Abbott and Belcher, 1986; Apichai, 1988; Dodds et al., 1992; Coleman et al., 2001).

Previous studies have shown that micropropagation of potato seed tubers depends on the biological value of cultivars, explant type (leaf, nodal segments, shoot tip), type of culture medium, season, temperature, photoperiod and balanced combination of plant growth regulators (PGRs) in the culture media (Akhtar et al., 2006; Dhital et al., 2010). GA_3 participates in cell elongation and GA_3 addition in MS medium enhances shoot growth (Camara et al., 2018; Rizza et al., 2017).

Osmotically active solutes have shown that sucrose acts as a carbon source and osmotic regulator. Sucrose and sucrose concentration are important factors for potato microtuberization and they have a profound effect on tuber growth (Azar et al., 2013). It acts as an energy for growth and biosynthetic processes and may influence growth in *in vitro* conditions (Ferreira et al., 2011). Sucrose is also closely related to stomatal density and photosynthetic pigment content, as well as development induction in some plant tissues, such as vascular and support tissues (Mohamed and Alsadon, 2010; Iarema et al., 2012). The rise of sucrose concentration in medium can enhance the microtuber production to some extent (Khan et al., 2018). However, high concentration of sucrose in the medium may decrease the photosynthetic ability of *in vitro* potato plants (Fuentes et al., 2005).

Potato starch has some unique physicochemical characteristics compared to starches from other sources as high phosphate content, absence of internal lipids and proteins in granules (Burlingame et al., 2009; Romano et al., 2016).

MATERIAL AND METHODS

The research was conducted in the Laboratory of Plant Biotechnology, Faculty of Agriculture, Goce Delcev University – Stip, Republic of North Macedonia. As starting material seed tubers from the potato varieties Agata, Marabel, Ultra, Sunshine and Agria were used.

***In vivo* treatment of potato seed tubers with GA_3**

The tubers from different varieties were treated with GA_3 with concentration of 10, 20 and 30 ppm. Control treatment, where the tubers were not treated with GA_3 was used to determinate whether GA_3 had effect on emergence of sprouts (Fig. 1).

The GA_3 treatment was used for induction of germination and rapid emergence of sprouts. After GA_3 treatment, one week old sprouts were detached from the potato tubers, and they were used as starting explants for further *in vitro* cultivation on MS medium supplemented with different concentrations of phytohormones.

Figure 1. The effect of 30 mg/l GA_3 treatment for rapid sprouting and de novo proliferation of sprouts in variety Agata compared to the control.

Sterilization of initial explants (sprouts)

The sprouts were surface cleaned by washing them under tap running water for 10-15 minutes and rinsing them in distilled water several times followed by surface sterilization of sprouts surface by immersion in:

– - 70% C_2H_5OH for 2 minutes.
– - 0.5% $HgCl_2$ for 3-5 minutes or 0.1% NaClO for 10 minutes and
– - several times rinsing with sterile water.

Initial explants – sprouts cultivated in *in vitro* conditions

The sprouts as initial explants were cultivated in MS medium supplemented with 30 g/l sucrose, 0.7% agar, 100 g/l myoinozitol, 200 g/l casein enzymatic hydrolysate, 0.1mg/l thiamine, 1.0 mg/l pyridoxine and 0.5 mg/l nicotinic acid. The MS medium pH was adjusted to 5.8.

The MS media supplemented with different concentration of cytokinins and/or auxins were used for induction of shoots culture from different potato varieties:

- Sprouts > MS + 2mg/l BAP
- Sprouts > MS + 2mg/l KIN
- Sprouts > MS + 2mg/l BAP + 1mg/l NAA
- Sprouts > MS + 2mg/l KIN + 1mg/l NAA

The sprouts developed into shoots with different number of nodes within a month. The shoots were divided into nodal segments and subcultured on:

- Shoot nodal segments > MS + 3mg/l BAP + 1mg/l NAA
- Shoot nodal segments > MS + 3 mg/l KIN + 1mg/l NAA

These media were used for stimulation of nodal segments growth.

When the explants have reached 15-20 mm length, they were divided into nodal segments and subcultured on MS supplemented with different concentration of BAP, NAA, and sucrose for induction of microtubers. The following media were used for induction of microtubers in different potato varieties:

- Nodal segments > MS + 2mg/l BAP + 2mg/l NAA + 4% sucrose
- Nodal segments > MS + 4mg/l BAP + 2mg/l NAA + 6% sucrose
- Nodal segments > MS + 6mg/l BAP + 2mg/l NAA + 8% sucrose

Maintenance of cultures in the climate chamber

All explants, sprouts and nodal segments, were incubated in a climate chamber under the following conditions: temperature 25 ± 10^{0}C; relative humidity 50%; photoperiod: 16/8 hours light/dark; illumination of 50 cd.

Data analysis

All data were subjected to statistical analysis with statistical package IBM SPSS Statistical 29, one-way ANOVA and Duncan post hoc test, with the level of significance 0.05%.

RESULTS AND DISCUSSION

All tubers treated with gibberellic acid GA_3 resulted in *de novo* germination of sprouts from the tuber eyelets. The treatment with 30 ppm GA_3 was the most effective for all potato varieties. The application of 30 ppm GA_3 as the highest dose of gibberellic acid resulted in 100% formation of sprouts from the tubers of potato varieties Ultra, Sunshine and Agria. The results presented in Table 1 show that all potato varieties have good response to gibberellic acid treatments, regardless of applied concentration. The variety Sunshine has shown 100% of formation of sprouts when treated with 10, 20 and 30 ppm GA_3.

The initiation of sprouts was the key factor to induce microtuberization. The subcultured sprouts on MS medium supplied with different concentrations and combinations of auxins and cytokinins proliferated into shoots. The nodal segments from regenerated shoots were used for induction of microtubers (Fig. 2a).

The nodal segments from shoots were subject of subcultivation on MS medium supplied with cytokinins and auxins and sucrose in concentration of 4, 6 and 8%. The sucrose was added in order to initiate higher rate of formation of microtubers. The results of microtuberization of seed potatoes are shown in Table 2.

Different researchers agreed that higher percent of sucrose in the medium had positive results on microtuberization process and increased the number and quality of microtubers (Farran and Mingo-Castel, 2006; Motallebi-Azar and Kazemiani, 2012; Ahmed et al., 2013). This confirms our findings during this research.

The culture of nodal segments was incubated in controlled climate chamber under dark conditions to initiate formation of microtubers. (Fig. 2b).

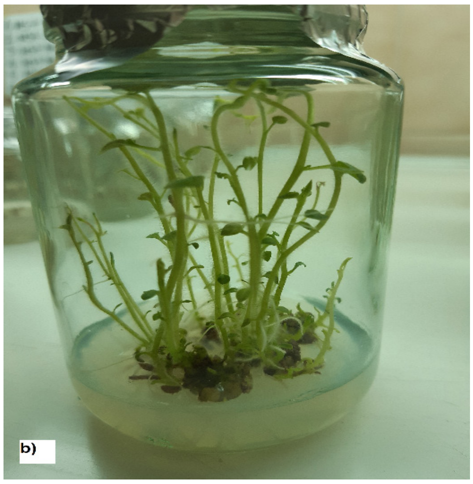

Figure 2. a) Culture of shoots b) Culture of nodal segments.

Table 1. Varieties of potato tubers treated with GA_3 and control.

Production of sprouts								
Variety	Treatment with GA_3	Number of tubers	Number of eyelets per tuber	Number of sprouts per tuber	Length of sprouts (mm)	Width of sprouts (mm)	Number of sprouts per eyelet	% of sprout proliferation
Agata	Control	20	1.89a	0.93ab	2.31b	2.04b	1.37ab	85
	10 ppm	22	1.47a	1.00a	2.41b	2.02a	1.40bc	100
	20 ppm	22	1.66a	1.00a	2.49b	2.00b	1.35a	100
	30 ppm	22	1.52b	0.96a	2.64bc	2.15ab	1.21ab	90.90
Marabel	Control	18	1.45a	0.94ab	2.50a	2.09b	1.52a	83.33
	10 ppm	18	1.67a	1.00a	2.40b	2.05a	1.50a	55.55
	20 ppm	18	1.34b	0.89b	2.64b	2.02b	1.30a	77.77
	30 ppm	18	1.32b	0.95a	2.65bc	2.02b	1.22ab	94.44
Ultra	Control	17	1.47a	1.00a	3.37a	2.23a	1.08b	100
	10 ppm	18	1.47a	0.94a	3.38a	2.14a	1.13bc	88.88
	20 ppm	18	1.45ab	1.00a	3.47a	2.33a	1.13ab	100
	30 ppm	18	1.24b	1.00a	3.28a	2.24a	1.32a	100
Sunshine	Control	15	1.81a	0.97ab	2.57a	2.00b	1.18ab	93.33
	10 ppm	15	1.45a	1.00a	2.71b	2.07a	1.05b	100
	20 ppm	15	1.46ab	1.00a	2.60b	2.02b	1.04b	100
	30 ppm	15	1.31b	1.00a	2.97ab	2.09ab	1.04b	100
Agria	Control	17	1.87a	0.87b	2.22b	2.00b	1.40ab	88.23
	10 ppm	17	1.50a	0.94a	2.62b	2.09a	1.14bc	88.23
	20 ppm	16	1.42ab	0.96a	2.57b	2.27a	1.28a	93.75
	30 ppm	16	2.00a	1.00a	2.35c	2.10ab	1.21ab	100

Means within each column having different letters are significantly different according to Duncan's test at $p<0,05$.

The rise of sucrose concentration in MS medium from 40g/l to 80 g/l increased the percentage of formation microtubers from 42.85% (4% sucrose) to 58.33% (8% sucrose) in the variety Ultra (Fig. 3a).

The variety Agata resulted with 100% microtuberization of nodal segments when cultivated on MS medium with 40g/l and 80 g/l sucrose. Higher microtuberization rate of the variety Sunshine was achieved with 80 g/l sucrose in MS medium (33.33%) as compared to 40 g/l (16.16%).

The developed microtubers were detached from nodal segments and subcultured on the new MS medium enriched with BAP 4mg/l, KIN 4mg/l and 8% of sucrose in order to increase their weight (Fig. 3b).

Table 2. Effect of different concentration of BAP, NAA, and sucrose on microtuberization in potato nodal segments.

Explants – nodal segments						Formation of microtubers			
Variety	MS medium with cytokinins and auxins	% of sucrose	Number of nodal segments	Length of nodes (mm)	Thickness of nodal segments (mm)	Number of microtubers per explant	Length of tubers (mm)	Width of tubers (mm)	Microtuberization (%)
Agata	2mg/l BAP + 2mg/l NAA	4%	4	7.50b	1.62a	4	3.50a	3.25a	100
Agata	6mg/l BAP + 2mg/l NAA	8%	3	13.33a	1.50a	3	5.00a	3.00a	100
Ultra	2mg/l BAP + 2mg/l NAA	4%	7	13.00a	1.07b	3	2.14a	1.42ab	42.85
Ultra	6mg/l BAP + 2mg/l NAA	8%	36	11.21a	1.14a	21	1.94b	1.44b	58.33
Sunshine	2mg/l BAP + 2mg/l NAA	4%	6	12.50a	1.16b	1	1.00a	0.33a	16.66
Sunshine	MS+6mg/l BAP + 2mg/l NAA	8%	24	11.91a	1.43ab	8	1.33b	0.75b	33.33

Means within each column having different letters are significantly different according to Duncan's test at $p<0,05$.

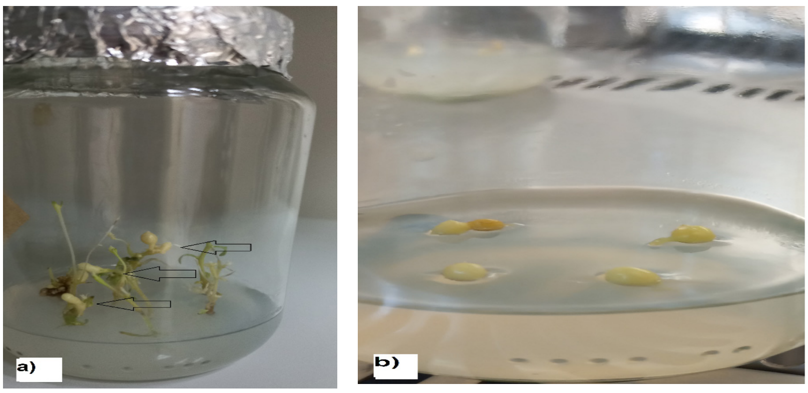

Figure 3. a) Microtuberization b) Culture of microtubers.

CONCLUDING REMARKS

Micropropagation is an alternative method for conventional breeding of potatoes. Methods of *in vitro* propagation using sprouts and nodal segments are more reliable to maintain the integrity of the genetic and breeding material.

Microtuberization is an important process for the production and storage of potatoes. Microtubers obtained by *in vitro* culture of nodal segments are suitable for manipulation, storage and distribution of healthy germplasm.

The results presented in this paper have proven that potato seed tubers have regenerative power and good potential for microtuberization.

Nodal segments of the variety Agata cultured on medium MS+6mg/l BAP + 2mg/l NAA+8% sucrose responded with 100% microtuberization.

The high concentration of sucrose acts as a stimulation signal leading to the accumulation of starch in microtubers.

The nodal segment culture of the variety Ultra resulted with 42.85% microtuberization when cultured on MS medium supplemented with 40 g/l sucrose, while rising the concentration of sucrose from 40 g/l to 80 g/l resulted in increase of microtuberization from 42.85% to 58.33%.

Microtuberization of the variety Sunshine was stimulated with higher concentration of sucrose 80 g/l in the medium and it resulted in microtuberization response of 33.33% of nodal segments.

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МИКРОПРОПАГАЦИЈА НА СЕМЕНСКИ КОМПИР (*Solanum tuberosum* L.) ВО *IN VITRO* УСЛОВИ

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Резиме

Во овој труд се прикажани резултатите од влијанието на фитохормонот гиберелинска киселина GA_3 врз формирање на ʻртулци во *in vivo* услови и *in vitro* микротуберизацијата на неколку генотипови компир Agria, Agata, Sunshine, Ultra и Marabel. Клубените од сите генотипови користени во овој експеримент беа серитифициран семенски компир.

Експериментот во *in vitro* услови беше поставен со ʻртулци и нодии на MS медиум (Murashige & Skoog, 1962) со додавање на различни концентрации и комбинации на ауксини и цитокинини. Микротуберизацијата беше стимулирана со зголемување на процентот на сахароза во MS медиумот од 40 g/l сахароза на 60 и 80 g/l.

Третирањето на клубените во *in vivo* услови со 30mg/l GA_3 се покажа како најефикасен за сите испитувани семенски генотипови за добивање на ʻртулци. Кај сите генотипови третирани со GA_3 резултираше со *de novo* ʻртулци од окцата на клубените.

Генотипот Agata резултираше со 100% микротуберизација од нодиите на MS медиум со 40 g/l и 80g/l сахароза. Микротуберизацијата кај генотипот Sunshine беше стимулирана со додавање на 80 g/l сахароза во MS медиумот.

Формираните микроклубени беа одделени од нодалните сегменти и пасажирани на нов MS медиум збогатен со BAP 4mg/l, KIN 4mg/l и 8% сахароза за зголемување на нивната тежина.

Клучни зборови: *фитохормон, микротуберизација, гиберелинска киселина, сахароза, генотипови на семенски компир*