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

Irena Petrova¹*, Liljana Koleva Gudeva¹
Faculty of Agriculture, Goce Delcev University, Stip, Republic of North Macedonia¹
*Corresponding author: irena_stojkova_7@hotmail.com

Abstract
In this paper the results of influence of phytohormone gibberellic acid GA₃ on sprout formation in in vivo conditions and in vitro microtuberization of the potato varieties Agría, 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 GA₃ was the most effective treatment for all potato varieties in proliferation of sprouts. All tubers that were treated with GA₃ 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). GA₃ participates in cell elongation and GA₃ 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 GA3**

The tubers from different varieties were treated with GA3 with concentration of 10, 20 and 30 ppm. Control treatment, where the tubers were not treated with GA3 was used to determinate whether GA3 had effect on emergence of sprouts (Fig. 1).

The GA3 treatment was used for induction of germination and rapid emergence of sprouts. After GA3 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 GA3 treatment for rapid sprouting and de novo proliferation of sprouts in variety Agata compared to the control.](image)

**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% C2H5OH for 2 minutes.
- 0.5% HgCl2 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 + 3mg/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°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 GA₃ resulted in de novo germination of sprouts from the tuber eyelets. The treatment with 30 ppm GA₃ was the most effective for all potato varieties. The application of 30 ppm GA₃ 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 GA₃.

The initiation of sprouts was the key factor to induce microtuberization. The subcultured sprouts on MS medium supplied with cytokinins and auxins and sucrose in concentration of 4, 6 and 8% 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 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).
Table 1. Varieties of potato tubers treated with GA$_3$ and control.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment with GA$_3$</th>
<th>Number of tubers</th>
<th>Number of eyelets per tuber</th>
<th>Number of sprouts per tuber</th>
<th>Length of sprouts (mm)</th>
<th>Width of sprouts (mm)</th>
<th>Number of sprouts per eyelet</th>
<th>% of sprout proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agata</td>
<td>Control</td>
<td>20</td>
<td>1.89a</td>
<td>0.93ab</td>
<td>2.31b</td>
<td>2.04b</td>
<td>1.37ab</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>22</td>
<td>1.47a</td>
<td>1.00a</td>
<td>2.41b</td>
<td>2.02a</td>
<td>1.40bc</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>20 ppm</td>
<td>22</td>
<td>1.66a</td>
<td>1.00a</td>
<td>2.49b</td>
<td>2.00b</td>
<td>1.35a</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30 ppm</td>
<td>22</td>
<td>1.52b</td>
<td>0.96a</td>
<td>2.64bc</td>
<td>2.15ab</td>
<td>1.21ab</td>
<td>90.90</td>
</tr>
<tr>
<td>Marabel</td>
<td>Control</td>
<td>18</td>
<td>1.45a</td>
<td>0.94a</td>
<td>2.50a</td>
<td>2.09b</td>
<td>1.52a</td>
<td>83.33</td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>18</td>
<td>1.67a</td>
<td>1.00a</td>
<td>2.40b</td>
<td>2.05a</td>
<td>1.50a</td>
<td>55.55</td>
</tr>
<tr>
<td></td>
<td>20 ppm</td>
<td>18</td>
<td>1.34b</td>
<td>0.89b</td>
<td>2.64b</td>
<td>2.02b</td>
<td>1.30a</td>
<td>77.77</td>
</tr>
<tr>
<td></td>
<td>30 ppm</td>
<td>18</td>
<td>1.32b</td>
<td>0.95a</td>
<td>2.65bc</td>
<td>2.02b</td>
<td>1.22ab</td>
<td>94.44</td>
</tr>
<tr>
<td>Ultra</td>
<td>Control</td>
<td>17</td>
<td>1.47a</td>
<td>1.00a</td>
<td>3.37a</td>
<td>2.23a</td>
<td>1.08b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>18</td>
<td>1.47a</td>
<td>0.94a</td>
<td>3.38a</td>
<td>2.14a</td>
<td>1.13bc</td>
<td>88.88</td>
</tr>
<tr>
<td></td>
<td>20 ppm</td>
<td>18</td>
<td>1.45ab</td>
<td>1.00a</td>
<td>3.47a</td>
<td>2.33a</td>
<td>1.13ab</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30 ppm</td>
<td>18</td>
<td>1.24b</td>
<td>1.00a</td>
<td>3.28a</td>
<td>2.24a</td>
<td>1.32a</td>
<td>100</td>
</tr>
<tr>
<td>Sunshine</td>
<td>Control</td>
<td>15</td>
<td>1.81a</td>
<td>0.97ab</td>
<td>2.57a</td>
<td>2.00b</td>
<td>1.18ab</td>
<td>93.33</td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>15</td>
<td>1.45a</td>
<td>1.00a</td>
<td>2.71b</td>
<td>2.07a</td>
<td>1.05b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>20 ppm</td>
<td>15</td>
<td>1.46ab</td>
<td>1.00a</td>
<td>2.60b</td>
<td>2.02b</td>
<td>1.04b</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>30 ppm</td>
<td>15</td>
<td>1.31b</td>
<td>1.00a</td>
<td>2.97ab</td>
<td>2.09ab</td>
<td>1.04b</td>
<td>100</td>
</tr>
<tr>
<td>Agria</td>
<td>Control</td>
<td>17</td>
<td>1.87a</td>
<td>0.87b</td>
<td>2.22b</td>
<td>2.00b</td>
<td>1.40ab</td>
<td>88.23</td>
</tr>
<tr>
<td></td>
<td>10 ppm</td>
<td>17</td>
<td>1.50a</td>
<td>0.94a</td>
<td>2.62b</td>
<td>2.09a</td>
<td>1.14bc</td>
<td>88.23</td>
</tr>
<tr>
<td></td>
<td>20 ppm</td>
<td>16</td>
<td>1.42ab</td>
<td>0.96a</td>
<td>2.57b</td>
<td>2.27a</td>
<td>1.28a</td>
<td>93.75</td>
</tr>
<tr>
<td></td>
<td>30 ppm</td>
<td>16</td>
<td>2.00a</td>
<td>1.00a</td>
<td>2.35c</td>
<td>2.10ab</td>
<td>1.21ab</td>
<td>100</td>
</tr>
</tbody>
</table>

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.
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.

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

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.

REFERENCES


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

Irena Петрова1*, Лилјана Колева-Гудева1

1Земјоделски факултет, Универзитет „Гоце Делчев - Штип“, Република Северна Македонија

*Kонтакт-автор: irena_stojkova_7@hotmail.com

**Резиме**

Во овој труд се прикажани резултатите од влијанието на фитохормонот гиберелинска киселина GA3 врз формирање на ‘ртулци 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 сертифициран семенски компир.

Експериментот во in vitro услови беше поставен со ‘ртулци 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 GA3 се покажа како најефикасен за сите испитувани семенски генотипови за добивање на ‘рутцел. Каж сите генотипови третирани со GA3 резултираше со del novo ‘рутци од окцата на клубените.

Генотипот Agata резултираше со 100% микротуберизација од нодите на MS медиум со 40 g/l и 80g/l сахароза. Микротуберизацијата кaj 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.

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