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EFFECT OF PLANT GROWTH REGULATORS AND SUCROSE ON MICROTUBERIZATION OF POTATO (*SOLANUM TUBEROSUM* L.)

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ABSTRACT

This paper presents results on the effect of different growth regulators on microtuberization induction in several varieties of seed and commercial potato (*Solanum tuberosum* L.) *in vitro*. The seed potatoes of the varieties Dido, Marabel, Agria and Agriko and commercial potatoes of the varieties Agria SR, Agria BE and Andrea were used in the experiment. Experiments *in vitro* were set using two types of explants: sprouts and nodal explants, on the MS medium supplemented with several different combinations and concentrations of cytokinines and auxins. Microtuberization was stimulated by increasing the percentage of sugar in MS medium from 3% to 9% sucrose.

On the MS+4 mg/l BAP+2mg/l NAA+6% sucrose, microtuberization reached up to 86.66% in node culture of the variety AgriaSR.

Key words: micropropagation, *in vitro*, nodes, sprouts, shoots, roots, microtubers.
Abbreviations: GA_3 (gibberellic acid), KIN (kinetin), BAP (6-Benzylaminopurine), NAA Naphthaleneacetic acid).

INTRODUCTION

Potato is the fourth important crop in the world after wheat, rice and maize. Potatoes are thought to have originated from high - mountain ranges of the Andes in South America. This crop is grown in 180 countries worldwide. According to the FAO statistic (https://faostat.fao.org), the largest producer of potatoes is Asia, then Europe, South America and North and Central America. The very early beginning of potato cultivation in Macedonia is dating back 150-170 years ago. Today in the country, potatoes are grown on more than 13,000 hectares with an average yield of 20-40 t/ha, and every year the area of potato cultivation is extended (Statistical Yearbook of Republic of Macedonia, 2014).

The formation of the tubers is a very complex process, but it can be stimulated under *in vitro* conditions known as microtuberization (Abbot and Belcher, 1986; Apichai, 1988; Dodds et al., 1992; Coleman et al., 2001). Mi crotubers have a huge advantage in terms of storage, transportation and manufacturing practices, because of their small size and weight. They can be planted directly into the soil or can be produced as bulk at any time of year. They have similar morphological and biochemical features of tubers compared with conventionally produced potatoes. Therefore, mass production of potato through microtuberization is already revolutionising the world in potato production (Kanwal et al., 2006).

To stimulate microtuberization, many researchers used different growth regulators for *in vitro* induction of microtubers (Tovar et al., 1985; Simko, 1993; Tugrul and Samanci, 2001). A number of extensive physiological studies have shown that *in vitro* tuberization is controlled by several factors, such as hormonal composition and concentration of phytohormone, ratio of photoperiod, composition and concentration of nutrients in the media etc. (Coleman et al., 2001; Zobayed et al., 2001; El-Sawy et al., 2007; Anoop and Chauhan, 2009). This technology is used to produce virus free seed potato in many countries in the world with great success (Wang and Hu, 1982; Khan et al., 2003). Lately protocol for mass production of microtubers has been automated using a bioreactor (Xuan, et al., 2003).

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The techniques of plant tissue culture are used worldwide to produce pre-basic virus free seed, known as microtubers. They are sowed in protected areas in order to produce mini-tubers (basic seed). Basic seed enters the chain of production of certified seed potatoes, to be distributed to end users, mainly farmers.

The main objective of this research was to study the effect of different growth regulators for induction of microtuberization. The research was focused on setting the culture of sprouts and nodes as initial explants from several varieties of seed and commercial potatoes under *in vitro* conditions. During this experimental work several parameters were followed: the development of explants, organogenesis, the effect of various hormones in the development of different starting explants and the ability for microtuberization.

MATERIAL AND METHODS

The experiment was conducted in the Laboratory of Plant Biotechnology, Faculty of Agriculture, Goce Delcev University – Stip, Macedonia. The following potato varieties were used as starting material for the experiment:

- seed potatoes: Dido, Marabel, Agria, Ambition and Agriko;
- commercial potatoes: Agria SR, Agria BE and Andrea.

The variety Agria SR is cultivated in Strumica region, while the variety Agria BE is cultivated in Berovo region. The two regions differ in altitude, soil types and climate, thus the commercial potatoes of the same variety were treated as different starting material.

In vivo treatment of potato tubers with GA_3

Tubers of different potato seed and commercial varieties used in the experiment were treated with different concentrations of GA_3: 2, 12 and 22 ppm. To determine whether GA_3 had effect on sprouts emergence, a control K was used, where the tubers were not treated with GA3 (Figure 1).

The GA3 treatment was used for induction of rapid emergence and germination 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 enriched with different concentrations of phytohormones.

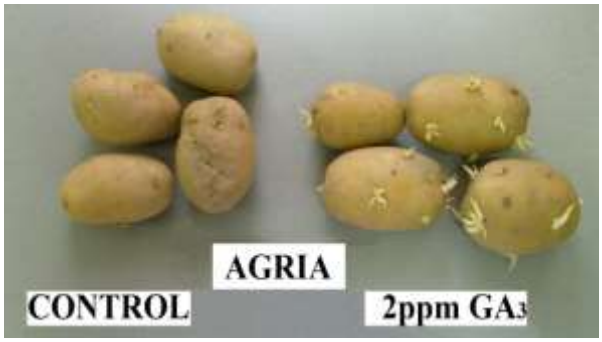

Figure 1. The effect of treatment with 2 ppm GA_3 for rapid sprouting and *de novo* production of sprouts in variety Agria compared to the control

Sterilization of initial explants (sprouts)

The sprouts were surface sterilised by washing under running water about 10-15 minutes and then washed in distilled water several times. After that, the sprouts were surface sterilized by immersion in:

- 70% C_2H_5OH for 2 minutes;
- 0.1% $HgCl_2$ for 3-5 minutes and then several times washed with sterile water.

Explants set under *in vitro* conditions

The sterilised sprouts as initial explants were placed on MS (Murashige and Skoog, 1962) solid medium (Figure 2A). The MS was supplemented with 0.7% agar, 100 g/l myo-inozitol, 200 g/l casein enzymatic hydrolysate, 0.1mg/l thiamine, 1.0 mg/l pyridoxine and 0.5 mg/l nicotinic acid, 2 mg/l BAP or 4 mg/l KIN as follows:

Sprouts → MS + 2 mg/l BAP (varieties: Dido, Marabel, Agria SR, Agria BE);

Sprouts → MS + 4 mg/l KIN (varieties Dido, Marbel, Agriko, Agria SR).

The MS medium pH was adjusted to 5.8.

Within a month, the sprouts developed into shoots with different number of nodes. The shoots were cut into nodes and subcultured on:

Sprout nodes → MS + 2 mg/l BAP + 1 mg/l IAA.

This medium was used for stimulation of nodes growth (Figure 2B).

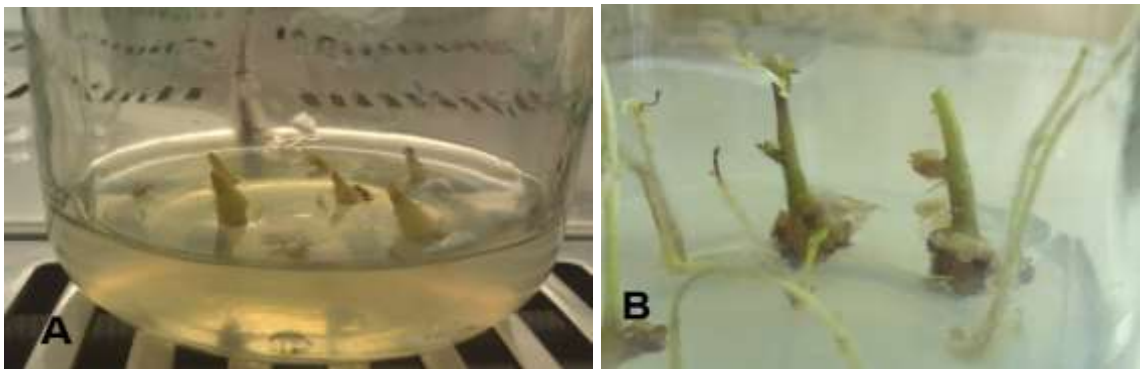

Figure 2. A) Culture of potato sprouts, B) Culture of node explants

When the explants reached certain length, they were cut into nodes and subcultured on MS supplemented with different concentration of BAP, NAA and sucrose for induction of microtubers (Figure 3A, 3B).

The following media were used for induction of microtubers in different potato varieties:

Nodes → MS + 2 mg/l BAP + 2 mg/l NAA+ 3% sucrose;
Nodes → MS + 1 mg/l BAP + 0.5 mg/l NAA+ 4% sucrose;
Nodes → MS + 4 mg/l BAP + 2 mg/l NAA+ 6% sucrose;
Nodes →MS + 6 mg/l BAP + 2 mg/l NAA+ 9% sucrose.

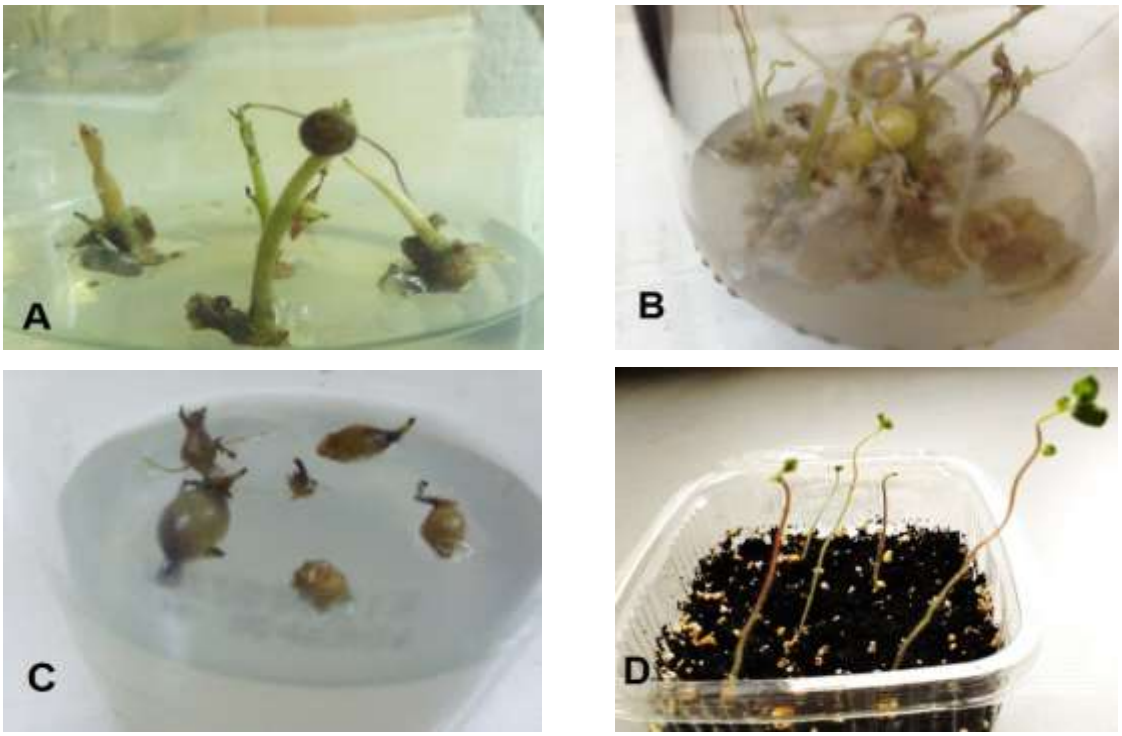

Figure 3. A) Formation of microtubers in node culture; B) Microtuberization on MS + 4 mg/l BAP + 2 mg/l NAA + 6% sucrose; C) Culture of microtubers in MS + 0.5 mg/l BAP + 1 mg/l KIN + 5% sucrose; D) Germinated microtubers into a sterile mix of peat : perlite (1:1)

Maintenance of cultures in the climate chamber

All explants, sprouts and nodes, were incubated in a climate chamber under the following conditions:

Temperature 25 ± 1°C;
Relative humidity 50%;
Photoperiod:16/8 hours light/dark;
Illumination of 50*cd.*

In vivo development of microtubers

Formed microtubers were subcultured on MS + 0.5 mg/l BAP + 1 mg/l KIN + 5% sucrose. This medium was used for microtuber growth (Figure 3C).

The formed microtubers in culture *in vitro* were planted in a sterile mixture of peat: perlite (1:1) for the purpose of forming the mini-tubers and later formation of the seed tubers of potato. Microtubers were adapted to non-sterile conditions and formed shoots (Figure 3D).

Data analysis

All data were subjected to statistical analysis with IBM SPSS Statistical 21, one-way ANOVA and Duncan *posthoc* test, with the level of significance 0.05%.

RESULTS AND DISCUSSION

During the research, the effect of different KIN and BAP concentrations on potato initial explants (sprouts) from different potato varieties was observed (Table 1).

Table 1. Effect of BAP and KIN on formation of shoots and roots from potato sprout explants

Initial explants – sprouts						Formation of shoots and roots						
Variety	MS medium (mg/l)	Number of explants	Length (mm)	Thickness (mm)	% of germination	Length of shoots (mm)	Thickness of shoots (mm)	Number of shoots	Number of roots	Length of roots (mm)	% of rooting	% of sprouting
Seed potato												
Dido	2BAP	25	10.80b	2.06bc	100	30.00a	1.00a	17	8	15.00a	26.90a	80.95a
Marabel	2BAP	36	13.52a	1.62c	100	18.68b	1.18a	16	2	15.00a	31.25a	86.66a
Dido	4 KIN	24	8.91b	2.29a	100	No shoots and roots induction						
Marabel	4 KIN	38	15.15a	1.80b	100	No shoots and roots induction						
Agriko	4 KIN	57	9.98b	1.22a	100	No shoots and roots induction						
Commercial potato												
Agria SR	2 BAP	19	6.63c	3.89a	100	27.65a	1.01a	20	2	3.50b	29.58a	82.50a
Agria BE	2 BAP	46	3.73d	2.29b	100	22.31ab	1.10a	44	14	8.00ab	30.03a	69.41a
Agria SR	4 KIN	24	7.62c	1.70b	100	No shoots and roots induction						

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $p \leq 0.05$.

The results showed that the medium MS + 2 mg/l BAP gave the best initiation in production of roots and shoots in all tested varieties, regardless if they originated as seed or commercial potato variety. The variety Marabel showed the best reaction to the medium MS + 2 mg/l BAP with 31.25% rooting explants and 86.66% sprouting explants. The initial explants of the variety Agria BE gave the highest number of shoots (44) when cultivated MS + 2 mg/l BAP The medium MS + 4 mg/l KIN did not have effect on neither root or shoot formation in tested varieties. This results showed that the medium MS + 2 mg/l BAP directed the initial explants towards rhizogenesis and shoots formation in all varieties tested in the experiment, regardless if they were seed or commercial potato.

The combination of cytokinin and auxin showed positive results on organogenesis in different potato varieties (Koleva Gudeva et al., 2012), thus we examined the influence of BAP and IAA for callus and root formation in different potato varieties (Table 2). The influence of MS + 2 mg/l + 1 mg/l IAA was tested for the shoot development, callus formation and rhizogenesis. The percentage of

root formation was between 26.90% for Dido and 31.55% for Marabel, without significant difference. The highest percentage of callus formation gave the variety Marabel (93.75%) which was significantly different from the variety Agria BE (50.44%).

Table 2. Effect of MS + 2 mg/l BAP + 1 mg/l IAA on formation of callus and roots from sprout nodes

Sprout nodes				Formation of callus and roots						
Variety	Number of explants	Length of shoots (mm)	Thickness of shoots (mm)	Height of callus (mm)	Thickness of callus (mm)	Number of calli	Number of roots	Length of roots (mm)	% of rooting	% of callus formation
Seed potato										
Dido	17	30.00a	1.00a	1.45a	1.38a	18	8	15.00a	26.90a	72.61ab
Marabel	16	18.68b	1.18a	0.94b	1.43a	16	2	15.00a	31.25a	93.75a
Commercial potato										
Agria BE	44	22.31ab	1.10a	1.18ab	1.25a	22	14	8.00ab	30.03a	50.44b
Agria SR	20	27.65a	1.01a	0.71b	0.59b	11	2	3.50b	29.58a	80.00ab

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $p \leq 0.05$.

Growth regulators BAP and NAA applied in concentration of 4-5 mg/l has important role in callus formation of node explants, while microtuber formation is always in relation with callus formation (Iqbal et al., 2014).The results of influence of MS medium and BAP, IAA and sucrose on callus formation and microtuberization are presented in Table 3 and Table 4.

Agria SR did not respond to the induction medium MS + 2 mg/l BAP + 2 mg/l NAA + 30% sucrose, neither with callus formation nor microtuberization.

Table 3. Effect of different concentrations of BAP, NAA and sucrose on formation of callus in potato node explants

Node explants						Formation of callus			
Variety	MS medium (mg/l)	Sucrose (%)	Number of explants	Length of node (mm)	Thickness of node (mm)	Height of callus (mm)	Thickness callus (mm)	Number of callus	% of callusing
Agria SR	2 BAP + 2 NAA	3	13	5.84d	0.86b	No callus induction			
Dido	1 BAP + 0.5 NAA	4	13	17.61c	0.97b	0.68a	0.85a	6b	43.33b
Agria BE	1 BAP + 0.5 NAA	4	13	23.15b	1.00b	0.65a	0.81a	6b	46.66b
Agria SR	4 BAP + 2 NAA	6	14	31.78a	1.42a	0.73a	0.82a	8ab	58.33ab
Agria BE	4 BAP + 2 NAA	6	14	30.21a	1.07b	0.80a	0.87a	8ab	58.33ab
Agria SR	6 BAP + 2 NAA	9	14	32.14a	1.50a	0.89a	0.90a	10a	80.00a

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $p \leq 0.05$.

Table 4. Effect of different concentrations of BAP, NAA and sucrose on microtuberization in potato node explants

Node explants						Microtuberization			
Variety	MS medium (mg/l)	Sucrose (%)	Number of explants	Length of node (mm)	Thickness of node(mm)	Length of tubers (mm)	Width of tubers (mm)	Number of tubers	% of tuberization
Agria SR	2 BAP + 2 NAA	3	13	5.84d	0.86b	No microtuber formation			
Dido	1 BAP + 0.5 NAA	4	13	17.61c	0.97b	5.00a	2.77a	8	58.33b
Agria BE	1 BAP + 0.5 NAA	4	13	23.15b	1.00b	4.94a	3.50a	9	78.33ab
Agria SR	4 BAP + 2 NAA	6	14	31.78a	1.42a	5.16a	3.50a	12	86.66a
Agria BE	4 BAP + 2 NAA	6	14	30.21a	1.07b	5.00a	3.80a	10	70.00ab
Agria SR	6 BAP + 2 NAA	9	14	32.14a	1.50a	5.47a	3.64a	17	83.33a

Mean separation in columns by Duncan's multiple range test. In each column, values followed by the same letter do not differ significantly at $p \leq 0.05$.

The lowest percentage of callus formation gave Dido and Agria S cultivated on MS + 1 BAP +0.5 NAA + 4% sucrose, 43.33% and 46.66% respectively. The highest percentage of callus formation 80% showed Agria SR on MS + 6 mg/l BAP + 2 mg/l NAA + 9% sucrose.

The highest percentage of microtuberization showed node explants from the variety Agria SR cultivated on the media MS + 4 mg/l BAP + 2 mg/l NAA + 6% sucrose (86.66%) and MS + 6 mg/l BAP + 2 mg/l NAA + 9% sucrose (83.33%). The lowest percentage of microtuberization showed node explants of Dido cultivated on the medium MS + 1 mg/l BAP + 0.5 mg/l NAA + 4% sucrose.

The highest number of microtubers gave Agria SR on MS + 4 mg/l BAP + 2 NAA + 6% sucrose and MS + 6 mg/l BAP + 2 mg/l NAA + 9% sucrose, 17 and 12 tubers respectively. Thee lowest number of microtubers (8) was obtained for Dido variety cultivated on MS + 1 mg/l BAP + 0.5 mg/l NAA + 4% sucrose. These results are in line with Dieme et al. (2013) who found that media enriched with BAP, KIN and sucrose gave better microtuber formation. 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.

From the presented results it is obvious that the utilisation of appropriate medium significantly improve the induction of microtuberization in potato. The shoot nodes from the varieties Dido, Marabel, Agria SR и Agria BE gave good results for induction of callus and roots when cultured on MS + 2 mg/l BAP + 1 mg/l NAA. The sucrose concentration had a great influence on microtuber formation and the variety Agria SR showed the highest potential for microtuberization compared to all other tested varieties.

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