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Micropropagation of Potato *Solanum tuberosum* L.

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Abstract

The effect of cytokinins and combination of cytokinins and auxins on *in vitro* microtuber formation and growth of two potato cultivars of potato (*Solanum tuberosum* L.) were evaluated.
In the present study sprouts and nodal explants of potato, cultivars Agrija and Andrea, were cultured on MS medium, supplemented with different hormonal combinations. For sprouts as an initial explants were used MS + 4 mg/l KIN and MS + 2 mg/l BAP, and for nodal explants were used MS + 4mg/l KIN + 1mg/l IAA and MS + 2 mg/l BAP+1 mg/l NAA. For rapid sprouting clean popato tubers were *in vivo* treated with 2 ppm GA_3. Between the two different explants (nodal segment and sprout) nodal cutting showed the better microtuber formation. The cultivar Agrija showed greater ability for *in vitro* propagation, with 2.14 tubers per shoot and 13.33% microtuber formation.

Keywords: *in vitro*, sprouts, nodal segments, microtuberisation, shoot formation, root formation.

1. Introduction

Potato (*Solanum tuberosum* L.) is a very important crop in agricultural production in the Republic of Macedonia and around the world. It is grown in 180 countries worldwide. According to FAO data [1], the most potatoes are produced in Asia, then in Europe; South America; then in North and Central America. Europe's largest manufacturers of potatoes are Ukraine, Poland, Belarus, Germany, Romania, the Netherlands, and France. The beginning of potato cultivation in Macedonia dates 150-170 years. Today in the Republic of Macedonia potatoes are produced on over 13000 hectares with average yields 20-40 t/ha and almost every year the area expand.

Tuberisation process in potato is a very complex, but it can be induced under *in vitro* condition. Because of their small size and weight, microtubers have tremendous advantages in terms of storage, transportation and production practices. They can be directly sown into the soil and can be produced in bulk in any season. They have similar morphological and biochemical characteristics compared to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production [2].

Many researchers used different growth regulators for *in vitro* induction of microtubers in potato [3-6]. A number of extensive physiological research has revealed that *in vitro* tuberisation is controlled by several factors, such as hormonal combination, ratio of photo period, nutrient compositions etc. [7-10]. This technology has been used for disease-free potato seed production in many countries [11-13]. Nowadays a protocol for mass propagation of potato microtubers is automated by using bioreactor system [14].

Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. The microtubers are sown in a protected environment to produce minitubers (basic seed). The basic seed enters the seed production chain to produce the certified seed to be sold to the farmers. The main objective of the present study was to standardize the media for potato plant growth and microtuber induction.

The main goal of this research was to set up a culture of sprouts and nodal segments as initial explants of two potato cultivars Agrija and Andrea in *in vitro* conditions. Explants development, organogenesis stage of explants on different hormonal media and possibilities for microtubrisation were followed during this experimental work.

2. Materials and Methods

The experiment was conducted at the Department of Plant Biotechnology, Faculty of Agriculture, Goce Delcev University – Stip, Republic of Macedonia. The well establish potato cultivars Agrija and Andrea were used as plant material for evaluation for their response to *in vitro* microtuberisation. Clean tubers were treated with 2 ppm GA_3 for rapid sprouting. One week old sprouts were used as initial explants (Fig. 1).

2.1 Sterilization of initial explants - sprouts

The sprouts were surface sterilized by washing under flow of tab water for 10-15 minutes. After washing the sprouts are surface sterilized by dipping in

- 70% C_2H_5OH for 2 minutes,
- 0.1 $HgCl_2$ solution for 3-5 minute, and
- then washed several times with autoclaved distilled water.

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These initial explants were cultured in Murashige and Skoog (MS) [15] solid medium (pH = 5.8) in test tubes supplemented with cytokinins.

Sprouts → MS + 4 mg/l KIN
Sprouts → MS + 2 mg/l BAP

2.2 *In vitro* culture conditions

The bud sprouted into plantlet having 4-5 nodal segments within one month. Nodal cuttings were collected from regenerated plantlets and used as explants for *in vitro* tuberisation of potato. MS media were prepared with 3% sucrose, 0.7% agar, myo-inositol 100g/l; casein enzymatic hydrolysate 200g/l, thiamine HCl 0,1 mg/l, pyridoxine HCl 1,0 mg/l, nicotinic acid 0.5 mg/l and supplemented with cytokinins and auxins.

Nodal explants → MS + 4mg/l KIN + 1mg/l IAA
Nodal explants → MS + 2 mg/l BAP+1 mg/l NAA

The cultures were incubated under the following culture chamber conditions:

- temperature 25±1°C;
- relative humidity 50%;
- photoperiod 16/8 hour light/dark and
- illumination of 50 $\mu mol \cdot m^2 \cdot s^{-1}$.

3. Results and Discussions

The influence of gibberellic acid on stimulation of sprout formation is presented in Table 1. The treatment with 2 ppm GA_3 was efficient for the two cultivars. All treated tubers resulted with *de novo* a sprout, which shows effect of 100.00% in sprouts formation. A higher number of sprouts were formed from the cultivar Agrija with average of 9.66 sprouts per tuber (Figure 1).

Table 1. The effect of GA_3 treatment on *in vivo* sprout formation in potato tubers *in vivo*.

Cultivar treated with 2 ppm GA_3	Nr. of treated tubers	Nr. of obtained sprouts per tuber	Sprouts formation %	Length of sprouts x̄ mm	Thickness of sprouts x̄ mm
Andrea	34	6.87	100.00	10.03	4.68
Agrija	32	9.66	100.00	8.27	1.83

Figure 1. The effect of treatmeant with 2 ppm GA_3 for rapid sprouting.

In the Table 2 is presented the effect of cytokinins KIN and BAP on *in vitro* shoots, roots and plantlets formation from sprout explants. The results show that the cultivar Agrija has maximum potential for rooting and shoots formation. On MS + 2 mg/l BAP both parameters show 100.00% rooting and formation of start explants (Figure 2).

Figure 2. Shoot formation from initial sprouts explants: **A.** after 2 weeks; **B.** after 3 week; **C.** after 6 weeks.

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Table 2. The effect of KIN and BAP on *in vitro* shoots, roots and plantlets formation form sprouts explants.

Cultivar on MS media	Initial explants				Shoot and root formation						
	Nr of explants	Type of explant	Length x̄ mm	Thickness x̄ mm	Shoot length x̄ mm	Shoot thickness x̄ mm	Nr of shoots per explant	Nr of root per shoot	Root length x̄ mm	% rooting	% shoot formation
MS + 4 mg/l KIN											
Andrea	33	sprout	6.22	3.87	20.07	2.50	1.25	3.00	10.66	11.75	42.42
Agrija	36	sprout	6.94	4.07	20.82	2.47	2.47	3.75	20.02	16.25	100.00
MS + 2 mg/l BAP											
Andrea	36	sprout	6.15	3.64	25.30	3.00	1.56	2.56	10.83	17.64	30.26
Agrija	34	sprout	7.82	2.87	26.36	2.72	2.37	2.37	20.33	100.00	100.00

Table 3. The effect of KIN + IAA and BAP + NAA on *in vitro* shoots, roots and plantlets formation form nodal segments explants.

Cultivar on MS media	explants				Shoot and root formation						
	Nr of explants	Type of explant	Length x̄ mm	Thickness x̄ mm	Shoot length x̄ mm	Shoot thickness x̄ mm	Nr of shoots per explant	Nr of root per shoot	Root length x̄ mm	% rooting	% shoot formation
MS + 4 mg/l KIN + 1 mg/l IAA											
Andrea	30	Nodal segments	18.07	2.50	29.18	2.60	3.60	2.90	26.72	100.00	100.00
Agrija	30		18.82	2.47	29.93	2.57	4.00	8.50	28.59	100.00	100.00
MS + 2 mg/l BAP + 1 mg/l NAA											
Andrea	30	Nodal segments	23.30	3.00	30.32	3.00	3.90	3.00	27.44	100.00	100.00
Agrija	30		24.36	2.72	33.05	2.82	4.24	8.20	31.03	100.00	100.00

Table 4. The effect of KIN + IAA and BAP + NAA on *in vitro* microtuber formation in potato.

Cultivar on MS media	explants				microtuber formation			
	Nr of explants	Type of explant	Length x̄ mm	Thickness x̄ mm	Shoot length x̄ mm	Shoot thickness x̄ mm	Nr of microtuber per explant	% microtuber formation
MS + 4 mg/l KIN + 1 mg/l IAA								
Andrea	30	Nodal segments	18.07	2.50	29.18	2.60	0.00	0.00
Agrija	30		18.82	2.47	29.93	2.57	1.50	10.00
MS + 2 mg/l BAP + 1 mg/l NAA								
Andrea	30	Nodal segments	23.30	3.00	30.32	3.00	0.00	0.00
Agrija	30		24.36	2.72	33.05	2.82	2.14	13.33

Figure 3. Shoot and microtuber formation from nodal explants: **A.** after 2 weeks; **B.** after 3 week; **C.** microtuberisation after 6 weeks.

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The effect of cytokinins and auxins in MS medium and their influence on organogenesis *in vitro* of nodal segments is presented in Table 3. The specific combination of cytokinin with auxin was effective for the both cultivars. The percentage of rooting and shoot formation is maximum 100 for the both cultivars.

Microtubersation is examined in MS medium with combination of cytokinin and auxin and the results are presented in Table 4. The cultivar Andrea result with no microtuberisation on all media, while the cultivar Agrija resulted with microtuberisation on the MS + 4 mg/l KIN + 1 mg/l IAA and MS + 2 mg/l BAP + 1 mg/l NAA with 10% microtubers and 13,33% microtubers respectively (Figure 3).

However, the effect was variable depending on the genotype and cytokinin concentration. The response of potato cultivars studied was in general agreement with previously reported findings by a number of investigators using different cultivars [15-17].

Sucrose has been extensively used to induce potato microtubers. It was refereed by many studies that explants maintained in medium devoid of sucrose responded negatively with respect to tuber formation regardless of the incubation periods. In contrast, the use of sucrose at concentrations range between 6-12% resulted in earlier microtuber formation and the intensity of microtuber formation increased progressively with the length of incubation period. These results confirmed the importance of sucrose on *in vitro* microtuber development as described by a number of investigators [16, 18, 19, 20].

In this experiment was used 3% sucrose in MS medium. Probably the low formation of tubers in the cultivar Agria (10.00% and 13.33%) is due to the low present of glucose used in the MS medium.

4 Conclusions

In conclusion, the results of present research suggested that standardisation of culturing media significantly improve the potato plant growth and microtuber induction.

Micro propagation is the alternative to conventional propagation of potatoes. *In vitro* propagation methods using sprouts and nodal cuttings are more reliable for maintaining genetic integrity of the multiplied clones.

Microtubers are the first generation of potato seed from tissue culture: they are used to solve the problems of transplanting the plantlets from *in vitro* to in vivo conditions.

The microtubers have a lot of advantages, thanks to their small size and reduced weight, upon storage, transport and mechanization. They can be planted on the soil and can be produced at any time of the year. They have similar morphology and biochemical features with traditional tubers. *In vitro* microtuber production is very important for the production and storage of a potato valuable stock. Potato microtubers obtained through *in vitro* culture from single-node cuttings are convenient for handling, storage and exchange of healthy germplasme. The nodal segments as starting explants demonstrated higher efficiency compared to the sprouts. The composition of MS with cytokinin and auxin has shown the best effect, especially MS + 2 mg/l BAP + 1 mg/l NAA where the cultivar Agrija formed 13.33% microtubers. High sucrose concentrations may act as inducing signal leading to starch accumulation, so to increase the present of microtuberisation the concentration of sucrose must be higher.

On the tested media the cultivar Agrija has higher potential for *in vitro* micropropagation and microtuberisation.

The study also demonstrates that *in vitro* tuberization capacity of potato depends on the genotype.

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