

# Influence of Indole-3 butyric acid and 6-benzylaminopurine with Sucrose on in vitro Potato Microtuber Formation

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## Abstract

The research aim was to study the influence of sucrose with a combination of growth regulators: Indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) on potato in vitro micro tuber formation.

Under two levels of sucrose, three potato cultivars, "Nevsky", "Riviera" and "Zefira" were studied for in vitro tuberization response: Murashige and Skoog (MS) media supplemented with 80 g.L<sup>-1</sup> sucrose (8% MS medium) and 100 g.L<sup>-1</sup> (10% MS medium). Two concentration (0.05 mg.L<sup>-1</sup> and 0.1 mg.L<sup>-1</sup>) of growth regulators: indole-3-butyric acid (IBA) and 6-benzylaminopurine (BAP) were tested in this work.

Potato varieties "Zefira", "Nevsky" reacted positively on high concentration of growth regulators (0.1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA) on in vitro tuberization process, while the concentration of IBA increased from 0.05 mg.L<sup>-1</sup> to 0.1 mg.L<sup>-1</sup> potato variety "Riviera" had a negative results on the microtuber germination rate.

Finally, the optimal concentration of sucrose (10 g.L<sup>-1</sup>) and growth regulators (0.1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA) for tuberization of potato varieties "Zefira" and "Nevsky" has been selected. The best protocol (8% MS medium + 0.1 mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA) for in vitro tuber induction of varieties "Riviera" was developed.

**Keywords:** Potato, MS medium, sucrose, micro-tuber, indole-3-butyric acid, 6-benzylaminopurine

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## INTRODUCTION

Potato (*Solanum tuberosum* L.) production can't supply global human's consumption. One of the variety reasons is the usage of contaminated tubers in crop production [4]. One way to enhance the production of virus-free seed potatoes is employing the tissue culture techniques all over the world. [31]; [7]. The reasons for potato degeneration are infection diseases, asexual micropropagation of potato in in vitro condition is the method for potato growers to produce disease-free tubers and has some advantages: in vitro microtubers are more stable and require less care for acclimatization in the open field. The characteristics of microtubers are controlled by sucrose level, growth regulators, a regulated condition in phytotron, etc. [21]; [13].

An efficient potato production model is being developed by several research groups throughout the world [17]; [34]. Microtuber technology in seed tuber production technique has been considered an effective alternative to a propagule [5]; [21]. Mass production of potato microtubers became more widely used in the world than the conventional method of micropropagation [6].

The genetic stability of multiplied clones is improved using in vitro micro propagation methods [26]. Microtubers demonstrate a higher yield (84%) potential, than conventional tubers for field planting, due to small size and weight of micro tubers they are easily handled, stored, and distributed [6];[7].

Micro tuberization is a hard and long-delayed process, it need highly growth regulators, temperature, photoperiod, light intensity and cultivars [1]; [20]; [3]; [14]; [11]. The formation of potato microtubers depends on a combination of cytokines and hormone growth factors [24];[15].

The role of the combination of hormones and sucrose in the tuberization process was extensively studied and well documented [11]. For in vitro development of microtubers in potato tuberization, many researchers used various growth regulators [24]; [29]. In some cases hormones have a residual effect on some varieties [9]. The media culture which is characterized by several aptitudes to in vitro tuberization according to potato varieties has been improved by several authors [25]; [28];[8].

The goal of this study was to find the optimal MS medium and the appropriate hormonal/sucrose combination for three varieties of potato (*Solanum tuberosum* L.): “Nevski”, “Riviera”, and “Zefira” for their in vitro microtuber formation.

## Materials and Methods

### 2.1. Sample collection

The experiment has been conducted at the Georgian Technical University, Biotechnology Center, Tbilisi, Georgia. These varieties were chosen because of their adaptability to Georgia’s agroclimatic conditions. The research was conducted with in vitro potato collection created by apical meristem technics of Georgian Technical University Biotechnology Center during 2019-2021year.

### 2.2. Propagation of in vitro potato

Murashige and Skoog medium (MS) was used to cultivate explants [32]. MS medium with 30 g.L<sup>-1</sup> sucrose; 7 g/L-1 agar and autoclaved to 121°C during 20 min at 15 psi, pH was adjusted to 6.1. 2-3 cm long single nodes were separated from 4-5 weeks old explants with 5-6 nodal segments under laminar flow chamber and used as explants for in vitro tuberization in potato.

### 2.3. Experimental site

The observation of micro tuber formation of potato varieties: “Nevsky”; “Zefira”;”Riviera” were conducted in phytotron under controlled condition (temperature: 23-240C; humidity- 75%; Lux-4.5000; 16h photoperiod). Control- Murashige and Skoog (MS) (1962) medium with 30 g.L<sup>-1</sup> sucrose (3% MS medium);

Six Modified MS medium (Murashige and Skoog (MS) (1962)) were used in experiment:

- MS+ 80 g.L<sup>-1</sup> sucrose (8% MS medium);
- 8% MS medium +1 mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA;
- 8% MS medium +1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA;
- MS+ 100 g.L<sup>-1</sup>sucrose (10% MS medium);
- 10% MS medium +1 mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA;
- 10% MS medium +1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA.

Microtubers were collected after 55-60 days of development. Data were recorded on days to shoot formation, a number of shoots per explant, days to micro tuber formation per explant, and the average weight of microtuber.

### 2.3. Statistical analysis:

Before harvest, the number of potato microtubers in each explant was examined. Digital Caliper was used for measuring the diameter (in millimeters) of each microtuber. Microtubers were weighed on an analytical balance directly after harvest to determine the microtuber weight in grams. The data were analyzed using the SAS program [30]. Growth parameters were analyzed and means were compared using Duncan's Multiple Range Tests.  $P \leq 0.05$  after culture with 3 replicates per treatment per culture condition.

According to Kikuta and Okazawa the organ formations were measured using the following parameters [33]. Microtuber formation index = (number microtuber formed × number of explant with microtuber)/ (number of produced explant).

## Results And Discussion

Potential of micro tuber production of all in vitro potato cultivars depending on sucrose/growth hormone combination [17]; [16]. It was revealed that growth regulators have a positive influence on potato in vitro microtuber formation. The combined effect of IBA and BAP on three potato varieties “Nevsky”; “Zefira”; ”Riviera” was studied in the presence of two concentrations of sucrose (80 g.L<sup>-1</sup> and 100 g.L<sup>-1</sup>). The highest rate of microtubers germination (86.66%) for “Nevsky” and “ Zefira” was obtained with the 10 % MS medium + 0.1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA while the combination 8 % MS medium + 0.1 mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA allows a significantly better germination rate (63.33%.) for “Riviera” (Figure 1.2).

Fig. 1: In vitro tuberization of potato cultivars: A, B-“Nevsky”; C-“Riviera”.



Fig. 2: In vitro tuberization of potato cultivars “Zefira” A-10 % MS medium; B-10 % MS medium +0.1 mg.L<sup>-1</sup> BAP+0.1 mg.L<sup>-1</sup> IBA.



According to the results, the cultivar “Nevsky” revealed maximum microtuber formation ability (Microtuber number-  $3.98 \pm 0.04$ ; Diameter-  $9.9 \pm 0.02$ ; Weight-  $0.09 \pm 0.002$ ) only on 10% MS medium +  $1 \text{ mg} \cdot \text{L}^{-1}$  BAP+ $0.1 \text{ mg} \cdot \text{L}^{-1}$  IBA; on the other medium "Nevsky" did not develop any microtuber (Table 1).

TABLE 1: Effects of sucrose/hormone combination on in vitro tuber formation of potato cultivar "Nevsky".

Potato cultivar “Nevsky”				
Sucrose/hormone combination(g.L <sup>-1</sup> )	Microtuber number (mm)	Microtuber diameter (mm)		Microtuber weight (g)
8%MS medium	-	-		-
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.05 mg.L <sup>-1</sup> IBA	-	-		-
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.1 mg.L <sup>-1</sup> IBA	-	-		-
10 % MS medium	-	-		-
10 % MS medium + 0.1 mg.L <sup>-1</sup> BAP + 0.05 mg.L <sup>-1</sup> IBA	-	-		-
10 % MS medium +0.1g.L <sup>-1</sup> BAP + 0.1g.L <sup>-1</sup> IBA	$3.98 \pm 0.04$	$9.9 \pm 0.02$		$0.09 \pm 0.002$

"Riviera" did not develop any microtuber in the presence of  $80 \text{ g} \cdot \text{L}^{-1}$  of sucrose, the germination process begins by increasing the sucrose concentration from  $80 \text{ g} \cdot \text{L}^{-1}$  up to  $100 \text{ g} \cdot \text{L}^{-1}$ , but increasing the concentration of kinetin

from 0.05 to 0.1 mg.L<sup>-1</sup> IBA negatively affected the tuber germination process of “Riviera”. In compared other media culture 8 % MS medium +0.1 mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA was more favorable for the implantation of microtubers formation of this variety. (Table 2).

TABLE 2: Effects of sucrose/hormone combination on in vitro tuber formation of potato cultivar "Riviera".

Potato cultivar „Riviera“			
Sucrose/hormone combination (g.L)	Microtuber number (mm)	Microtuber diameter (mm)	Microtuber weight (g)
8%MS medium	-	-	-
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.05 mg.L <sup>-1</sup> IBA	2.4±0.03	6.2±0.04	0.038±0.003
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.1 mg.L <sup>-1</sup> IBA	1.6±0.02	3.9±0.03	0.021±0.002
10 % MS medium	1.2±0.02	3.4±0.03	0.017±0.002
10 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.05 mg.L <sup>-1</sup> IBA	-	-	-
10 % MS medium +0.1 mg.L <sup>-1</sup> BAP + 0.1 mg.L <sup>-1</sup> IBA	-	-	-

For „Zefira” increasing of sucrose concentration from 80 g.L<sup>-1</sup> to 10 g.L<sup>-1</sup> did not show a significant difference on microtuber formation process between microtuber number (from 1.3±0.03 to 1.4±0.02) as well as diameter (from 4.8±0.02 to 4.4±0.03) and weight (0.032±0.002 to 0.038±0.002). Increasing concentration of kinetin from 0.05 to 0.1 mg.L<sup>-1</sup> helped to raise slightly the germination rate: tuber number from 1.3 to 1.9; diameter - from 4.8 to 6.2 mm; weight from 0.032 to 0.048 g.

However, increasing the concentration of combination sucrose (from 80 g.L<sup>-1</sup> up to 100 g.L<sup>-1</sup>) with IBA/BAP (from 0.05 to 0.1mg.L<sup>-1</sup>) have a positive effect on the potato in vitro tuber formation: tuber number from 1.3 to 3.2; diameter from 4.8 to 9.9 mm; weight from 0.032 to 0.087 g (Table 3).

TABLE 3: Effects of sucrose/hormone combination on in vitro tuber formation of potato cultivars “Zefira”.

Potato cultivar „Zefira“			
Sucrose/hormone combination (g.L <sup>-1</sup> / mg.L <sup>-1</sup> )	Microtuber number (mm)	Microtuber diameter (mm)	Microtuber weight (g)
8% MS medium	1.3± 0.03	4.8±0.02	0.032±0.002
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP+0.05 mg.L <sup>-1</sup> IBA	1.5±0.02	5.2± 0.03	0.039±0.002
8 % MS medium +0.1 mg.L <sup>-1</sup> BAP+0.1 mg.L <sup>-1</sup> IBA	1.9±0.03	6.2±0.04	0.048±0.003
10 % MS medium	1.4±0.02	4.4±0.03	0.038±0.002
10 % MS medium +0.1 mg.L <sup>-1</sup> BAP+0.05 mg.L <sup>-1</sup> IBA	1.8±0.03	5.6±0.02	0.047±0.002
10 % MS medium +0.1 mg.L <sup>-1</sup> BAP+0.1 mg.L <sup>-1</sup> IBA	3.2±0.02	9.9±0.02	0.087±0.002

Improvement of in vitro microtuber formation system is important for potato quality seed production technology. Sucrose has a two major role in vitro tuberization as an energy source and as a signal for microtuber development [10].

Under in vitro environment, 30 g.L<sup>-1</sup> sucrose was estimated to be beneficial for vegetative growth and shoot development without tuber formation [21]. It is also suggested that the maximum number of microtuber from explants growing in the dark condition provided brown and with no sprout [27].

Even in the absence of growth regulators sucrose in MS medium can enhance the quantity and weight of microtubers and induce tuber development [23]. Our researched varieties were characterized by sick development of microtubers without hormones even in the increase of sucrose concentration from 80 g.L<sup>-1</sup> to 100 mg.L<sup>-1</sup>. According to some studies, large sucrose concentrations decreased germination rates by up to 55 percent [2].

The influence of hormones/ sucrose on in vitro potato propagation is different and depends on potato varieties due to their genetic diversity [26].

The microtuber formation of the potato varieties "Nevsky" and "Zefira" significantly enhances in 10% MS medium+0.1 mg.L<sup>-1</sup>BAP+0.1 mg.L<sup>-1</sup> IBA treated plants followed by 8% MS medium +0.1mg.L<sup>-1</sup> BAP + 0.05 mg.L<sup>-1</sup> IBA for "Riviera" whereas the minimum growth of the plantlets were recorded on 8% MS medium (Figure 1,2).

Using 0.1mg.L<sup>-1</sup> BAP+0.1 mg.L<sup>-1</sup> IBA in the MS medium resulted in a significantly larger number of microtubers, while an increase in sucrose concentration reduced the number of microtubers in the potato variety "Riviera," but no induction was observed by increasing the concentration of the hormone (Tab.3). The involvement of kinetin is ethylene biosynthesis impacts on cell elongation and tuber formation and depends on the amount of sucrose in the culture medium [23]. Some studies suggested that BAP/IBA combination increased the weight and diameter of microtubers [12]; [22]. Microtubet developments can be induced with a relevant combination of kinetin and BAP [15]; [19].

## Conclusion

Finally, the best culture medium for in vitro tuber production from single nodal explants of the potato types "Zefira," "Nevsky" and "Riviera" has been developed. The research showed that potato in vitro tuber formation was strongly dependent on the mixture of sucrose and hormones as well as genotype interaction. Increased sucrose concentration in the medium can help potato cultivars generate more microtuber. Positively evaluated the influence of IBA/BAP increased concentration (0.1 mg.L<sup>-1</sup> BAP + 0.1 mg.L<sup>-1</sup> IBA) on in vitro tuberization process of potato varieties. The selection of optimal MS medium for in vitro tuber production will help to eliminate the shortage of potato seed improve the social welfare of local farmers and the development of agriculture field in Georgia.

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## References

1. A. Badoni, and J. S. Chauhan, "Effect of Growth Regulators on Meristem-tip Development and in vitro Multiplication of Potato Cultivar 'Kufri Himalini'" *Indian Journal of crop Science*, vol. 4, pp. 155-157, 2009.
2. A. Dieme, M. A. N. Sambe, E. C. Agbangba, and M.O. Sy, "Residual effects of sucrose and hormonal treatments of the tuberization medium on In vitro germination of potato (*Solanum tuberosum* L.) microtubers", *Am. J. Plants Sci*, vol.4, pp.182-1878, September 2013.
3. A. El-Sawy, S. Bekheet, and U. I. Aly, "Morphological and molecular characterization of potato microtubers production on coumarininducing medium", *In J Agri Biol*, vol.9 (5), pp. 675-680. 2007.
4. A. Hirpa, M. P. M. Meuwissen, A. Tesfaye, W. J. M. Lommen, A. O. Lansink, A. Tsegaye, and P. C. Strik, " Analysis of seed potato systems in Ethiopia", *Am. J. Potato Res*, vol. 87, pp. 537-552, November-December 2010.
5. A. J. Haverkort, M. Van de Waart, and J. Marinus, "Field performance of potato microtubers as a propagation material", *Potato Res*, vol.34, pp. 353-364, September 1991.
6. A. Kanwal, A. Ali, and K. Shoaib, "In vitro microtuberization of potato (*Solanum tuberosum* L.) cultivar Kuroda a new variety in Pakistan", *International Journal of Agriculture and Biology*, vol. 8(3), pp.337-340, February 2006.
7. A. M. Khalafalla, "Effect of plant density and seed size on growth and yield of *Solanum* Potato in Khartoum State, Sudan", *Afr. Crop Sci. J*, vol. 9(1), pp.77- 82. January 2001.
8. C. L. Le, "Factors influencing the in vitro tuberization of potato microcuttings (*Solanum tuberosum* L., var. *Agria*)", *Revue Suisse Agric*, vol. 22, pp.115 – 116, 1990.
9. D. Abraham, A. N. S. Mame, C. A. Emile, and O.S. Mame, " Residual Effects of Sucrose and Hormonal Treatments of the Tuberization Medium on in vitro Germination of Potato (*Solanum tuberosum* L.) Microtubers", *American Journal of Plant Sciences*, vol. 4 pp.1872-1878, September 2013.

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10. D. J. Donnelly, W. K. Coleman, and S. E. Coleman, "Potato micro tuber and performance: A review", *American Journal of Potato Research*, vol. 80, pp. 103-115, March 2003.
11. D. Vreugdenhil, and P. C. Struik, "An Integrated view of the hormonal regulation of tuber formation in potato (*Solanum tuberosum* L.)", *Physiol. Pl.* vol. 75 (4), pp.525-31, April 1989.
12. E. Aryakia, and Y. Hamidoghli, "Comparison of kinetin and 6-Banzyl amino purine effect on in vitro microtuberization of two cultivars of Potato (*Solanum tuberosum* L.)", *Environ Sci*, vol.8, pp. 710-714, January 2010.
13. P. Ranalli, F. Bassi, G. Ruaro, P. Del Re, M. Di Candilo and G. Mandolino, "Microtuber and minituber production and field performance compared with normal tubers". *Potato Res*, volume 37(4): pp.383-391. December 1994
14. G. Hussey and N. J. Stacey, "Factors affecting the formation of in vitro tubers of potato (*Solanum tuberosum* L.)", *Ann. Bot.* vol. 53(4): pp.565-578, April 1984.
15. G. A. Romanov, N. P. Aksenova, T. N. Konstantinova, S. A. Golyanovskaya, J. Kossomann, and L. Willmitzer, "Effect of indole - 3-acetic acid and kinetin on tuberization parameters of different cultivars and lines of potato in vitro", *Plant Growth Reg.* vol. 32, pp.245-251, November 2000.
16. H. Mehraj, K. Momena, R. Adeeba, A. F. M. Jamal Uddin, S. Islam, and L. Rahman, "In vitro Microtuberization of Potato (*Solanum Tuberosum* L.) Cultivar through Sucrose and Growth Regulator". *Journal of Bioscience and Agriculture Research*, vol. 02 (02), pp.76-82. October 2014.
17. J. Gopal, A. Chamail, and D. Sarkar, "In vitro production of microtuber for conservation of potato germplasm: effects of genotypes, abscisic acid, and sucrose." *In vitro Cellular and Developmental Biology-Plant*, vol. 40, pp. 485-490, September 2004.
18. J. Kawakami, K. Iwama, T. Hasegawa, and Y. Jitsuyama, "Growth and yield of potato grown from microrubers in fields". *Am. J. Potato Res*, vol. 80(6), pp.371-378, November 2003.
19. J. Liu, and C. Xie, "Correlation of cell division and cell expansion to potato microtuber growth in vitro". *Plant Cell, Tissue and Organ Culture*, vol. 67, pp.159-164, November 2001.
20. K. W. Coleman, J. D. Danielle, and S. E. Coleman, "Potato microtuber as research tools: A Review", *Am J Potato Res*, vol. 78(47), pp. 47-55, January 2001.
21. M. E. Hoque, "In vitro tuberization in potato (*Solanum tuberosum* L.)" *Plant Omics Journal*. Vol. 3(1), pp. 7-11, January 2010.
22. M. Ebadi, and A. Iranbakhsh, "The Induction and Growth of Potato (*Solanum tuberosum* L.) Microtubers (Sante Cultivar) in Response to the Different Concentrations of 6-Benzylaminopurine and Sucrose", *African Journal of Biotechnology*, vol. 10 (52), pp.10626-10635, May 2011.
23. M. J. Alix, S. Savvides, J. Blakes, R. Herrmann, and R. Hornung, "Effects of illumination sources, culture ventilation and sucrose on potato (*Solanum tuberosum* L.) microtuber production under short days", *Annals of Applied. Biology*, vol.139 (2), pp. 175-187, October 2001.
24. M. J. Hossain, "In vitro Microtuberisation in Potato Obtained from Diverse Sources", *Plant Tissue Cult. & Biotech*, vol.15 (2), pp. 157-166, January 2005.
25. M. L. Fung, B. R. Irvine, and W.G. Barker, "In vitro tuberization of common potato (*Solanum tuberosum*) is not a response to the osmotic concentration of the medium", *Canadian Journal of Botany*, vol. 50, pp. 603-605, March 1972.
26. N. Khan, and A. Bano, "Role of plant growth promoting rhizobacteria and Ag-nanoparticles in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation", *Int. J. Phytoremediation*, vol.18 (3), pp.211-221. October 2016.
27. P. Wang, and C. Hu, "In vitro mass tuberization and virus free seed potato production in Taiwan". *Am. Potato J*, vol.59, pp.33-37, January 1982.
28. P. Ranalli, F. Bassi, G. Ruaro, P. Del Re, M. Di Candilo and G. Mandolino, "Microtuber and minituber production and field performance compared with normal tubers". *Potato Res*, volume 37(4): pp.383-391. December 1994.
29. S. Ali, N. Khan, and F. Nouroz, "Effects of sucrose and growth regulators on the microtuberization of CIP potato (*Solanum tuberosum*) germplasm", *Pakistan Journal of Botany*, vol. 50(2), pp.763-768, 2018.
30. S.P. Joyner, *Introductory Guide for Personal Computers*, 6<sup>th</sup> ed., SAS Institute Inc, SAS/STAT Software, Cary, North Carolina statistical, pp.1-378.
31. T. B. Jha, and G. Biswajit, *Plant Tissue Culture: Applied and Basic*, Universities Press (India) Ltd, India, 2005, pp.1-206.
32. T. E. Murashige, and F.A. Skoog, "Revised medium for rapid growth and bio assays with tobacco tissue cultures", *Physiologia Plantarum*, vol.15 (3), 473-497. 1962.
33. Y. Kikuta, and Y. Okazawa, "Control of root and shoot-bud formation from potato tuber tissue cultured in vitro", *Physiol. Plant*, vol. 61(1), pp 8-12, April 2006.
34. Z. Zhang, W. Zhou and H. Li, "The role of GA, IAA and BAP in the regulation of In vitro shoot growth and microtuberization in potato", *Acta Pys. Planta*, vol. 27(3), pp.363-369, September 2005.