Auxin and Cytokine Treatment Effect in Combination with Sucrose on *in vitro* Potato Regeneration

Iveta Megrelishvili, Ekaterine Bulauri, Tamar Chipashvili, Maia Kukhaleishvili

Georgian Technical University, Biotechnology Center, Tbilisi, Georgia
ivetamegerelishvili@gmail.com

Abstract: In this study we focused on *in vitro* potato regeneration under different hormonal (auxin/cytokine) treatment with combination of sucrose in three potato varieties, "Nevsky, Riviera, Zefira". Three parameters were observed in response to treatment, number of shoots development, size of shoots, and root length. *In vitro* tissue culture propagation of three potato cultivars (*Solanum tuberosum* L.) "Nevsky", Zefira" and "Riviera" were studied on modified MS medium supplemented with hormones (IBA/BAP) concentration: MS + 30g/l sucrose (6% MS medium), 6%MS medium +1mg/l BAP + 0,05mg/l IBA and 6% MS medium +1mg/l BAP + 0,1mg/l IBA. Morphological characterization of all three *in vitro* potato cultivars on modified MS medium was variable depending on varieties. It was revealed that high concentration of IBA has negative effect on plants development, respectively 6% MS medium + 1mg/l BA + 0,1mg/l IBA was not optimal neither cultivars of potato. According to the results cultivars Zefira" and "Riviera" had maximum potential for *in vitro* rooting (correspondingly: 82.97% and 100%) and shoots (100% and 87.34%) formation on 6% MS medium + 1mg/l BA + 0,05mg/l IBA, but "Nevsky" gave maximum development (rooting-82% and shoot formation -87%) on 6% MS medium.
[Megrelishvili I, Bulauri E, Chipashvili T, Kukhaleishvili M. **Auxin and Cytokine Treatment Effect in Combination with Sucrose on *in vitro* Potato Regeneration.** *Nat Sci* 2016;14(8):24-27]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 5. doi:10.7537/marsnsj14081605.

Keywords: *in vitro*, de novo shoot formation, root formation, auxin, cytokine, Indole-3 butyric acid (IBA), and 6-Benzylaminopurine (BAP)

1. Introduction

Potatoes are a widespread culture. According to FAO data potatoes are grown in 180 countries through the world, in line with 2005 data 202 974 000 tons of potatoes were consumed in the world. From 1991 to 2007 years potato production increased 267.99-325.30 million tons (FAOSTAT Agriculture (2016).

In terms of consumption potato is the major crop in Georgia. In spite of diverse climatic conditions of Georgia, potato is high spreading culture mountain regions in Georgian, drought –resistant ability of potato enable us to produce as food (early and late) as seed potatoes. Recently *in vitro* tissue cultures become wider and significant in Georgian agriculture production and development. *In vitro* potato plantlets derived meristem culture technique are free from the risk of spreading bacteria, fungi and insect transmitted virus, *in vitro* regeneration protocol was establish in many laboratories in the world but still there is (or more study needs to be done about which potato genotypes are more efficient for in vitro tissue culture propagation) less information about which potatoes genotypes is more efficient for propagated vegetatively by *in vito* tissue culturettt technique need to be study (Naik and Sarkar, 2000, Karim, 2009).

In vitro plant reproduction widely used in agriculture and biotechnology and has a great advantage compared to traditional breeding methods: A) allows to obtain get virus-free planting material in a short-term. B) plant reproduction can be conducted throughout the year, without a break for transformation t (Khan et al., 2003, Wang and Hu, 1982, Islam and Chowdhury, 1998, Espinoza at al., 1986, Liljana at al., 2012).

For potato *in vitro* regeneration mainly are used Murashige-Skooge (MS) medium. one group of researchers adopt hormone free MS medium for *in vitro* plant production *in vitro* plants are grown on hormone free MS medium on the other hand studies are done on the effect of different combination of growth hormones for *in vitro* potato reproduction (Badoni and Chauhan, 2009).

Growth regulators play an important role in potato regeneration, it should be noted that growth hormones on potato sprout explants are characterized different effects according to potato varieties, it depends on their genetics and endogenous hormones concentration (Khadiga at al., 2009, Kaur at al., 2015, Kumlay, 2014).

Our research group is working *in vitro* tissue culture potato plant breeding, we have 52 different varieties in the center that's allows as to study genotype differences on *in vitro* potato production and farther adaptation in the field, the aim of the research was to find out and set a new protocol for test tube potato production for the varieties that are most used in Georgia agriculture production.

Our research center is working successfully on test-tube potato plant breeding, we have 52 varieties in our *in vitro* collection.

The present investigation was carried out to select best MS medium protocol and identify perfect hormonal/sucrose combination for three varieties of potato: "Nevski", "Riviera" and "Zefira" for their *in vitro* cultivation.

2. Material and Methods

Plant material and growth conditions

Three potato variety Nevsky", Riviera" and "Zefira was used for *in vitro* propagation experiment at tissue culture laboratory of Georgian Technical University Biotechnological Center**.** These diversities of potatoes have been selected in the interests of the Georgian farmers. The three varieties were tested for *in vitro* response under high levels of sucrose and two different combinations of growth hormones:

Medium composition and hormonal treatment

1. MS medium+ 30g/l sucrose (6% MS medium).
2. 6 % MS medium +1mg/l BA + 0,05mg/l IBA.
3. 6 % MS medium +1mg/l BA + 0,1mg/l IBA.

As a control we use basal MS medium ((Murashige and Skoog, (MS) 1962), the pH of the medium was adjust 5.8, agar (7g/l) was added into the medium and the medium was autoclaved at 121^0C for 20 minutes at 15 psi. MS basal medium containing 30g/l sucrose. All the surface sterilization procedures were carried out under sterile condition of laminar flow chamber, 4 week old explant were dissected into single nodes (2-3 cm long) on a sterile plate. The excised explants were cultured into culture medium in tube and incubated under 16 hour photoperiod at 25-26^0C with a light intensity of 2500 lux. The sprouts were allowed to grow into plantlets having nodal segments for 3 to 4 weeks.

Best combination of medium for each potato cultivars was selected for their stem height, thickness, amount of nodes per plant and rooting (%) after 4 weeks.

3. Results

In vitro cultivation of potato varieties was studied on three different MS medium, sucrose, IBA and BAP concentration were examined in detail.

The sucrose influences on three *in vitro* potato cultivar's (Nevsky, Riviera, Zefira) rooting and shoots formation is presented in Table 1.

Table 1. The effect of sucrose on *in vitro* potato shoots and roots regeneration

potato Cultivars	Plant type	Shoot length (mm)	Shoot thickness (mm)	Nodes per plant	Root length (mm)	Rooting (%)	Shoot formation (%)
MS medium+ 30mg/l sucrose(6% MS medium)							
Nevsky	*in vitro* plant	140	2	9	22	100	98
Riviera	*in vitro* plant	50	0.8	2	0.5	18	15
Zefira	*in vitro* plant	110	1.8	7	14	82	87

The results showed that the Nevsky has maximum potential for *in vitro* rooting and shoots formation on basal MS medium+ 30mg/l sucrose (6% MS medium) among the three different genotype of potato, All parameters revealed optimal formation: 100.00% rooting, high and strength shoot formation (98%) with 9 nodes per plant, but Riviera had a weak *in vitro* development (rooting-18% and shoot formation- 15% with only 2 nods) on 6% MS medium (Figure 1). It is known that auxin/ cytokine combination has varies result on *in vitro* morphogenetic response (Morariu at al., 2010). On next stage of research on 6%MS medium was supplemented cytokine 6-Benzylaminopurine (BAP) and auxin Indol-3-butiric acid (IBA).

Only one concentration of BAP was used (1mg/l), but two concentrations of IBA (0,05mg/l and 0,1mg/l) was selected for experiment.

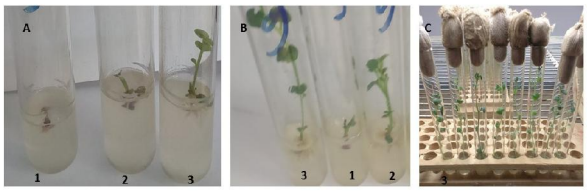

Figure 1. Sucrose effect on three potato cultivar's(Nevsky, Riviera, Zefira) *in vitro* propagation A- after 2 weeks (1-Riviera, 2-Zefira, 3-Nevsky) B-after 3 weeks (1-Riviera, 2-Zefira, 3-Nevsky) C-after 4 weeks (3- Nevsky), 1-Riviera, 2-Zefira, 3- Nevsky.

There is presented influence of IBA (0.05mg/l) and BAP (1mg/l) on *in vitro* roots and shoots formation which depends on potato cultivars in the table 2.

As we can see on Figure 2. De novo shoot formation and rooting percentage in "Nevsky" genotype. Cytokine/ auxin combination (1mg/l BAP / 0,05mg/l.

Table 2. The effect of cytokine IBA (0.05mg/l) and auxin BAP (1mg/l) *in vitro* potato cultivar's shoots and roots formation

potato Cultivars	plant type	Shoot length (mm)	Shoot thickness (mm)	Nodes per plant	Root length(mm)	rooting (%)	shoot formation(%)
6% MS medium+ 1mg/l BAP + 0,05mg/l IBA							
Nevsky	*in vitro* plant	91	1.21	5	16.42	68.5	66.74
Riviera	*in vitro* plant	112.14	1.57	6	12.23	83	87.34
Zefira	*in vitro* plant	147	2.67	9	23.56	100	100

IBA) was not effective to compare 6 % MS medium without growth hormones, whereas *in vitro* regeneration of potato cultivars- Zefira and Riviera testified improved on 6% MS medium containing BAP and IBA, Zefira showed maximum rooting and shoot formation(both parameters -100% and 100%) and strength shoots with 9 nods. Riviera revealed *in vitro* rooting and shoot formation correspondingly: 83% and 87.34% with 6 nods. Concentration of IBA was increased on the next stage (from 0.05mg/l to 0.1mg/l), High concentration of IBA was not effective on *in vitro* development in all three cultivars: Zefira, Nevsky, Riviera (Table 3).

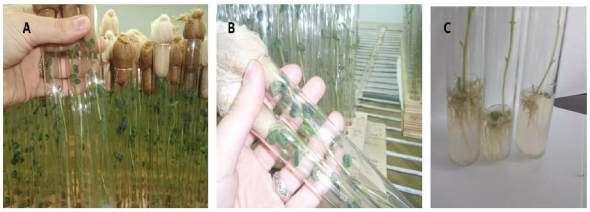

Figure 2. Effect of growth hormones combination IBA (0.1mg/l) /BAP (1mg/l) on two potato cultivar's (Zefira, Riviera) *in vitro* propagation. A-Root and shoot formation after 4 weeks (Zefira); B-Root and shoot formation after 4 weeks (Riviera); C-Root formation after 4 weeks (Zefira)

Table 3. The effect of cytokines IBA (0.1mg/l) and auxin BAP (1mg/l) *in vitro* potato shoot and root formation

potato Cultivars	Type of plant	Shoot length (mm)	Shoot thickness (mm)	Nodes per plant	Root length (mm)	Root formation (%)	Shoot formation (%)
6% MS medium+ 1mg/l BA + 0,1mg/l IBA							
Nevsky	*in vitro* plant	46.15	0.77	3	9.56	38.7	24.89
Riviera	*in vitro* plant	42.76	0.69	3	8.24	38	23.56
Zefira	*in vitro* plant	100	1.64	6	12.3	79.7	78.4

According to the results three potato cultivars had variable *in vitro* growing on all experimental MS medium. 6% MS medium + 1mg/l BA + 0,1mg/l IBA wasn't effective neither of potato cultivars, Zefira and Riviera gave their maximum *in vitro* regeneration per explant on 6% MS medium + 1mg/l BAP + 0,05mg/l IBA. Nevsky revealed perfect *in vitro* development on MS medium+ 30g/l sucrose without growth hormones.

4. Discussions

Auxin/ cytokine combinations effect on plant growth depend on the plant genotype, their chemical consistent and physiological statement. Auxin/ and cytokine interaction may be interrelated, simultaneous or antagonistic, in most cases their molecular interaction mechanism is not fully studied (Coenen and Lomax, 1997).

Cytokines and auxins possess (cross talk) stimulation action and they play leading role in plant micro propagation, but sucrose together with phytohormones have an important role in regulation of morphogenesis (Negrutiu at all., 1987).

The result of research suggested that 1mg/l BAP had a positive effect and an important role in plant differentiation process, which was expressed in great formation (shoots and root) of *in vitro* plant. High concentration of IBA (0.1mg/l) results in plant root thickness, it is undesirable for plant. When we used auxin low concentration of (0.05mg/l) actively growing of root meristem, took place, this is confirmed by our result (normal and excellent formation of root).

In conclusion, both hormones combination presented in experiment with 30 g/l sucrose showed optimal result on *in vitro* growing potato cultivars Zefira and Riviera, but best *in vitro* cultivation of Nevsky was revealed MS medium supplemented only 30 g/l sucrose without growth hormones, probably this result depends on Nevsky's genotypes, it seems Nevsky has ability to produce itself the amount of hormones which is necessary it's normal growing.

Acknowledgements:

The authors acknowledged to the PhD student Nino Murvanidze Plant Production Faculty of Bioscience Engineering Ghent University Coupure links 653, 9000 Gent, Belgium for professional support to carry out this work.

Corresponding Author:
Dr. Iveta Megrelishvili
Georgian Technical University, Biotechnology Center
77, Kostava Str, 0175, Tbilisi Georgia
Telephone: (995 77) 387222
E-mail: ivetamegerelishvili@gmail.com

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5/29/2016