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Plant Growing

Influence of Exogenous Phytohormones Concentrations on Potato Morphogenesis *in vitro* Culture

Avtandil Korakhashvili[*], Maia Kukhaleishvili[**], Iveta Megrelishvili[**], Tamara Shamatava[**], Ekaterine Bulauri[**]

[*] *Academy Member, National Academy of Sciences of Georgia, Tbilisi, Georgia*
[**] *Biotechnology Center, Georgian Technical University, Tbilisi, Georgia*

Abstract. Potato (*Solanum tuberosum* L.) is one of the most important crops in the world, it is grown approximately in 100 countries worldwide. Globally world's second produced crop by mass - potato support to food availability of planet population. It can be achieved through improved productivity, either by increasing yields or expanding production areas, combined with advance and new technologies, reducing clean seed materials for cropping and production. distribution of viruses-free seed yields and the high yield levels is the main issue in potato cultivation, as the modern agriculture developing world, where actual yield observed as a food stability and safety. Leading technologies for the viruses-free potato seed production in Georgia is cell molecular cloning method, which needs additional adaptation and improvement. Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. The aim of the study was to estimate the influence of different concentration of exogenous phytohormones (6-benzylaminopurine (BAP) and Indole-3-acetic acid (IAA) on the potato variety "Jelly" *in vitro* morphogenesis. As a result of the activation of meristem cells, the growth of the plants in length and the rapid formation of the roots took place**.** The optimal concentration of growth regulators for *in vitro* germination of potato variety "Jelly" has been selected. Accelerates the process of morphogenesis, which is of great importance for obtaining a large number of *in vitro* test plants in a short time. Therefore, both cytokinins and auxins should be applied in the nutrient area at a concentration that will accelerate the micropropagation process. © *2025 Bull. Natl. Acad. Sci. Georg.*

Keywords: 6–benzylaminopurine, indole-3-acetic acid, microclonal propagation, exogenous phyto-hormones

Introduction

For the understanding of the effects of *in vitro* propagation on potato plants, consider the following points: Enhances rapid multiplication of released potato varieties under controlled conditions; Reduces the risk of disease transmission compared to traditional propagation technologies; Allows for the preservation of clean genetic material from genetic banks and traits of elite potato cultivars; Enables the selection of disease-resistant and high-yielding clones of released in specific regions of country (from foothills to highlands); Facilitates year-round production, independent of seasonal constraints (short vegetation period); Supports research on potato plant development and response to environmental factors (Korakhashvili, 2015).

High quality, healthy and vigorous seed potatoes are of utmost importance for the health, vigor, and yield of resulting potato crops in any traditional potato cultivated regions of Georgia. Production of high-quality seed potatoes as well, has always been an important issue in potato-growing areas and was gradually improved thanks to improved processes and new technologies This article presents objectives of seed potato multiplication schemes, the diversity of one of ring of seed production systems from informal to advanced technologies such as tissue culture for fast and healthy multiplication methods (Kukhaleishvili et. al., 2018).

For the obtaining of virus disease free potato seed, the specific composition of phytohormones and their concentrations in the medium are particular importance for the development of cultural plant *in vitro* morphogenesis. The regulation of morphogenesis is significantly dependent on exogenous phytohormones, which underlies microclonal propagation of this crop (Marija et al., 2023).

Murashige and Skoog (MS) media supplemented with different concentracions of growth regulators: indole-3-Acetic acid (IAA) and 6-benzylaminopurine (BAP) were tested in this work.

Studies have shown that the combination of 1.0 mg/L BAP + 1.5 mg/L Indole-3-acetic acid (IAA), effected positively on the explants *in vitro* growth, developments while increased concentration of 1.5 mg/l BAP + 1.5 mg/l IAA had a negative results, meristem cells was inhibited and developed of small, unbranched plants and reducing the concentration of BAP in the MS medium to 1.0 mg/l and combining with 2.0 mg/l of IAA potato plants showed deformation and hardening of the stem, 90% of the leaves were yellow, in most cases degenerated (Murashige & Skoog, 1962; Book, 2004).

The authors build a conceptual framework around how complementary, complex, and collaborative advance technologies contribute to the generation of enabling cell technologies in potato crop. Then they apply it to global issues related sectors to study those enabling technologies and their trajectories (Megrelishvili et al. 2022).

Condition Preface

The tissue culture advance technologies recently are based on the *in vitro* cultivation of cells, tissues and organs, controlling various morphogenetic processes, and providing the ability to store, propagate, and reproduce (Nalini et.al, 2021).

Murashige-Skoog medium is mainly used to obtain *in vitro* plants of various cultures For the cultivation of isolated plant cultures and tissues, MS medium is required that contains essential substances needed by the plant, including phytohormones: cytokinins, auxins, gibberellins, abscisic acid and ethylene. All of them are the base of researching materials *in vitro* potato germination of potato (Murashige & Skoog, 1962).

In potato culture the phytohormones cytokinins are synthesized mainly in the roots and transported upward along the stem, while auxins are formed in the apical meristem and move nonpolarly throughout this annual plant. They play a primary role in the differentiation process leading to cell division. However, neither alone can stimulate cell division, it requires the presence of both phytohormones (Korakhashvili et.al., 2024).

Cytokinins resolve apical dominance and induce the development of axillary buds, as well as regulate the growth of somatic embryos and plant formation. Auxins affect cell division, differentiation, and elongation. The most important organogenic effect of auxins is the stimulation of root formation (Kacharava et.al., 2018).

At the onset of axillary bud growth, the accumulation of cytokinins is explained by the synthesis of cytokinins. Auxins stimulate cell division in the stem parenchyma, leading to differentiation of stem root germs (Taras & Unterweger, 2016).

The concentrations of phytohormones introduced into the medium are important during plant micropropagation. The basis of micropropagation is the development of axillary buds, through the

selection of appropriate mediums and cultivation conditions. Most scientists uses modify medium during tissue culture technology in various crops especially synthetic phytohormones (Shirin et al., 2015).

6-Benzylaminopurine-BAP, belonging to the cytokinin group, is a synthetic substance that is often used in cytokinin nutrient media during micropropagation of various crops (Kukhaleishvili et. al., 2018). Indole-3-acetic acid belongs to the auxin group and is one of the important phytohormones in plant micropropagation technology (Spaepen et al., 2011). The morphogenetic response that occurs during plant micropropagation has been studied mainly in the *in vitro* propagation of cereals, berries, and ornamental plants, however, it remains unknown for most crops (Schaller et al.,, 2015).

Materials and Methods

The general goal of this study was to find the optimal MS medium and the appropriate hormonal combination for the cultivar of potato (*Solanum tuberosum* L.) variety "Jally" for its *in vitro* development.

Samples collection. The experiment has been implemented in Biotechnology Center of the Georgian Technical University. Released varieties of potato were chosen because of their adaptability to Georgian soil and climatic conditions. The research was conducted with *in vitro* potato collection (created by apical meristem technics) of same Georgian Technical University Biotechnology Center.

Apical meristem method. Meristem cell culture involves the isolation of apical (tip) meristems, which are located at the tips of vegetative organs and are free from viral infections. The tissue culture method is based on the *in vitro* cultivation of organs, tissues, cells, and isolated protoplasts. The isolation of the apical meristem is carried out in laminar-sterile boxes (Blom van Staden et al., 2018).

Propagation of *in vitro* potato Murashige and Skoog medium (MS) was used to cultivate these explants (Hajare et al., 2021). Medium with 30g/L-1 sucrose, 7 g/L-1 agar, 0.04 mg/l Kinetin + 1.5 mg/l Indole-3-acetic acid (IAA), Salts of micro and macro elements in different concentrations, vitamins, autoclaved to 121°C during 20 min at 15 psi, pH was adjusted to 6.4. 6-8 cm long single nodes were separated from 4-5 weeks old explants with 6-7 nodal segments under laminar flow chamber and used as explants for *in vitro* propagation. We have prepared 4 types of food areas, where kinetin has been replaced by BAP (different concentrations).

1. MS medium 0.04 mg/l Kinetin+1.5mg/l IAA
2. 1.0 mg/l BAP + 1.5 mg/l IAA
3. 1.5 mg/l BAP + 1.5 mg/l IAA
4. 0.1 mg/l BAP + 2.0 mg/l IAA

Explants were placed in Phytotrone regulate condition (25-27°C; 4500-5500 lux, 70-75% humidity, 16/8 h).

Results and Discussion

In this experimental work the potato *in vitro* collection of Georgian Technical Unievrosty Biotecnology Center was used as the main source base and development.

The potato variety "Jally" were propagated in 2019-2024 years for further study of the influence of the growth regulators BAP and IAA.

Conducted studies have shown that different concentrations of phytohormones have a different effect on potato *in vitro* propagation.

As the results showed, the combination of 1.5 mg/l BAP + 1.5 mg/l IAA to the MS medium have a negative effect of *in vitro* potato micropropagation. After 22-23 day of incubation in phytotrone undeveloped plants were formed with seek root system (2-3 roots), the leaves and stem were thin and yellowed. Plants growth inhibition is associated with the effect of high concentrations of BAP on the regenerative processes occurring in the plant, in particular, high concentrations caused

inhibition of the stems of the explants (Kukhaleishvili et. al., 2018).

Reducing the concentration of BAP in the MS medium to 1.0 mg/l and combining with 2.0 mg/l of IAA potato plants showed deformation and hardening of the stem, 90% of the leaves were yellow, in most cases degenerated.

Above mentioned combination of phytohormones caused severe disturbances in morphological processes in the plant: inhibition of axillary buds and root formation on cuttings, thickening and shortening of roots.

Potato explants on the Murashige-Skoog medium (MS medium control) developed during 22-23 days. 64.2% of the *in vitro* plants had normal, green leaves, medium-thick stems, a root system with 15-17 roots with 5-6 nodes. Same conclusion had some authors in estimations of plant growing intensively *in vitro* conditions (Mohapatra et. al., 2017; Fathi, 2008).

In compared to other combination of phytohormones, 1.0 mg/l BAP + 1.5 mg/l IAA was more favorable for the implantation of *in vitro* formation of this potato variety.

Fig. Effect of 1.0 mg/l BAP+ 1.5 mg/l IAA combination on *in vitro* potato development (96.7 of developed plants).

After 17 day of the explants inoculation formed a stem with 8-9 internodes, well-developed large, green leaves, a strong root system with 22-25 roots. The formation process of axillary buds and elongation of the plants in the stem were actively. 96.7% of the obtained plants were strong, which is a good basis for their further cultivation (Figure).

Conclusion

Based on the obtained results, following conclusion have achieved: during microclonal propagation of plants, It is necessary to determine optimal concentrations of exogenous phytohormones in the medium, the use of which ensures the optimal course of plant propagation in *in vitro* culture and the regulation of morphogenesis at all stages of microclonal propagation.

By this scientific work *in vitro* micro propagation technology obtained element of influence of exogenous phytohormones concentrations on potato morphogenesis. As it's important aspect of an alternative to conventional (vegetative) propagation of potatoes whereas aseptically meristem cultures were used for the pathogen free potato plants. Different sterilization protocols were used for the isolating potato sprouts from potato genotype named Murashige and Skoog. It was found that the suitable exogenous phytohormones concentrations giving high percentages of survived individuals.

The potato variety "Jally" were propagated showed greater ability for *in vitro* propagation. The aim of the study was the presentation of suitable protocol of exogenous phytohormones concentrations on potato morphogenesis for *in vitro* induction of potato plantlets stocks free of pathogens. The results of the present study showed that the highest record of tuberization percentage, weight and number of microtubers per shoot were obtained, with significantly improve the potato plant growth. This protocol will help for mass propagation of virus free plants and *in vitro* germplasm.

მემცენარეობა

ეგზოგენური ფიტოჰორმონების კონცენტრაციის გავლენა *in vitro* კარტოფილის მორფოგენეზზე

ა. კორახაშვილი*, მ. კუხალეიშვილი**, ი. მეგრელიშვილი**, თ. შამათავა**, ე. ბულაური**

* *აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია, თბილისი, საქართველო*
** *საქართველოს ტექნიკური უნივერსიტეტი, ბიოტექნოლოგიის ცენტრი, თბილისი, საქართველო*

თანამედროვე პირობებში ტუბერიანი კულტურების მიკროგამრავლების *in vitro* ტექნოლოგიამ მოიპოვა ეგზოგენური ფიტოჰორმონების კონცენტრაციის გავლენის ელემენტი კარტოფილის (*Solanum tuberosum* L.) მორფოგენეზზეც. ეს ტექნოლოგია კარტოფილის ტრადიციული (ვეგეტატიური) გამრავლების ალტერნატივის მნიშვნელოვანი ასპექტია, ხოლო პათოგენებისგან თავისუფალი უვირუსო კარტოფილის მცენარეების მისაღებად ადრე ასეპტიკურად მერისტემული კულტურები გამოიყენებოდა. კვლევებში კარტოფილის ყლორტების იზოლირებისთვის გამოყენებულ იქნა სტერილიზაციის სხვადასხვა პროტოკოლის (Murashige-Skoog) გენოტიპები. შედეგად დადგინდა, რომ შესაფერისი ეგზოგენური ფიტოჰორმონების კონცენტრაცია გადარჩენილი ინდივიდების მაღალ პროცენტს იძლევა. ამ მეთოდით გამრავლებული კარტოფილის ჯიშმა „ჯალი“ აჩვენა ბევრად უფრო მეტი უნარი *in vitro* გამრავლებისათვის. კვლევის მიზანი მიღწეულ იქნა – კარტოფილის მორფოგენეზზე ეგზოგენური ფიტოჰორმონების კონცენტრაციის შესაფერისი პროტოკოლის წარდგენა პათოგენებისგან თავისუფალი კარტოფილის ნერგების *in vitro* ინდუქციისთვის დასაბუთებულია, რაც მნიშვნელოვან პრაქტიკულ დახმარებას გაუწევს მეკარტოფილე ფერმერებს უვირუსო კარტოფილის თესლის წარმოების გასაზრდელად, შესაძლებელს გახდის კვლევების შედეგების კომერციალიზაციას.

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