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EFFECT OF GROWTH REGULATORS ON THE FORMATION OF POTATO (*SOLANUM TUBEROSUM* L.) MICRO-PLANTS *IN VITRO*

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ABSTRACT: Effect of growth regulators IAR (indole-3-acetic acid) and kinetin (6-furfuryl aminopurine) on length and mass formation of micro-plants of different maturity table potatoes (*Solanum tuberosum* L.) varieties was evaluated. The varieties of different maturity group included were very early 'VB Venta', early 'Goda', medium early 'VB Meda' and very late 'VB Aista'. Microplant explants were grown in modified MS nutrient medium with different concentrations of growth regulators.. The concentration of 1.75 ml/l of auxin resulted in significant increase in length and weight of the micro-plant with highest micro plant growth (10.03± 0.96 cm) and mass (0.218 ± 0.09 g) formation in var 'VB Meda' compared to least growth in VB Venta' (6.2 ± 0.75 cm). Kinetin at concentration of 0.8 ml/l resulted in longest micro-plants formation by 'VB Meda' variety (9.03 ± 0.18 cm) and the smallest by 'VB Venta' (5.20 ± 0.15 cm) explants.

KEYWORDS: growth regulators, *Solanum tuberosum* L., micro-plants, *in vitro*, genotype

INTRODUCTION

Potatoes are able to easily adapt to various growing conditions, are distinguished by high yields and nutritional value, therefore they are very widespread and are considered the most important non-grained agricultural plant in the world. Potatoes are also one of the main raw materials for starch extraction, and the starch content of potatoes is usually between 9% and 25% (Acharjee *et al*., 2018). Because potato tubers cannot be stored for a long time, the use of potatoes in the form of starch and modified products has become an excellent alternative toa (Fernie and Willmitzer, 2001; Sonnewald and Sonnewald, 2014). According to the Food and Agriculture Organization of the United Nations (FAO), potatoes rank fourth after corn, rice, and wheat in terms of the volume of world production in tonnes (FAO, 2011). Due to the nutritional value of potatoes since ancient times, they are called the second bread. Potato tubers, depending on the variety, accumulate 15-35% of dry matter, of which starch 17-29 %, protein - 1-2%, mineral salts - about 1%. When comparing vegetable proteins, potatoes are inferior only to wheat and buckwheat. Almost three times more dry matter can be obtained from a unit of area for potatoes than from grain fodder plants. In addition, potato tubers contain potassium, sodium, calcium, magnesium, phosphorus, iron. According to the total amount of mineral compounds accumulated in potatoes, they exceed many types of fruits and vegetables. 18 amino acids are found in potato proteins. Potatoes are characterized by high nutritional value and are a source of vitamins C, A_1 (carotene), group B, PP_1 and K. By eating an average of 300 g of boiled potatoes every day, we provide ourselves with the necessary carbohydrates, phosphorus, vitamin B_2 10-12%, amino acids – 30-40%, vitamin C – 40-50%, vitamin B_1 – 20-25%, potassium – 55-60%, calcium and iron – 25-30% and carotene – 1-2%. Due to the excellent taste, nutritional and culinary

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qualities, potatoes have become a product of almost daily use. Potatoes also have healing properties (Leonel *et al*., 2017).

The meristemic method of potato seeding is currently the most promising and fastest for seed potato reproduction. Meristem culture may have been the first biotechnological method used to remove viruses from systematically infected potato clones. Over the years, this technique has been successfully combined with the method of microproduction to bring out potato seeds without diseases. The rapid reproduction of these disease-free clones using microproduction in combination with conventional propagation methods has now become an integral part of seed production in many countries (Barrell *et al*., 2013). The technique of plant tissue culture has become an alternative in recent years and has become popular as an alternative method of vegetative propagation of plants (Hajare *et al*., 2021). As a modern technology, plant tissue culture has great potential to meet the ever-growing demand of the world. Healthy, thermotherapeutically cleaned of viruses, without bacterial and fungal beginnings, potatoes are grown from the meristemic cells of the seedling growth cone. In this way, not only good quality is achieved, but conditions are created for the significantly faster reproduction of potatoes of new varieties (Roosher, 1988; Hajare *et al*., 2021).

Microdescence is a method of vegetative propagation *in culture in vitro*, designed to multiply quickly and in large quantities by plants. When propagated in this way, many plants in a small space are regenerated from a small explant. In this work, microvegetative reproduction was used. This method is aimed at cloning plants, while maintaining their genetic and physiological characteristics. The optimal composition of the medium and the growing conditions for each species may be fundamentally different, so they are determined experimentally.

Phytohormones are needed to control *in vitro* plant cells, tissues, organs, or entire plant crops according to the desired results: maintain vitality, grow, initiate organogenesis (the formation of organs, for example, the formation of a stem with leaves or roots), and so on. Without phytohormones, plant growth could not be controlled (Ohnuma *et al*., 2020; Hajare *et al*., 2021).

The purpose of the work. To study the effect of growth regulators on micro-plants of potatoes (*Solanum tuberosum* L.) *in vitro*.

The work has the following tasks: 1. To study the effect of indoleyl-3-acetic acid (IAR) on different microgrowths of potatoes; 2. Determine the most appropriate concentration of indoleyl-3-acetic acid (IAR) for micro-plants *in vitro*; 3. To study the effects of 6-furfurylaminopurin (kinetin) on different microgrowths of potatoes; 4. Determine the most appropriate concentration of 6-furfurylaminopurin (kinetin) for micro-plants *in vitro*.

MATERIALS AND METHODS

The experiment was prepared and carried out at the Biotechnology Laboratory of the Lithuanian Center for Agrarian and Forest Sciences and at the Institute of Agriculture of Vokė brancho (Vilnius) in 2021.

The purpose of the work. To study the effects of growth regulators on micro-plants of potatoes (*Solanum tuberosum* L) *in vitro*.

The object of res earch. Microgrowths of edible potatoes (*Solanum tuberosum* L.) of different varieties (Table 1) of different maturity: very early 'VB Venta', early 'Goda', medium early 'VB Meda' and late 'VB Aista', as well as growth regulators IAR (indolyl-3-acetic acid) (Figs. 2 and 3) and kinetin (6-furfurylaminopurin).

Table 1: Pedigree information of Lithuanian potato cultivars

Varieties	Registration LTU	Maturity	Parentage
'VB Venta'	2009	Very early	'Priekuliu visagrie' × 'Pirmunes'
'Good'	2004	Early	'Ausonia' ×'Franzi'
'VB Meda'	2019	Medium	'Matilda' × No.3093
'VB Aista'	2006	Very late	No.263 -× No.476-9

Murashige & Skoog (MS) medium (Murashige and Skoog, 1962; Saker *et al*., 2012) is one of the most widely used nourishing media for tissue cultures. An important feature of this medium is the high concentration of nitrates, potassium, and ammonium ions. The nourishing medium is sterile in an autoclave at 120 °C at 0.75–1.00 atm. sfor pressure, 30 min with a pH = 5.7-5.8. Microgrowths are cut and planted in a nutrient medium of a certain concentration of phytohormones (5 ml). The planted explants were kept *in vitro in a* tumor, where the photoperiod is automatically regulated - during the day for 16 hours the seedlings grow in the light (12 thousand km). Lx), 8 a.m. in the dark. The air temperature during the day is up to 24 °C, at night it is about 21 °C, and the humidity is 86-90%. Length (cm) and mass (g) of micro-plants measured during the tests.

Test 1: The influence of IAR on microgrowths.

2-factor (AxB) test: A – explantants of potato varieties ('VB Venta', 'Goda', 'VB Meda' and 'VB Aista'); B – 3-indolylacetic acid (IAR) concentrations of 0.0, 1.0, 1.25, 1.5, 1.75 and 2.0 ml/l(solutions prepared by dilution method). Potato explants were planted in mediums of different concentrations of January 5-7 mention year. Biometric indicators were measured 4 weeks later (Fig. 5), when microgrowths formed new 4-7 leaflets. For 4 genotypes and 6 concentrations (one option planted 50 pieces explants), during the entire test – 1200 explants.

Test 2: Effect of kinetins on microgrowths.

2-factor (AxB) test: A – explantants of potato varieties ('VB Venta', 'Goda', 'VB Meda' and 'VB Aista'); B – kinetin concentrations – 0.0, 0.4, 0.5, 0.6, 0.7 and 0.8 ml/l (solutions prepared by the dilution method). Potato explants were planted in mediums of different kinetin concentrations on February 9-10 mention year. Biometric indicators were measured 4 weeks later, when microgrowths formed new 4-7 leaflets. For 4 genotypes and 6 concentrations (one option planted 50 pieces explants), during the entire test – 1200 explants.

Statistics of Figure 1-4 show means and their standard deviations (x ± SD). The reliability of differences between samplings was assessed by the dispersion analysis with further evaluation of the least significant difference (LSD) using the Statistica 5 software package.

RESULTS AND DISCUSSION

Description of Lithuanian potato varieties

'VB Venta' (2009). The maturity of the variety is very early. Tubers are round, with medium-deep eyes. Skin and flesh color is light yellow. Cooking characteristics are perfect. There is no browning after peeling. Taste is excellent. The variety is salad type. Starch content is up to 14-16%. The variety is resistant to wart disease, field resistance to the black legand potato leaf roll virus. Storage characteristics under controlled conditions are excellent.

'Goda' (2004). The variety is early. Tubers are oval round, medium in size, with shallow eyes. Flesh and skin colour is light yellow.

Sugar content is low. No discoloration after peeling and cooking. Suitable for chips industry. Starch content is up to 16-17%. The variety is resistant to potato cyst nematode Ro1 and wart disease. Foliage is resistant and tubers fully resistant to the late blight. Storage is good.

'VB Meda' (2019). The plant is compact and medium. The flower colour is white with short flowering period. Tubers are oval, shallow eyes, yellow skin, and flesh, and rough. Potato variety has low reducing sugars. Table varieties suitable for baking. Tubers do not change colour when baking and boiling. Great taste and other culinary qualities. Starch content is up to 14-17%. Resistant to potato cyst nematodes Ro_1 patotype, immune to potato cancer, has good resistance to viruses. Tubers have resistant to late blight. Variety has good storage conditions. Potato variety grows all soils. Due to its high resistance to potato diseases, it is suitable for ecological farming.

'VB Aista' (2006). This is a late, food potato variety. The bush is stretched out, the stems are tall. The flowers are white, of medium size. The variety flowers abundantly and for a long time. The tubers are big, yellowish, flat round with shallow eyes. The output of commercial tubers is 90%. Starch content is high, up to 21%. The variety is good for starch industry. The potatoes are palatable. The boiled potatoes do not get dark, boil soft. The variety is resistant to wart disease, potato cyst nematode R_{o1}, late blight. The variety is resistant to drought, can be grown in all kinds of soil using proper cultivation techniques. The variety is resistant to mechanical injuries while harvesting; characterized by good storage quality.

The effect of all phytohormones on plants is quite polyvalent. All of them affect cell growth and division, adaptation and aging processes, substance circulation, respiration, nucleic acid and protein synthesis, etc. However, each group has its own specific effect (Ohnuma *et al.,* 2020). Potatoes, like other plants, have a whole known group of phytohormones. For example, the different hormones contained in potatoes (IAA, CK, OR, JA) stimulate some stages of tuberization, and GA inhibits them (Kolachevskaya *et al.,* 2018). Auxins play a very important role at almost all stages of the development of the plant, which require changes in the meristem. Studies *using* auxins in vitro highlight their importance for tubers (Roumeliotis *et al.,* 2012).

High concentrations of auxins can and without cytokinins cause mitosis in somatic plant cells. In general, it is necessary to determine the adequate levels of concentration of auxins and cytokinins for plant regeneration.

The influence of auxin (IAR) on the formation of microgrowths

The study used 4 potato genotypes, as the in *vitro* productivity of explants depends on the amount of IAR in the culture medium. Tested nourishing medium with IAR concentrations of 1.0, 1.25, 1.5, 1,75 and 2,0 ml/l.

The results of the studies showed that the highest microgrowths were the 'VB Meda' varieties (10. 03 ± 0.96 cm), the lowest – 'VB Venta' (6. 2 ± 0.75 cm) (Fig. 1 a). Most of the masses of micro-plants were formed by the varieties of 'VB Meda' (0. 218 ± 0.09 g) explants (Fig. 1, b).

Summing up the results of the length and mass of micro-plants, it can be said that for the explants of the 'VB Meda' variety, the effect of IAR was the greatest. The explants of the 'VB Aista', 'Goda' and 'VB Venta' varieties also developed well.

When using a golden concentration of 1.75 ml/l, a sharp increase in the length of

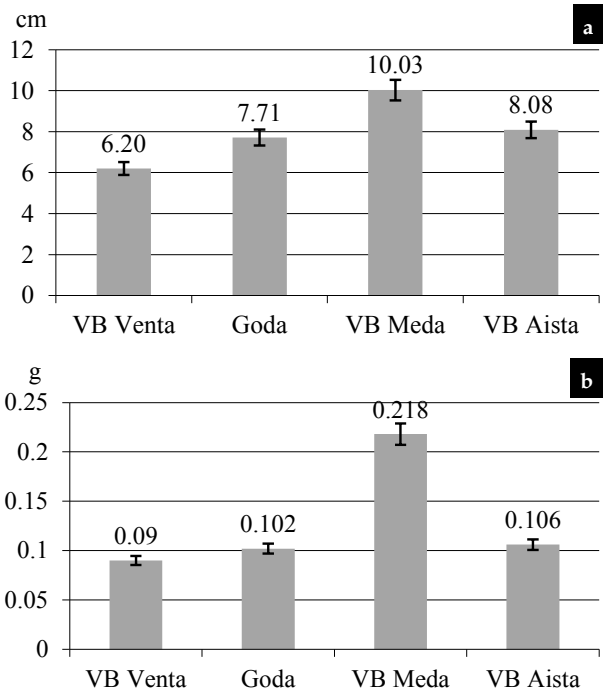


Fig. 1. *The influence of different potato genotypes on the length of micro-plants (a) ($LSD_{01(A)}$ = 0.521) and (b) for mass ($LSD_{01(A)}$ = 0. 037) using auxin (IAR)*

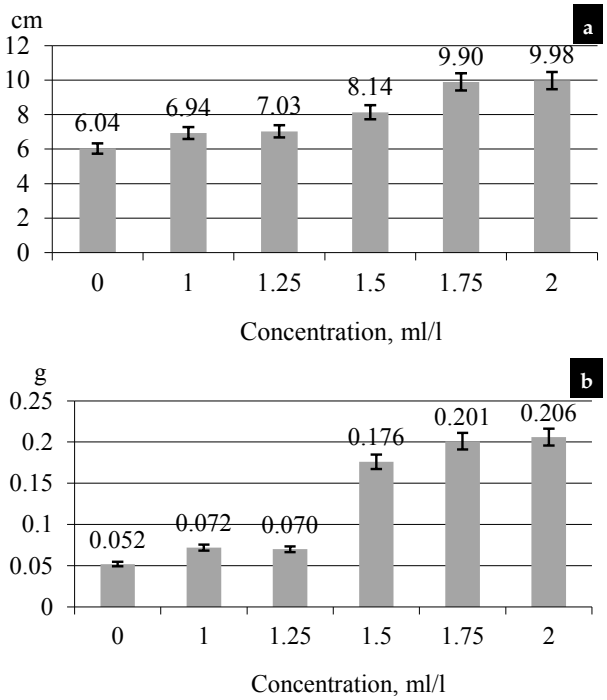


Fig. 2. *The effect of auxin (IAR) on the length of micro-plants (a) ($LSD_{01(B)}$ = 0. 675) and (b) for mass ($LSD_{01(B)}$ = 0.082)*

the micro-plants $LSD_{01(AxB)}$ = 1.430 (Fig. 2, a) and the mass $LSD_{01(AxB)}$ = 0.047 (Fig. 2, b) is observed.

Summarizing the results of the concentrations of the IAR compound of 1.75 and 2.0 ml/l had a positive effect on most tests. The scientific literature states that for stem morphogenesis, the optimal concentration of auxins is 1.0 ml/l (Ohnuma *et al*., 2020). Our study showed that the optimal concentration for microgrowths is 1.75 ml/l. Auxin (IAR) stimulated cell swelling and growth, in part and cell division.

The influence of kinetin on the formation of microgrowths

In the study, 4 potato genotypes were used, as the in *vitro* productivity of explants depends on the amount of kinetins in the culture medium. Tested nourishing medium with 0.4, 0.5, 0.6 in concentrations of 0.7 and 0.8 ml/l of kinetin.

The results of the test showed that the length of micro-plants in different potato genotypes is different (Fig. 3, a). The longest microgrowths were shaped by 'VB Meda' (9. 03 ± 0.18 cm) and the smallest ones are exhibitors of the 'VB Venta' variety (5.2 ± 0.15 cm).

Summarizing the results of the length and mass of micro-plants (Fig. 3, b), it can be said that the kinetin effect was greatest on the explants of the 'VB Meda' variety. The explants of the 'Goda', 'VB Aista' and 'VB Venta' varieties also developed well.

When using a concentration of kinetin 0.8 ml/l, an increase in the length of the micro-plants $LSD_{01(AxB)}$ = 1.286 (Fig. 4, a)) and the mass $LSD_{01(AxB)}$ = 0.021 (Fig. 4, b) are observed. Summarizing the results of the length and

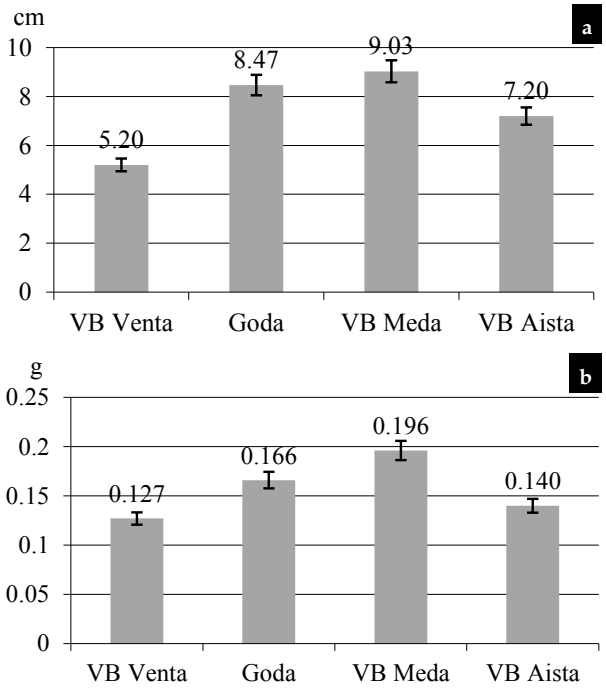


Fig. 3. *The influence of different potato genotypes on the length of micro-plants (a) ($LSD_{01(A)}$ = 0.341) and (b) for mass ($LSD_{01(A)}$ = 0.046) using kinetin.*

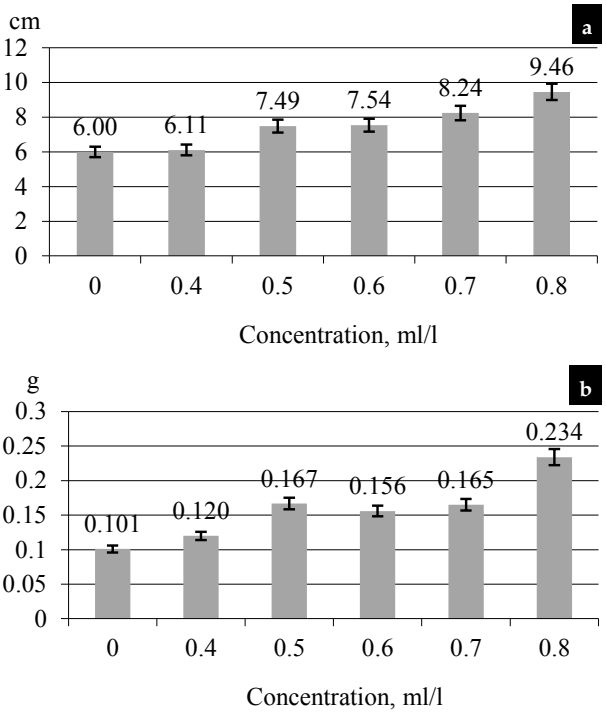


Fig. 4. *The effect of kinetin on the length of micro-plants (a) ($LSD_{01(B)}$ = 0.754) and (b) for mass ($LSD_{01(B)}$ = 0.031)*

mass of micro-plants, it can be said that the effect of 0.4, 0.5, 0.6, 0.7 and 0.8 ml/l of the kinetin compound concentrations of on most tests was positive.

It is believed that the formation and development of leaves *in vitro* had a significant positive influence on the IAR and kinetin. Perhaps the amount of endogenous phytohormones was determined by the genotype of the plant and thus influenced the dependence of organogenesis on the content of exogenous hormones. In the scientific literature it is indicated that most often the effect of one phytohormone on the metabolism of another is not direct but is a consequence of the functional effect on the activity of tissues.

CONCLUSION

After assessing the length and mass of the potato micro-plants using five concentrations of indolyl-3acetic acid (IAR), it was observed that the IAR effect was greatest on the explants of the 'VB Meda' variety. The explants of the 'VB Aista', 'Goda' and 'VB Venta' varieties also developed well. The study of the influence of the five concentrations of indolyl-3acetic acid (IAR) on microgrowths of the 'VB Venta', 'Goda', 'VB Meda' and 'VB Aista' varieties of potatoes *in vitro* showed that the optimum concentration for the length and mass of micro-plants is 1,75 ml/l. After assessing the length and mass of the micro-plants of potatoes using five concentrations of 6-furfurylaminopurin (kinetin), it was noted that for the explantants of the 'VB Meda' variety, the kinetin effect was greatest. The explants of the 'Goda', 'VB Aista' and 'VB Venta' varieties also developed well. The study of the influence of five concentrations of 6-furfurylaminopurin (kinetin) on microgrowths of potatoes of the varieties 'VB Venta', 'Goda', 'Goda', 'VB

Meda' and 'VB Aista' in *vitro* showed the maximum length of micro-plants (9.46 ± 0.27 cm) and mass (0.234 ± 0.012 g) obtained using a concentration of 0.8 ml/l.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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