

# Factors of Increasing the Productivity of Potatoes

## *In vitro*

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**Abstract**— The promising trend in increasing the productivity of potatoes is efficient use of plant growth regulators and microfertilizers. The paper presents the assessment of the current study on the influence of various substances and defines the need to identify the influence of growth regulators on the net reproduction of plants *in vitro*. It also describes the utilized methods of microclonal propagation of potatoes, preparation of nutrient mediums of various modification. The promising Russian potato grades such as Charoite, Lomonosovsky, Mayflower, Hussar and Real were the objects of the study. The study was conducted in 2017-2018 in the laboratory of microclonal propagation of plants of the Velikie Luki State Agricultural Academy. Various influence of the used nutrient mediums on the studied grades and low influence of factors on plant height on the 21st day were established. On the 21st day of passage the studied potatoes positively reacted to added nutrient medium of growth regulators. The analysis of results demonstrates that the options with the use of MS+ Kafom K nutrient medium are more favorable for morphogenesis and rhizogenesis of microplants *in vitro*. At the same time the grade feature had no considerable influence on the final development of plants and more depended on the content of nutrient components.

**Keywords**— potatoes, growth regulators, *in vitro*, productivity, grade, morphogenesis, rhizogenesis

### I. PREAMBLE

In Russia potatoes represent one of the main food and commercial crops. However, the relevant problem of potato production in the entire territory is still the lack of qualitative seed material, which limits the potato productivity. Unfortunately, in the total amount of seed material the ratio of foreign material is quite high. Thus, for example, the planting areas of the Pskov region occupied with foreign grades make up to 65%. At the same time, low quality of planting material limits the distribution of domestic grades [13].

The experience of domestic and foreign researchers indicates that the use biologically active agents with growth-regulating, antistress and immune-protective activity in the

cultivation of various crops allows solving many issues related to the increase in culture resistance to adverse environmental factors and various pathogens. The average potato yield in Russia makes 25-27 t/ha, whereas the potential of this culture allows receiving 50-80 t/ha and more. In this regard, one of the promising trends in increasing the productivity of potatoes is efficient use of plant growth regulators and microfertilizers [2].

### II. INTRODUCTION

The role of microelements in plants is that they form part of many enzymes playing the role of catalysts of biochemical processes and increasing their activity. Microelements stimulate the plant growth and accelerate their development; have positive effect on the stability of plants against adverse environmental conditions; play an important role in fight against some plant diseases [6].

The *in vitro* conditions represent a good model system to study the effect of growth regulators on potato plants. Besides, the growth stimulation at the test-tube stage may considerably influence the outdoor productivity of plants [15].

It shall be noted that the cultivation conditions *in vitro* make their impact and the function of a mineral fertilizer in such conditions may significantly differ from that of the same agents in the field. Besides, physiological processes within plants free from viruses differ from similar processes within infected plants, which may also affect susceptibility of such plants to growth factors [9].

Shoot and root apical meristems, which form tissues and ensure the plant growth, make the main contribution to the formation of a vegetable organism.

Delicate regulation of balance of dividing cell in meristems is required for normal growth and development. The cell proliferation regulators represent complexes of cyclines and cycline-dependent kinases. Besides, hormones and some substances play an important role in the regulation. It has been generally accepted for a long time that the main growth

regulators are the so-called classical phytohormones or the “great five”. Today they are supplemented with brassinosteroids, jasmonates, salicylic acid and strigolactones.

Recently many studies are focused on CLE peptides, which representatives play a central role in the control of stem cells of all types of meristems and in communication between different meristems.

The tissue culture has been studied for quite a long time, however there are still many unknown factors influencing the growth and development of plants *in vitro*.

The important quantitative index of the tissue culture is the formation of internodes on the regenerated plant. It is found that the higher the yield, the more microplants may be received through propagation by cutting during accelerated reproduction. In many respects this indicator depends on varietal feature and is regulated by external and internal factors. The external factors include light, heat and moisture, while the internal ones – content of nutrient medium. The influence on the organogenesis of *in vitro* plants is also exerted by the latter one. The development of microplants of the studied grades was in direct dependence and content of nutrient medium [1].

All seed-production measures were linked to the maximum yield of healthy planting potatoes. Therefore, it was critical to define the influence of growth regulators on plant reproduction coefficient *in vitro* [10].

### III. METHODS

The study was conducted in 2017-2018 in the laboratory of microclonal propagation of plants of the Velikie Luki State Agricultural Academy.

#### A. Method of potato microclonal propagation

This method was used in the reproduction of the first healthy plants grown from apical meristems.

After plant growth from apical meristems before the formation of 5-8 leaves they were taken with forceps from a test tube in a sterile room and cut into culm segments including part of a stem with one leaf on the sterilized Petri dish with a sharp scalpel or a razor-blade. Part of a stem with a root system may be left in old nutrient medium for repeated growing. The culm segments were placed to the internode depth in test tubes with nutrient medium.

All tools and the Petri dish were sterilized prior to propagation by cutting of each plant. The plants were grown from culm segments at +20-23°C, relative moisture of 70-80%, fluorescent lighting with luminous intensity of 3-4 thousand lux and a 16-hour light period.

On the 4-7<sup>th</sup> day after planting the culm segments demonstrate the growth of a stem and roots. In 18-21 days the plants are completely grown and ready for repeated propagation by cutting.

For cultivation of plants from culm segments the Murashige and Skoog medium was used as modified by the Lorch Potato Research Institute and the Velikie Luki State

Agricultural Academy. The medium was prepared in double-distilled water. Certain volumes of concentrated mother solutions of components prepared in advance were used for mass preparation of mediums.

#### B. Method of preparation of nutrient mediums

The cultivation of plants *in vitro* is based on the basics of physiology, therefore we created special conditions to obtain the entire plant from the tissue or through restoration of a missing part. The nutrient mediums include all elements necessary for a plant.

The mother or concentrated solutions of macro-, microsalts, vitamins and phytohormones were prepared to accelerate the preparation of the necessary medium. We applied the Murashige and Skoog medium (MS) in various modifications.

The promising Russian potato grades such as Charoite, Lomonosovsky, Mayflower, Hussar and Real were the objects of the study.

Experiments were conducted in quadruplicated manner (20 plants each) according to the tissue culture technique. The number of roots and their length, number of internodes, height of plants were measured at various plant development stages. Statistical processing was carried out according to a technique on a personal computer using the STRAZ software [11].

The Murashige and Skoog medium (MS) was used as modified by the Velikie Luki State Agricultural Academy for the cultivation of plants from culm segments (Table 1).

TABLE I. OPTIMIZATION OF MICROCLONAL PROPAGATION S

Option	Medium composition	Amount of substance, ml/l; g/l	Test name
I	Macroelements	50	MS-control
	CaCl <sub>2</sub>	5	
	Microelements	1	
	Fe-chelate	5	
	Vitamins	1	
II	Vitanoll-NP	1	MS + Vitanoll-NP
III	MS+Vitanoll-PK	1	MS + Vitanoll-PK
IV	Kafom K	1	MS + Kafom K

The following microfertilizers of plants were used in the study:

VITANOLL – vitaminized fertilizer based on a polymer complex of macro- and microelements. The agent contains water-soluble copolymers, exerts the properties of surfactant, has high adhesive potential, exerts stimulating and antioxidant characteristics due to vitamins: ascorbic acid (0.01-0.05%), amber acid (0.1-0.2%), and humic acid (0.05-0.1%). Vitanoll ensures high and stable efficiency of plant protection agents due to considerable increase of the coverage area and

penetration of systemic agents inside plants via stomatal mechanism of a plant.

Vitanoll NP: nitrogen content – 9-12%, phosphorus content – 28-30%, pH =4.5-6.5. Microelements: magnesium, manganese, sulfur, zinc, boron, molybdenum.

Vitanoll PK: phosphorus content – 13-16%, potassium content – 16-20%, pH =4.5-6.5. Microelements: magnesium, manganese, sulfur, zinc, boron, molybdenum [5].

Kafom K: phosphorus content ( $P_2O_5$ ) – 30%, potassium content ( $K_2O$ ) – 20%.

The results obtained using the phosphite-based product, such as Kafom K, turned out so efficient that became a real discovery in the market of fertilizers for plant nutrition. The water-soluble phosphite-based agents are also perfectly acquired through leaf treatment. High mobility of phosphite ions ensures fast, efficient and even distribution of phosphorus and other elements in a plant. Besides, the Kafom fertilizers have immune-stimulating effect on plants. Due to stimulation of synthesis the phytoalexins of natural phytoncids, phosphites have good, preventive action and develop plant resistance to some fungous diseases. Thus, we get highly efficient fertilizer with fungicidal properties [3].

#### IV. EXPERIMENTAL PART

The growth of potato plants *in vitro* is defined as the process of a differentiation the organism structure due to

formation of new and increase of its old elements, exerting considerable influence on distribution, redistribution and use of organic substances formed through photosynthesis and metabolism, as well as absorbed mineral salts and water ensuring the formation of new organs, tissues and their regeneration, alongside with reserve deposition [8, 14].

One of the indicators of the tissue culture is the number of formed internodes on the regenerated plant. The higher the yield, the more microplants may be received through propagation by cutting during accelerated reproduction.

The study made it possible to obtain data on the development of potato plants *in vitro*. The studied potato grades demonstrated different behavior within the studied nutrient mediums (Table 2).

On the 7<sup>th</sup> day of passage the greatest number of internodes was formed by Charoite – 3.2 pieces and Real – 3.1 pieces in nutrient mediums with the addition of MS Vitanoll PK. The maximum number of internodes was formed on the 14<sup>th</sup> day by Real – 5.0 pieces in the medium with the addition of Kafom K. On the last day cultivation, the nutrient medium with the addition of Kafom K was positive for the formation of internodes of the studied grades. The highest number was observed for Mayflower and Hussar – 6.2 and 6.0 pieces respectively.

TABLE II. DYNAMICS OF GROWTH AND DEVELOPMENT OF POTATO MICROPLANTS IN VITR

Grade		Number of internodes, pcs.			Height on the 21 <sup>st</sup> day	Height ±St
		7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day		
Charoite	MS Control	1.7	4.4	4.7	65.2	-
	MS Vitanoll-NP	1.2	3.5	4.5	60.3	-4.9
	MS Vitanoll-PK	3.2	4.1	4.7	67.7	+2.5
	MS Kafom K	2.8	4.5	4.8	69.3	+4.1
Real	MS Control	2.8	4.5	5.0	74.0	-
	MS БИТ. NP	3.0	4.7	5.3	74.3	+0.3
	MS Vitanoll-PK	3.1	4.8	5.7	78.6	+4.6
	MS Kafom K	2.5	5.0	5.5	74.7	+0.7
Lomonosovsky	MS Control	1.6	2.9	4.7	65.9	-
	MS Vitanoll-NP	2.8	3.8	5.3	75.5	+9.6
	MS Vitanoll-PK	1.2	2.8	5.2	74.8	+8.9
	MS Kafom K	1.4	3.3	5.4	84.2	+18.3
Mayflower	MS Control	1.6	2.4	5.1	70.6	-
	MS Vitanoll-NP	2.0	3.0	5.3	75.2	+4.6
	MS Vitanoll-PK	2.2	3.0	6.0	75.3	+4.7
	MS Kafom K	2.5	3.5	6.2	80.6	+10.0
Hussar	MS Control	1.2	2.4	4.8	63.2	-
	MS Vitanoll-NP	1.5	2.3	5.1	70.6	+7.4
	MS Vitanoll-PK	1.8	2.6	6.0	77.5	+14.3
	MS Kafom K	2.5	3.0	6.0	79.3	+16.1

The study of the influence of factors on the number of internodes via the multivariate analysis of variance resulted in the following statistically-valid findings:

The grade has statistically-valid impact ( $p=0$ ); the medium

has statistically-valid impact ( $p=0$ ); the cultivation time has statistically-valid impact ( $p=0$ ); the grade and nutrient medium have statistically-valid joint impact ( $p=0$ ); the grade and cultivation time have statistically-valid joint impact ( $p=0$ ); the

nutrient medium and cultivation time have statistically-valid joint impact ( $p=0.020581$ ).

Low influence of factors on plant height on the 21<sup>st</sup> day was revealed ( $v=9.55 < 10$ ).

The objective of the study was the accelerate the growth and development of microplants. The important estimated figure in the production of test-tube material is the study of height of received regenerative agents. On average its formation reaches standard requirements for planting under cover on the 21<sup>st</sup> day of cultivation. However, this indicator is in direct dependence on biology of the studied grades [7].

The studied potato grades positively reacted to nutrient medium of growth regulators on the 21<sup>st</sup> day of passage. Thus, the Lomonosovky grade showed the maximum height indicator – 84.2 mm with the addition of Kafom K, which exceeded the standard option by 20.6 mm. Quite good results were obtained for Mayflower grade on the same nutrient medium – 80.6 mm, which exceeded the standard by 14.8; 10.0; 10.9; and 18.3 mm respectively.

The study of rhizogenesis showed that after replanting the test-tube plants in soil almost all roots die and grow new roots with a different morphostructure. The phenotype of a test-tube plant cannot be considered defective. It corresponds to conditions of closed environment, and hence, the strengthening of rhizogenesis is an important step in microclonal propagation. A key indicator of plant rhizogenesis *in vitro* is the number and length of roots. Well-developed root system exerts positive impact on the formation of plants *in vitro* [12].

Table 3 shows data on the development of culture rhizogenesis *in vitro*. Charoite grade showed the greatest number of roots on the 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day of passage in nutrient medium with the addition of Kafom K and equaled 2.2; 4.4; 5.5 pieces, which exceeded the control on the 21<sup>st</sup> day by 1.2 pieces.

Real showed the best indicators over all days in nutrient medium with the addition of Vitanoll NP by 3.4; 4.8; 6.3 pieces respectively, which is more than in the control by 0.8 pieces.

TABLE III. GROWTH DYNAMICS OF THE ROOT SYSTEM OF POTATO MICROPLANTS IN VITRO

Grade		Number of roots, pcs.			±control on the 21 <sup>st</sup> day
		7 <sup>th</sup> day	14 <sup>th</sup> day	21 <sup>st</sup> day	
Charoite	MS Control	1.9	3.5	4.3	-
	MS Vitanoll-NP	1.6	3.8	4.5	+0.2
	MS Vitanoll-PK	2.0	2.6	2.8	-1.5
	MS Kafom K	2.2	4.4	5.5	+1.2
Real	MS Control	2.4	4.2	5.5	-
	MS Вит. NP	3.4	4.8	6.3	+0.8
	MS Vitanoll-PK	1.6	2.8	3.2	-2.3
	MS Kafom K	2.9	4.4	4.7	-0.8
Lomonosovsky	MS Control	2.2	3.5	4.3	-
	MS Vitanoll-NP	2.6	3.6	3.9	-0.4
	MS Vitanoll-PK	1.9	3.4	3.6	-0.7
	MS Kafom K	2.5	5.2	5.4	+1.1
Mayflower	MS Control	1.8	4.0	5.3	-
	MS Vitanoll-NP	4.5	6.0	6.3	+1.0
	MS Vitanoll-PK	4.2	6.0	6.4	+1.1
	MS Kafom K	4.8	5.9	7.1	+1.8
Hussar	MS Control	1.5	2.3	3.5	-
	MS Vitanoll-NP	2.6	3.8	4.8	+1.3
	MS Vitanoll-PK	3.3	4.5	4.8	+1.3
	MS Kafom K	3.1	4.7	5.1	+1.6

The study of the influence of factors on the number of roots via the multivariate analysis of variance resulted in the following statistically-valid findings:

The cultivation time has statistically-valid impact ( $p=0$ ); the grade has statistically-valid impact ( $p=0$ ); the medium has statistically-valid impact ( $p=0$ ); the grade and cultivation time have no statistically-valid joint impact ( $p=0.39953$ ); the nutrient medium and cultivation time have no statistically-valid joint impact ( $p=0.909612$ ); the grade and nutrient medium have statistically-valid joint impact ( $p=0$ ).

On the 1<sup>st</sup> and 21<sup>st</sup> day of passage the Mayflower demonstrated a positive effect with the addition of Kafom K and formed 4.8 and 6.0 pieces of roots, which is more than in the control by 1.8 pieces, and on the 14<sup>th</sup> day the Vitanoll NP showed better result and made 6.0 pieces.

Hussar grade on the 7<sup>th</sup> day of cultivation showed the maximum number of roots in nutrient medium with the addition of Vitanoll PK. In the next days the bigger number of roots was created with the addition of Kafom K and equaled to 4.7 and 5.1 pieces, which is more than in the control option by 1.6 pieces.

## V. CONCLUSIONS

The obtained data make it possible to note that the options with MS+ Kafom K in the concentration of 1 g/l turned to be the most favorable for morphogenesis and rhizogenesis of microplants in vitro. The study shows that the grade feature had no considerable influence on the final development of plants and more depended on the content of nutrient components.

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