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In vitro micropropagation and *ex vitro* rooting of some potato varieties

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The article presents the results on selection of optimal concentrations of nutrient media components and nutrient solution at the stages of clonal micropropagation of potato varieties Lyubava, Kemerovochanin, and Tuleevskiy (actual reproduction, rooting *in vitro,* adaptation to *ex vitro* conditions). The influence of some components of the nutrient medium (sucrose, agar-agar, growth regulators, namely α-naphthyl acetic acid, β-indolyl acetic acid, and β-indolyl propionic acid) was studied at the stages of reproduction and rooting in order to obtain regenerants of the studied potato varieties. The best development of plants on nutrient medium with addition of 4 g L^{-1} of agar-agar was revealed. The addition of sucrose in the concentration of 3-5% contributed to the formation of more internodes. The influence of naphthyl acetic acid, β-indolyl oil, and β-indolyl propionic acids in different concentrations on the rhizogenesis of regenerating plants of three potato varieties at the stages of reproduction and establishment of different types and concentrations of auxins was studied. The researches have shown that a one-stage method of adaptation of *Solanum tuberosum* L. varieties of Lyubava, Kemerovochanin, and Tuleevskiy to the conditions of *ex vitro* cultivation with the use of hydroponic installation is characterized by efficiency and allows to receive plants with well-developed root system. The efficiency of using the hydroponic plant for adaptation of potato regenerants at the final stage of micropropagation is shown.

Key words: Healing planting material; Clonal micropropagation; Potatoes; Nutrient medium; Adaptation to *ex vitro* conditions

Introduction

The method of clonal micropropagation is the initial stage of accelerated reproduction of potato varieties (Lapshinov et al., 2013). This method has become widespread in the production of healing planting material of various plant species, including tuber potatoes *Solanum tuberosum* L. (Miakisheva et al., 2016). Good development of plants from cuttings is the main criterion for evaluation of micropropagation efficiency. The main factors influencing the parameters of growth and development of microplants are variety features and composition of the nutrient medium (Ryabtseva et al., 2017). Factors of nutrient environment is one of the important parameters that should be optimized to obtain successful plant regeneration. Nutrient medium is a substrate on which all morphogenetic processes, typical for the explant introduced into the culture *in vitro,* take place. The main requirement for this substrate for micropropagation is to provide a high reproduction coefficient. The environment developed by Murashige and Skoog (MS) in 1962 is the most common environment used for working with plant tissue cultures. Components of the cultural environment can be divided into six groups: macronutrients, trace elements, iron source, vitamins, carbon sources, and plant growth regulators.

When cultivated *in vitro,* regenerated plants cannot synthesize sugar, which is necessary for their normal development; therefore, carbohydrates, mainly sucrose, are used in nutrient media at all stages of micropropagation. This is because *in vivo* sucrose is the predominant transport form of sugars in phloem. Carbohydrates, on the one hand, are the elements of nutrition; on the other hand, they are substances that together with the salt composition create a certain osmotic pressure. Their importance increases at the stage of rooting in connection with the need to prepare the plant for autotrophic nutrition in non-sterile conditions (Demenko et al., 2010; Deryabin & Trunova, 2014).

For the normal passage of biochemical processes, plant organisms need vitamins. However, under certain conditions, plant tissues are not capable of synthesizing some of them (Otrozhy et al., 2009; Ewing, 2010). Vitamins are a group of low-molecular organic compounds. They participate in biochemical reactions, performing a catalytic function as part of the enzyme active centers (Caligari, 2007). Vitamins play an important role in metabolism. Thus, thiamine (vitamin B1) stimulates the development of the root system and is a coenzyme in the non-oxidative decarboxylation of α-ketoacids (Nugent, 2001).

According to studies of Dobranszki J. (2011), when cultivating individual plants under artificial conditions, their root system did not synthesize a sufficient amount of thiamine, pyridoxine, thus when these vitamins were added to the nutrient medium, the plant growth improved significantly. According to Sanders et al. (2002), in the absence or lack of thiamine, the decarboxylation reaction of pyruvic grape and some other acids in organisms is suppressed and acids accumulate in tissues. According to I.A. Tarchevsky (2002), one of the important functions of pyridoxine (vitamin B6) is to maintain the balance of potassium and sodium in the body, moreover, vitamin B6 plays an extremely important role in nitrogen metabolism. Nicotinic acid (vitamin PP) is a precursor to the synthesis of many essential compounds in plant metabolism, in the metabolic system is often part of coenzymes active in anabolic reactions (Cherevchenko et al., 2008). Thus, it is necessary to study the functions, concentrations and influence of vitamins on different varieties in the complex, because they interact with each other, give both a positive and negative effect on the studied crop, species or variety (Steudle, 2001). In connection with the development of biotechnology of agricultural plants, the importance of information about phytohormonal regulation of organogenesis increases. Phytohormones are the main tool in various manipulations with cell cultures and plant tissues *in vitro* (Jacobsen & Hutten, 2006). In the processes of rhizogenesis induction the main role is given to auxins (Chrevchenko et al., 2008). They control the development of plants at all stages: at the cellular level the growth of cells by stretching, division and differentiation of specific cell types. This group of growth regulators plays a key role in root development and the establishment of lateral organs of shoots (Petrasek & Friml, 2009; Caligari, 2007).

Research of Berry & Björkman (2008) have shown that auxins activate vital physiological processes of plants, providing an increase in their productivity, as well as resistance to diseases and adverse stressors of the environment. According to the experiments (Elaleem et al., 2009; Ewing, 2010), auxins stimulate the differentiation of meristematic or dedifferentiated cells into conductive tissue cells. Under its influence, conductive floemas and xylemas are formed in the callus tissue. Thus, the nutrient medium is the main factor determining the success of clonal micropropagation. The main processes occurring during tissue cultivation are largely regulated by the mineral components of the environment, their concentration and ratio. An important factor regulating the differentiation and morphogenesis of isolated tissues is the presence of growth regulators in the nutrient medium (Kalinin et al., 1992). When working with different plant species, a special selection of media components and their concentrations is required for normal morphogenesis of plant micropods. In this regard, the aim of the study was to select the optimal concentrations of nutrient media components at the stage of clonal micropropagation at the stage of actual reproduction, rooting and adaptation of potato plants of Lyubava, Kemerovochanin, and Tuleevskiy varieties. In our research we also planned: to study the influence of nutrient medium components (sucrose, vitamins, agar-agar) on morphological indicators of potato regenerating plants development; to consider the influence of different types of auxins and their concentrations on the rhizogenesis of potato regenerants at stages of reproduction and rooting; to compare the biometric parameters of plants (height, number of internodes, number of leaves) of several potato varieties before and after adaptation to the hydroponic plant.

Material and Methods

The experiment was conducted in the laboratory of plant biotechnology of the Altai Center of Applied Biotechnology of Altai State University. The object of our study was potato varieties (*Solanum tuberosum* L.): early maturing - Lyubava, early middle maturing Kemerovo, and middle maturing - Tuleevskiy. Lyubava is an early variety of table use and for processing into crispy potatoes and fries. Tubers are bright pink (Figure 1). Eyehole are red and of medium depth. Flesh is white. Wreath is pale and red violet. Yield is 33-45 t ha^{-1}, in the first unearthing 60 days after planting - 15-20 t ha^{-1}. Productivity is 90-96%. Weight of grocery-wire tuber is 90-120 g. Starchiness 13-16%. Taste qualities and safety are good. Resistant to viral diseases. Moderately susceptible to phytofluorosis 33, relatively resistant to common parsley, ring rot, and risoctoniosis. Weakly susceptible to alternative disease. The value of the variety is its early maturity, high yield, good taste and safety.

Figure 1. Potate tubers of Lyubava variety.

Kemerovchanin

It is a medium early table variety. Average early and medium height plant. The bush is compact. White flowers. Tubers and pulp yellow with rounded shape (Figure 2). Eyeholes of medium depth. Yield is 35-45 t ha^{-1}. Weight of the grocery-wire tuber is 100-170 g. Starch content is 16-17.5%. Taste qualities are good. Resistant to cancer and golden potato nematode. Relatively resistant to phytofluorosis, common parsley, and alternative.

Figure 2. Kemerovchanin potato tubers.

Tuleevskiy

It is a middle-ripening variety of canteen purpose. Medium height, intermediate type, semi-permanent plant. Medium size sheet, from intermediate to open type, dark green. Waviness of the edges is weak. Corona is white and very large. Commercial yield – 18.0-42.4 t ha^{-1}, maximum – 45.8 t ha^{-1}. The tuber is long with very small eyeholes. The skin is slightly rough and yellow (Figure 3). Flesh is yellow. Weight of grocery-wire tuber is 122-270 g. Starch content is 13.7-16.8%. The taste is good and excellent. Marketability 88-99%, storability is 90%. Resistant to potato cancer agent, susceptible to golden cyst-forming potato nematode. It is moderately resistant on the bottle and moderately susceptible on tubers to the pathogen phytofluorosis (Simakov et al., 2010).

Figure 3. Tuleevskiy potato tubers.

The research method of research was based on generally accepted classical methods of working with isolated tissue cultures and plant organs (Butenko, 1971)]. Microtraces with one axillary kidney were used as explants. Conditions of microsprouts growth: photoperiod of light/darkness is 16/8 hours, illumination is 2-3 kilolux, temperature 24 ± 1ºC. Passage duration was 25 days. The main nutrient medium was Murashige, Skoog (Ìs), supplemented with 100 mg L^{-1} meso-Inositol and 1 g L^{-1} casein hydrolysate.
To select the optimal micropropagation parameters, nutrient media with different agar-agar content (1; 3; 4; 5; 6; 9%), sucrose (1; 3; 4; 5; 6; 9%) and vitamins (0 (control) ml L^{-1}, 1.0 ml L^{-1}, 1.5 ml L^{-1}, 2.0 ml L^{-1}, and 2.5 ml L^{-1}) according to MS registration were used. Microplants were rooted in agarized media according to the Murashige and Skoog prescriptions, supplemented for the induction of rhizogenesis with auxins - α-naphthylacetic acid (NUK), β-indolyl oil acid, β-indolyl propionic acid in different concentrations (0.1, 0.5, 1.5, 3.0, and 5.0 µmol). In the experiment on adaptation of potato plants, all regenerants were obtained on agarized nutrient medium according to MS prescription. Adaptation of regenerated plants was carried out at the new improved hydroponic unit "Maxivit" developed in the Altai Center of Applied Biotechnology of Altai State University. Regenerants were taken out of test tubes, the root system was washed from agar in distilled water, plants were placed in cassettes, and then - in the

vegetation cell of the hydroponic installation, filled with nutrient solution according to 1/2 M" (Table 1), modified according to the content of $KN_{2P}O_4$ (510 mg L^{-1}).

Table 1. Nutrient solution for adaptation of potato regenerating plants.

Components	Concentration of mother solution, ml L^{-1}	Input volume (per 30 L of base solution), mL
Macronutrients	25	38
Micronutrients	1	8
$CaCl_2 \times 2H_2O$	1	8
Ferric chelate	10	15

The hydroponic system operates according to the set parameters (Table 2), during the first day of adaptation the plants are cultivated without lighting, then turn on the lighting and set the frequency of lighting.

Table 2. Adaptation conditions for potato regeneration plants on the hydroponic plant.

Adaptation parameter	Values
Photoperiod	1-2 days of adaptation with no lighting, since third day 16 hours daylight, 8 hours night
Lighting type	LED
Hydroponics solution mode	Solution feeding five min, break 20 min
Room temperature	+20–21°C
Hydroponics solution temperature	+18–20°C
Hydroponics solution pH	6.0
Hydroponics solution change periodicity	After 10 days
Duration of adaptation	25 days

The lighting sources were LED lamps. The total power consumption is 85 W. Spectral composition was: red, blue, and white light in the proportion of 9:5:4, respectively. The adaptation period of regenerated plants on the hydroponic plant was 25 days. After that, the regenerants removed from the cassettes of the vegetation ditch and analyzed 30 plants of each variant in triple repetition according to the following criteria: plant height (cm), number of internodes (pcs.), number of leaves (pcs.), number of roots (pcs.), average root length (cm). Statistical data were processed by Microsoft Office Excel 2007. The values in tables presented like average and confidence intervals. Confidence of the evaluated indicators was accepted at the level of significance P<0.05.

Results and Discussion

An important indicator that provides a high reproduction rate of plants is a well-formed internode. In clonal micropropagation, mainly solid media are used. Agar-agar is added in concentration of 7 g L^{-1}. However, liquid nutrient media increase the availability of its components for the conductive system of plants. Our studies have shown that a decrease in the concentration of agar (up to 4.0 g L^{-1}) increases the number of internodes (Table 3).

Table 3. Influence of nutrient medium components on internodes number in potato regeneration plants, pcs/explants.

Sucrose, (%)						Agar-agar, (%)					
1	3	4	5	7	9	1	3	4	5	7	9
Lyubava											
5.1 ± 0.6	6.3 ± 1.4	7.1 ± 0.8	7.0 ± 1.3	5.4 ± 1.8	5.1 ± 0.4	5.3 ± 1.1	6.8 ± 0.4	7.4 ± 1.5	6.2 ± 1.8	6.7 ± 1.0	6.1 ± 2.1
Kemerovchanin											
6.0 ± 1.3	8.9 ± 0.9	9.2 ± 1.1	7.1 ± 1.5	5.9 ± 2.1	4.1 ± 1.9	4.6 ± 0.3	8.6 ± 0.4	9.3 ± 1.4	6.4 ± 2.4	5.7 ± 0.2	4.9 ± 0.1
Tuleevskiy											
5.9 ± 0.7	6.3 ± 1.9	6.4 ± 0.6	5.8 ± 1.8	5.9 ± 2.1	5.2 ± 1.3	5.8 ± 1.6	6.6 ± 1.1	6.3 ± 0.8	5.7 ± 0.4	5.4 ± 0.7	5.1 ± 1.2

Sucrose in the nutrient medium is a major source of carbon. The role of this carbohydrate in root formation and microtuberculosis stimulation is important. Sucrose is a growing factor not only for the root system, but also affects other biometric parameters of plants *in vitro* (Borodaeva et al., 2017; Demenko et al., 2010). Formation of more internodes was observed when adding carbohydrates in concentration from 3 to 5%. Low and high concentrations of sucrose reduced the reproduction rate (to 6.0, 5.8, and 4.3 pcs/explant, respectively). When studying the influence of different concentrations of vitamins on the morphogenesis of potato regenerants, the optimal morphological parameters were recorded in the variety Kemerovochanin on the nutrient medium MS + vitamins 2.0... 2.5 ml L^{-1}. The number of internodes in this case was 7/8.8 pcs. per plant, plant height – 13/12.1 cm; total length of root system - 4.7/4.5 cm; number of roots - 13.6/15.4 pcs. per plant (Table 4).

Table 4. Influence of vitamins on morphogenesis of regenerated potato varieties Lyubava, Kemerovochanin, Tuleevskiy.

Variants	Сорта	Plant height, cm	Internodes number, psc/plant	Weight of leaves and stem, mg	Root number, pcs/plant	Root length, cm
MS+ vitamin 1.0 ml L^{-1}	Lyubava	8.9 ± 0.7	5.9 ± 1.1	439 ± 1.6	6.3 ± 1.7	3.9 ± 0.8
	Kemerovchanin	9.3 ± 0.9	6.2 ± 0.6	440 ± 1.2	8.0 ± 1.5	4.3 ± 1.7
	Tuleevskiy	9.7 ± 0.4	6.3 ± 0.9	420 ± 0.4	7.1 ± 0.8	3.7 ± 0.5
MS+ vitamin 1.5 ml L^{-1}	Lyubava	8.5 ± 1.0	5.8 ± 0.7	435 ± 1.5	6.4 ± 1.2	4.0 ± 0.7
	Kemerovchanin	10.4 ± 1.3	6.1 ± 0.4	440 ± 1.4	11.3 ± 1.4	4.1 ± 1.2
	Tuleevskiy	9.6 ± 0.2	6.2 ± 0.5	430 ± 0.9	6.9 ± 1.1	3.8 ± 0.4
MS+ vitamin 2.0 ml L^{-1}	Lyubava	8.9 ± 1.3	6.0 ± 0.9	445 ± 0.6	6.6 ± 0.2	4.4 ± 1.2
	Kemerovchanin	12.1 ± 1.2	7.0 ± 1.0	460 ± 1.3	13.6 ± 2.3	4.5 ± 1.8
	Tuleevskiy	9.8 ± 0.7	6.8 ± 0.4	430 ± 0.8	7.2 ± 1.5	3.7 ± 0.7
MS+ vitamin 2.5 ml L^{-1}	Lyubava	9.5 ± 1.1	6.4 ± 1.1	495 ± 1.3	6.5 ± 0.6	3.5 ± 0.8
	Kemerovchanin	13.2 ± 1.4	8.8 ± 0.7	520 ± 1.6	15.4 ± 1.3	4.7 ± 1.0
	Tuleevskiy	10.2 ± 0.9	7.4 ± 0.8	490 ± 1.2	7.9 ± 0.9	4.2 ± 0.9

We revealed that the variety Lyubava had a positive reaction to an increase in the concentration of vitamins in the nutrient medium (Table 4). This variety forms 5.9 internodes per plant of potatoes on MS + 1.0 ml L^{-1} media. The best result of this variety was on the nutrient medium MS + 2.5 ml L^{-1} of vitamin - 6.4 internodes per plant. Tuleevskiy variety forms 6.3 of internodes on a standard nutrient medium, the increase in the concentration of vitamin complex in the nutrient medium had a positive effect on the number of internodes, it increased from 6.2 to 7.4 per plant.

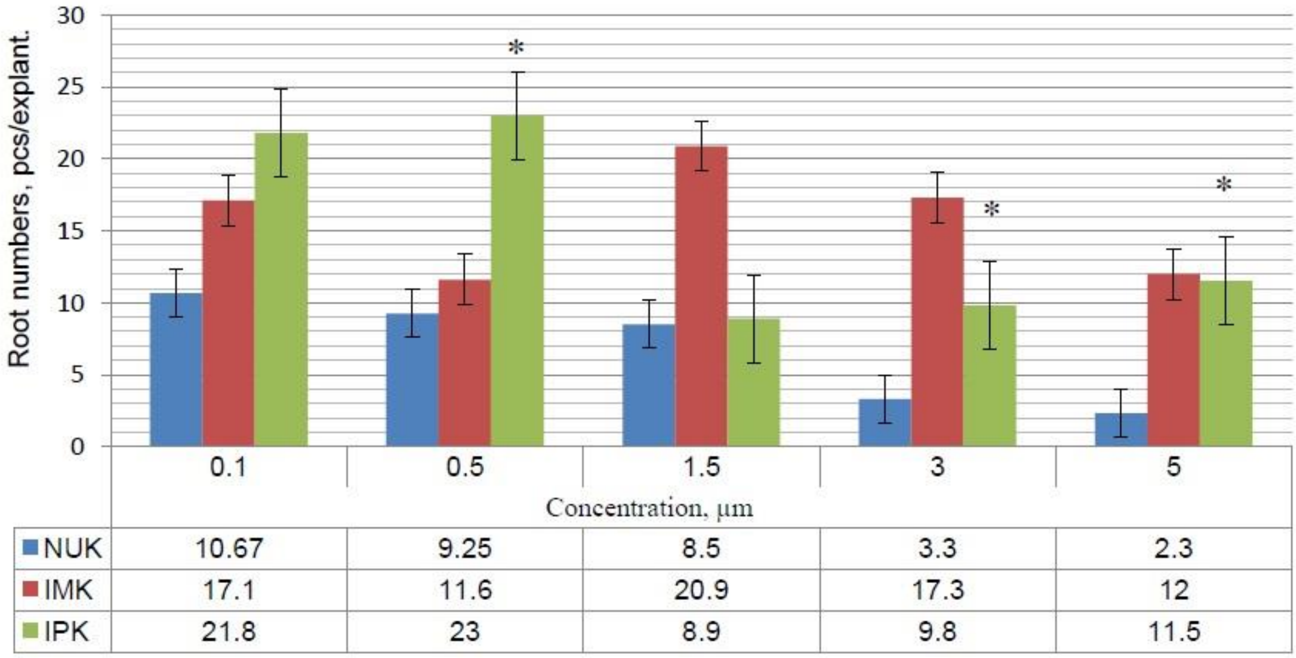
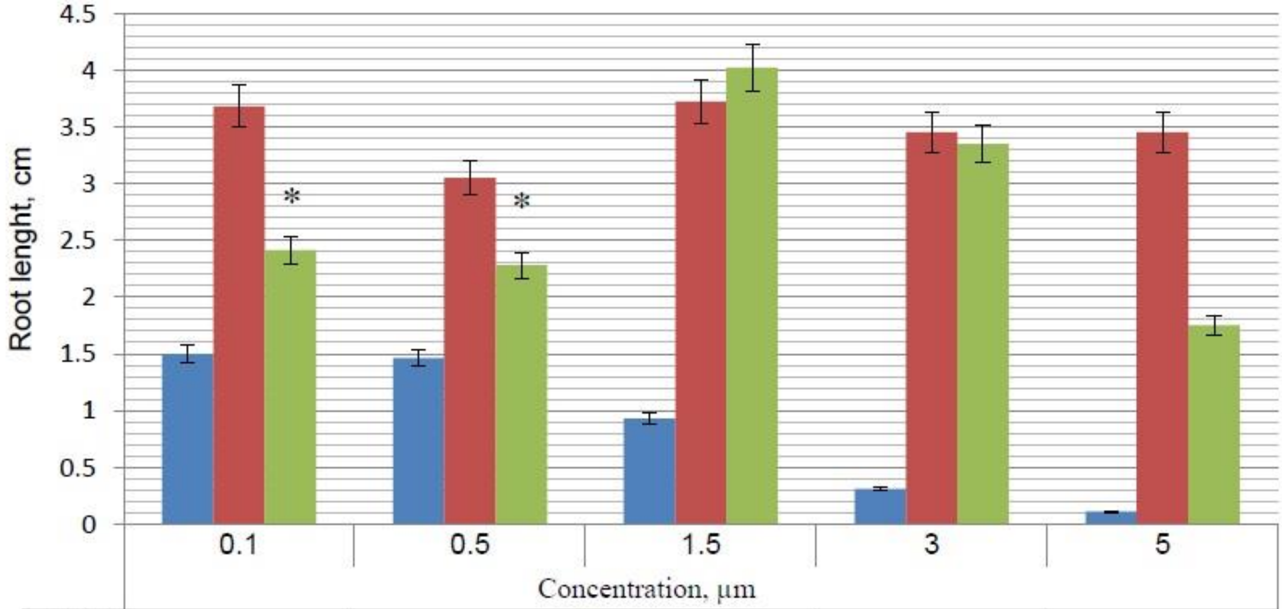


Figure 4. Influence of growth regulators on the number of roots of Lyubava potatoes. * - statistically significant differences, p<0.05. (NUK - Naphthyl Acetic Acid, IMK – Indole Butyric Acid, IPK - Ndole-3-Propionic Acid.

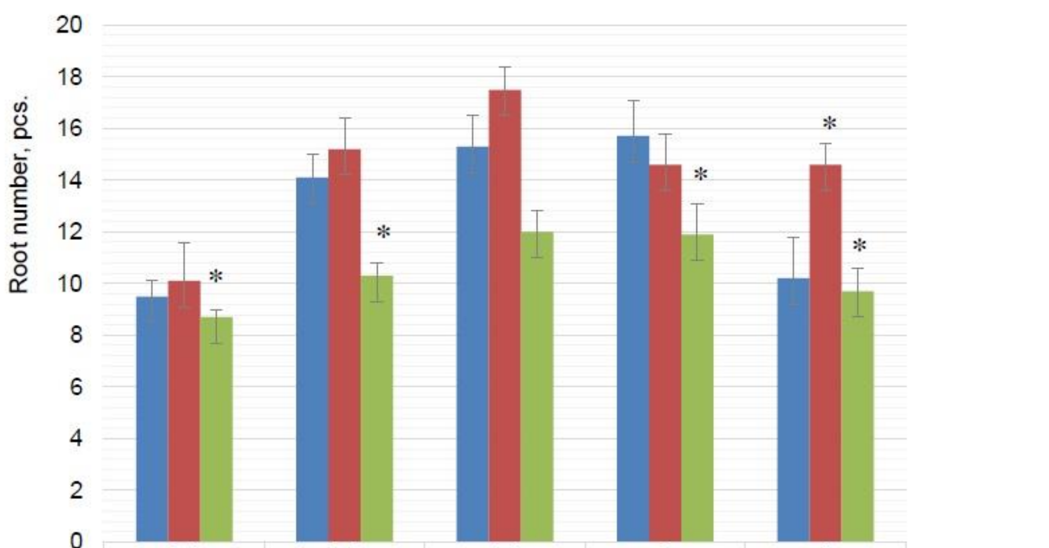
Analysis of the length of the formed roots revealed that the variety Lyubava in the variant with 1.5 µmol indole-3-propionic acid (IPK on the graphs) had the longest roots (4.02 ± 0.26 cm). Higher concentrations of auxin (1.5, 3.0, and 5.0 µmol) were less effective in promoting root growth of all potato varieties under study. Low concentration of indole acetic acid (0.1 µmol) weakly stimulated the increase of root length in all potato varieties (Figure 5).



	0.1	0.5	1.5	3	5
NUK	1.5	1.46	0.93	0.31	0.11
ИIMK	3.68	3.05	3.72	3.45	3.45
IPK	2.41	2.28	4.02	3,.5	1.75

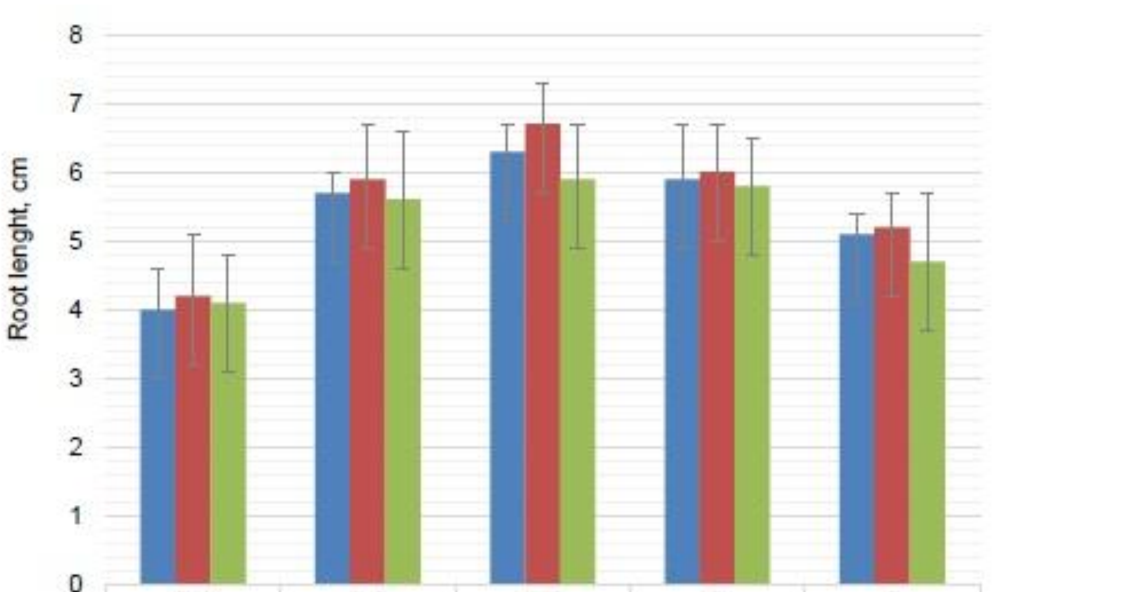
Figure 5. Influence of growth regulators on the root length of Lyubava potatoes.

The data obtained showed that the efficiency of root formation in potato regeneration depends on the type and concentration of the used auctioneer. Maximum rhizogenesis in the Kemerovchanin variety was recorded using 1.5 µmol of IMC. Thus, the number of roots applied to the nutrient medium at this concentration was 17.8 pcs/plant, the average length of the root was 6.7 cm. When adding 1.5 µM of IPK to the nutrient medium, there was a greater number of roots - 12.0 pcs/plant, about 16.0 pcs/plant, when adding 3.0 µM of NUK to the nutrient medium (Fig.6). The best indicators of rhizogenesis were noted at the introduction of the studied growth regulators in the nutrient medium at a concentration of 1.5 µM, the average length of roots was 6.3 cm at the introduction of 1.5 µmol of NUK and 5.9 cm - 1.5 µmol of IPK into the nutrient medium (Figure 6). Introduction of 5 µmol of the NUK growth regulator in the Lyubava variety was inhibited the root formation (0.5 ± 0.81 pcs/plant), while the introduction of 0.5 µmol IPK into the medium contributed to the formation of the largest number of roots (4.7 ± 3.57 pcs/plant) (Figure 7).



	0.1	0.5	1.5	3	5
NUK	9.5	14.1	15.30	15.7	10.2
IMK	10.1	15.2	17.5	14.6	14.6
IPK	8.7	10.3	12	11.9	9.7

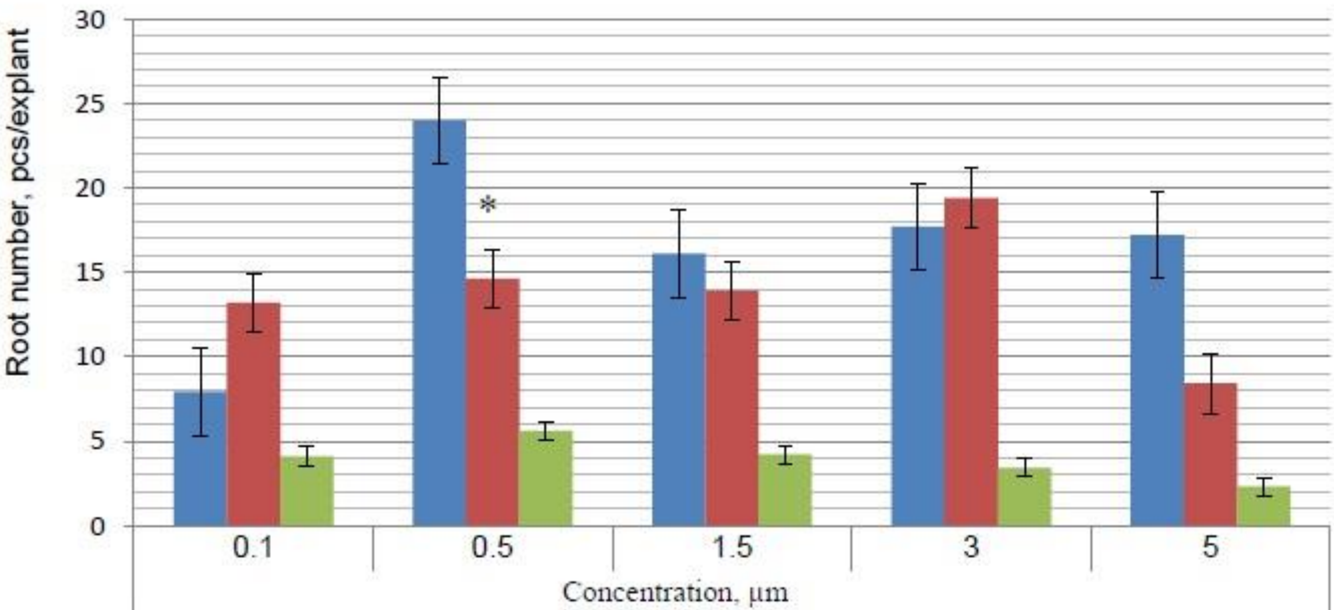
Figure 6. Influence of growth regulators on the number of roots of Kemerovo potatoes.



	0.1	0.5	1,5	3	5
NUK	4.0	5.7	6,3	5,9	5.1
IMK	4.2	5.9	6,7	6	5.2
IPK	4.1	5.6	5,9	5,8	4.7

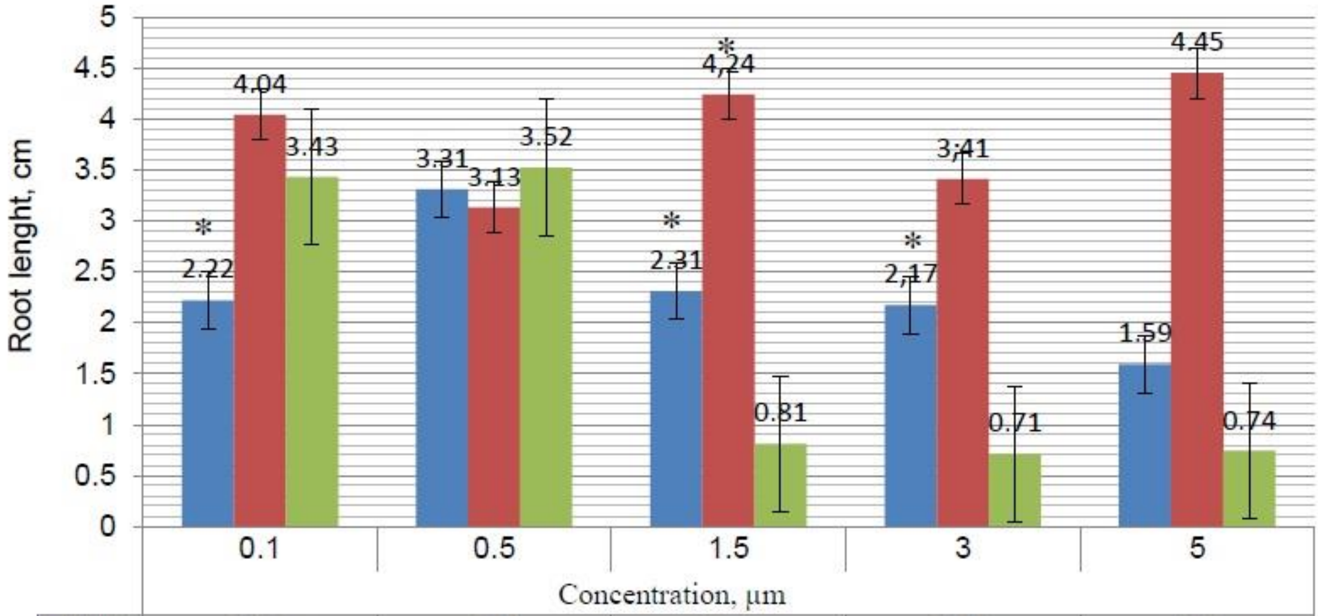
Figure 7. Influence of growth regulators on root length of Kemerovchanin variety.

The influence of NUK concentration (0.1, 0.5, 1.5, 3.0, and 5.0 µmol) on potato rhizogenesis *in vitro* was caused after 20 days the maximum number of roots (24.0 ± 4.31 pcs/explant) in Tuleevskiy variety on the nutrient medium with the addition of 0.5 µmol of NUK. The use of IMK as a rhizogenesis stimulator showed that the maximum number of roots (33.4 ± 1.06 pcs/explant) was observed in Tuleevskiy variety on the nutrient medium with 3.0 µmol of IMK (Figure 8). The analysis of the length of the formed roots revealed that the variety Tuleevskiy in the variant with 0.5 µM NUK had the longest roots (3.3 ± 1.2 cm). A similar picture was observed when using 1.5 µmol of NUK. Higher concentrations of auxin (3.0 and 5.0 µmol) inhibited the growth of formed roots (Figure 9).



	0.1	0.5	1.5	3	5
NUK	7.9	24	16.1	17.7	17.2
IMK	13.2	14.6	13.9	19.4	8.4
IPK	4.1	5.6	4.2	3.4	2.3

Figure 8. Influence of growth regulators on the number of roots in Tuleevskiy variety.



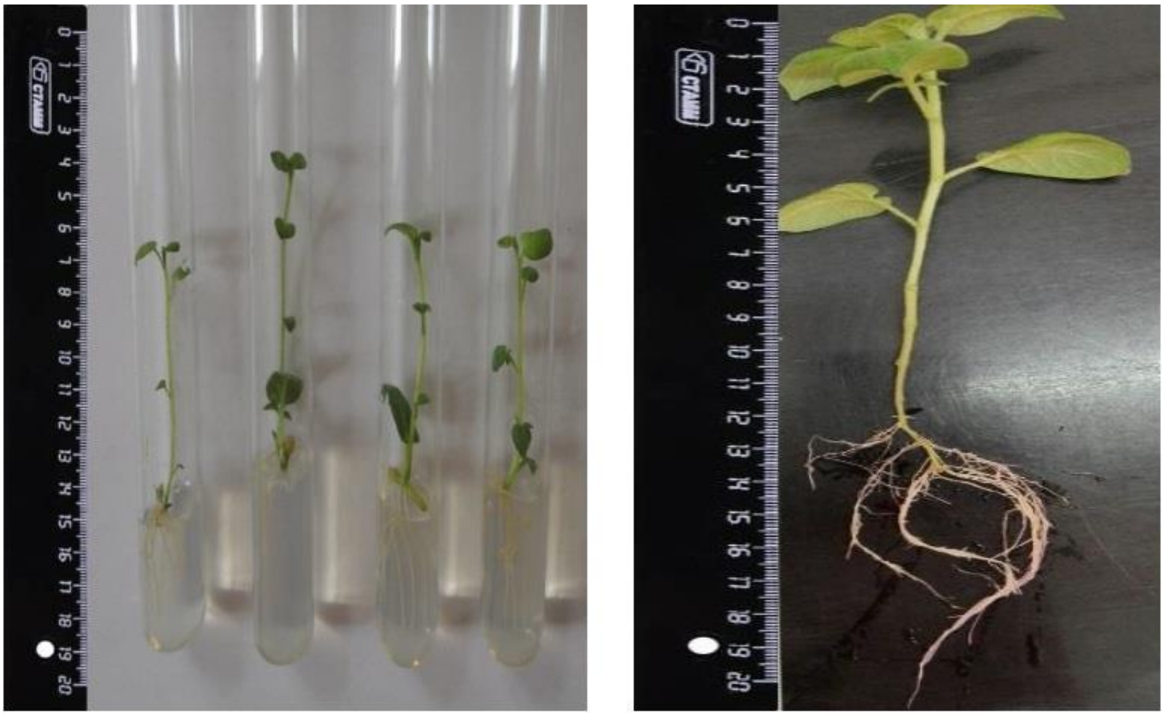
	0.1	0.5	1.5	3	5
NUK	2.22	3.31	2 .1	2.17	1.59
IMK	4.04	3.13	4.24	3.41	4.45
IPK	3,43	3.52	0.81	0.71	0.74

Figure 9. Influence of growth regulators on the root length in Tuleevskiy variety.

Adaptation of regenerating plants to *ex vitro* conditions is the final, key stage of clonal micropropagation of plants and is carried out on hydroponic plants (Figure 10). It is noted that the use of nutrient solutions with a lower concentration of mineral salts (1/2 and 1/4 of the composition) contributes to the better development of the regenerants, compared with the full composition. A two-stage method of adaptation of regenerating plants to the conditions of *ex vitro* cultivation on hydroponic plants has been developed: 1) nutrient solution 1/2 or 1/4 MS medium, modified according to the content of KN_2PO_4; 2) nutrient solution 1/2 or 1/4 MS medium, modified according to the content of NH_4NO_3 (Vechernina, 2006; Miakisheva et al., 2017b). However, only the first stage of potato regeneration was suitable for adaptation of potatoes. Solution replacement was performed every 10 days, pH measurements of the solution were performed every day, and was brought to the level of 5.7-6.0 with the help of NaOH or HCL normal solution.

Figure 10. Potato regenerated plants on an adaptation plant culturing unit.

The data presented in Table 5, indicate a natural increase in all parameters of growth and development of potato regenerants during their adaptation. The study showed that the survival rate of regenerated plants on hydroponics was 100%. The number of roots of the Lyubava variety after 25 days of adaptation increased by 1.4 times, for the rest of the varieties under study the indicator increased almost threefold. The average root length for all the varieties studied was three times higher after the adaptation of the regenerating plants. The height of shoots of Lyubava and Kemerovochanin varieties (Figures 11 and 12) was almost the same (14.1 ± 0.16, 14.2 ± 0.19). The most foliated shoots were formed in Tuleevskiy variety after adaptation (Figure 13).



a b

Figure 11. Lubawa potatoes: a) Test-tube plants; b) Regenerate after adaptation.

a b

Figure 12. Kemerovchanin potatoes: a) Test-tube plants; b) Regenerate after adaptation.

Table 5. Some indicators (before/after adaptation) of potato regeneration plants adapted to the hydroponic (n=30).

Variety	Lyubava	Kemerovchanin	Tuleevskiy
Survivability, %	100	100	100
Root number, pcs	4.2 ± 0.60/ 16.0 ± 0.09	7.8 ± 0.50/ 21.0 ± 0.11	5.3 ± 0.40/ 17.4 ± 0.23
Mean root length, cm	2.3 ± 0.15/ 7.8 ± 0.07	3.1 ± 0.13/ 9.4 ± 0.16	2.8 ± 0.07/ 8.6 ± 0.23
Shoot height, cm	7.9 ± 0.10/ 14.1 ± 0.16	8.3 ± /0.09 14.2 ± 0.19	5.6 ± 0.11/ 13.1 ± 0.12
Leaves number, pcs	4.3 ± 0.08/ 6.9 ± 0.12	4.0 ± 0.11/ 7.0 ± 0.18	6.5 ± 0.14/ 8.0 ± 0.08

Figure 13. Tuleevskiy potatoes: a) Test-tube plants; b) Regenerate after adaptation.

Conclusion

When using agar-agar in the concentration of 4 g L^{-1} there is an increase in the number of internodes of regenerated plants varieties of potatoes Lyubava, Kemerovochanin, and Tuleevskiy. An addition of vitamins (B1, B6, and PP) to the nutrient medium of 2.0-2.5 mL L^{-1} promotes the formation of higher plants, increases number of internodes and roots. At the stages of *in vitro* reproduction and establishment of potatoes, the type and concentration of auxins are of paramount importance. According to our research, at these stages the optimal use of 0.5-1.5 µmol of indole butyric acid for Kemerovochanin; 0.5 or 1.5 µmol for Lyubava - 0.1; 0.5 µM indole butyric acid and 0.1-3.0 µmol of indole butyric acid, for Tuleevskiy - 0.5 µmol of α-naphthylacetic acid and 0.1-5.0 of indole butyric acid. Adaptation of regenerating plants to *ex vitro* conditions using hydroponics filled with a nutrient solution of a certain media (1/2 MS+ KN_2PO_4) allows to obtain potato regenerants with a well-developed root system and above-ground part. Thus, the survival rate of regenerated plants on hydroponics was 100%. The height of the studied regenerants should be doubled after 25 days of adaptation. The value of the indicator "average root length" of the varieties Lyubava, Kemerovochanin, and Tuleevskiy after adaptation was three times higher than that of test-tube plants.

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