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Research Article

THE EFFECT OF VARIOUS SUBSTRATES OF THE NUTRIENT MEDIUM ON THE GROWTH AND DEVELOPMENT OF IN VITRO POTATO PLANTS

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ABSTRACT
One of the main factors that determines success of tissue culture is selection and preparation of the nutrient medium with regard to the effect of its main components on potato plants. Slightest inconsistencies in the nutrient medium entail significant changes in plants ontogenesis. In the laboratory of FSBEI HE (FederalState Budget Educational Institution of Higher Education) The Chechen State University and the Gorsky SAU (State Agarian University), research has been performed with various modifications of nutrient medium in order to detect reaction of varieties Zhukovsky Early, Meteor (the all-Russian R&D Institute of Potato Breeding), Aurora (the Vsevolozhsk Breeding Station), Pribrezhny(the Sakhalin Experimental Station of the all-Russian R&D Institute of Phytopathology), and Andra(Germany) to changes in plant nutrition in vitro. It has been established that the initial medium of the R&D Institute of Potato Breeding ensures the maximum growth and survival ability of plants of varieties Zhukovsky Early, Aurora, Meteor and Pribrezhny, and the nutritional medium modification I – of variety Andra.

Keywords: potatoes, variety, in vitro, nutrient medium, seed breeding, explant.

INTRODUCTION

Potato seed breeding is conducted worldwide according to the classical scheme: variety breeding, its periodic improvement in laboratories, reproduction in vitro, producing greenhouse minitubers (GMT) from test tubes, five-year reproduction cycle: GMT►1PP► SSE► SE► E►1p. However, unfortunately, it is very hard to make this scheme work in Russia[1].Original potato seed breeding involves maintaining a bank of healthy potato varieties (BHPV), obtaining and production of healthy (free of viral and other infections) source material (microplants, micro-and minitubers, basic clones), growing the first field generation of minitubers, and obtaining super elite potatoes. Achieving this goal also requires introducing clonal reproduction into the *in vitro* culture, which can ensure a guaranteed quality of seed material. According to scientists of the all-Russian R&D Institute of Potato Breeding, updating the *in vitro* collection no less than once in two years becomes a prerequisite for many regions of our country due to the high infectious background of carriers of viral and fungal diseases. Freeing seed material from viral and other infections requires *in vitro* source lines obtained on the basis of introducing base clones into the culture in BHPV field nurseries, which are multiplied up to the required amount during winter and spring using the method of grafting on artificial nutrient media in the laboratory conditions. They also offer making not more than 4 cycles of grafting of the original micro plants. High requirements apply to the micro plants intended for clonal propagation in the *in vitro* culture, which should be green, should have well-developed root system and leaves, and should have no less than four internodes. It is unacceptable to use non-typical varieties of plants, as well as underdeveloped (bad doers) or dried (with curved stems) plants[2,3].Given the above, we performed the research aimed at identifying the influence of various nutrient media on the growth and development of various varieties in the *in vitro* culture. One of the main factors that determines success of tissue culture is selection and preparation of the nutrient medium with regard to the effect of its main components on potato plants. Slightest inconsistencies in the nutrient medium entail significant changes in plants' ontogenesis. Moreover, varieties differ in their ability to develop in the *in vitro* culture, depending on the medium composition, i.e. various varietal reactions of plants to the nutrient medium are observed. Subsequently, this affects growth and development intensity in the in vitro plants (branching, dieback, callus formation, internodes formation, etc.), as well as the varying in wide limits survival ability of plants from the *in vitro* culture in the soil substrate[4].

Materials And Methods

Responsiveness of various potato cultivars to altered modifications of nutrition media was studied. Plants of varieties Zhukovsky Early, Aurora, Meteor, Pribrezhny, and Andra were tested. Surveys and observations were performed according to the methods of the all-Russian R&D Institute of Potato Breeding and the All-Union Academy of Agricultural Sciences[5-9]. The yield of plants from one initial

sample was determined, and further development was assessed in the laboratory. After sterilization and rinsing sprouts of potato tubers in sterile box, 100-250 μm apical meristems were extracted from them. For meristems isolation, 3-5 cm long etiolated or light green shoots were used. 100-150 μm meristems almost cannot be isolated, therefore the first leaf primordia are isolated along with them, then its size reaches 500 μm[4, 10].The regeneration ability of explants largely depended on its size. The smaller the explant was, the weaker was its ability to regenerate. The larger the explant was, the higher was its stability, but the risk of saving the virus in its cells increased[11].The results of many years of research by scientists from Russia and other countries have shown that when cultivating explants from the apical meristems of various sizes (150, 200, 250 μm), the frequency of regenerated plants formation ranged from 28 to 63%, depending on the biological features of potato varieties. In this regard, the optimal size of explants of 200 μm was practically chosen and experimentally substantiated[12].The meristem was isolated in a laminar box with microscope MBS-10 in aseptic conditions. Under the microscope, young and embryonic leaves (primordia) were pinched off by weak pressing on the needle, the 150-200 μm meristem was isolated and transferred to a test tube with the Murashige and Skoog nutrient medium with agar or with liquid medium with a supporting bridge. After isolation of each meristem, the table of the microscope was wiped clean with cotton wool soaked in alcohol; tools (needles and scalpels) were sterilized and exposed to the flame of a burner. Isolated explants from the apical meristem had been cultivated on the agar medium until 0.3-0.5 cm long sprouts were formed. They were then transplanted onto the fresh nutrient medium containing all components required for stimulating rhizogenesis and stem growth. Regenerated plants derived from the apical meristem were grown in a phytotron on special racks with 16-hour photoperiod (3000-4000 Lux), 22-25°C, and 70-80% relative humidity. Efficiency of the method of microclonal propagation largely depends on the nutrient medium which ensures the maximum yield of regenerated plants from the apical meristem. As a rule, for cultivating explants and regenerated plants, the Murashige and Skoog medium is used. The main role is played by the optimal ratio and concentration of the growth regulators introduced into the nutrient medium[10].Today there are many improved models of nutrient media. The nutrient medium for cultivating the apical meristem is somewhat different from the medium for growing plants after internodes' grafting. To facilitate the nutrient medium preparation, growth solutions are prepared beforehand and stored in a refrigerator. Reproduction of plants and their adaptation to the *in vitro* conditions requires a medium that would ensure stem growth and plants' rooting. As a rule, it has simpler composition, does not contain phytohormones, or contains auxin in a small amount. Undoubtedly, the use of minimal media causes plants' hardening against adverse environmental factors, and promotes their normal growth when planted in the soil. In this regard, it is necessary at this stage to reduce auxin and sucrose concentration without cytokinins. The content of mineral salts is the same as in the basic nutrient medium[2, 11].The studied explants of plants of various potato cultivars react differently to the nutrient medium, and studying the features of each biological object on specific nutrient mediums of *in vitro* plants is a necessary element in assessment and propagation of the initial samples. The most important controlling factor of the nutrient medium is sucrose. Many authors state that a significant increase in the dosage of sucrose in the nutrient medium, or its removal reduces the activity of cell division, results in retarded growth and development of plants in test tubes. For each variety, the dosage of sucrose should be chosen individually. In our experiments we used different modifications of nutrient medium to detect the reaction of the varieties to changes in the nutrition of *in vitro* plants (Table 1). The dosage of vitamins and growth regulator were changed. The most significant changes from the original nutrient medium were noted in modification 1, in which 10 mg of Ca pathotenate, 0.5 mg of folic acid, 1,000 mg of casein hydrolysate, 0.5 mg of riboflavin, 1.0 mg of biotin, and 0.015 mg of B_{12} were added.

Results And Discussion

Varieties' reaction to the changes in the nutrient medium varies. Thus, varieties Zhukovsky Early, Aurora, Meteor, and Pribrezhny on the original nutrient medium ensured the maximum plant growth, while variety Andra grew well on nutrient environment in modification 1, and reached the height of 13.2 cm (Figure 1).Equally important for in vitro growth and development of plants is rooting of cuttings (Table 2). Thus, variety Pribrezhny had weak root system with the minimum number of roots in all variants of the experiment. Roots' formation lagged by 1-2 days. In modifications 2 and 3, their very weak growth was noted. In our opinion, this variety is sensitive to the conditions of reproduction when the culture is introduced into *in vitro,* requires finding the optimal nutritional medium that would ensure high reproduction rate. In modification 2, all varieties, except for Andra, formed small root system (6.6-7.2 pcs/plant) with delayed start of their growth. In our case, this figure changed a little, depending on the composition of the nutrient medium. All varieties, except for Andra, formed the minimum number of roots on the nutrient medium of modification 2.

Table 1: Composition Of The Basic Nutrient Media Used For Cultivating Plants From Meristems And Cuttings, Mg/L

Main ingredients	The Murashige-Skoog medium (orig.)	Modification of media for cultivating plants from meristems and cuttings		
	0	1	2	3
Macrosalts				
NH_4NO_3	1,650	1,650	1,650	1,650
KNO_3	1,900	1,900	1,900	1,900
Ca $Ce_2 \times 2H_2O$	440	440	440	440
$MgSO_4 \times 7H_2O$	370	370	370	370
KH_2PO_4	170	170	170	170
Na_2 EDTA	37.3	37.3	37.3	37.3
$FeSO_4 \times 7H_2O$	27.8	27.8	27.8	27.8
Microsalts				
H_3BO_3	6.2	6.2	6.2	6.2
$MnSO_4 \times 4H_2O$	22.3	22.3	22.3	22.3
$ZnSO_4 \times 4H_2O$	8.6	8.6	8.6	8.6
KJ	0.83	0.75	0.75	0.83
$CuSO_4 \times 5H_2O$	0.025	0.025	0.025	0.025
$Na_2MoO_4 \times 2H_2O$	0.25	0.25	0.25	0.25
$CoCl_2 \times 6H_2O$	0.025	0.025	0.025	0.025
Vitamins				
Meso-inosite	100	100	-	-
Nicotinic acid	0.5	2.0	-	-
Pyridoxine	0.5	1.0	1.0	1.0
Thiamine	1.0	1.0	0.2	1.6
Ascorbic acid	-	-	-	3.0
Ca pantothenate	-	10.0	-	-
Sucrose	30,000	30,000	30,000	30,000
Casein hydrolysate	1,000	-	-	-
Folic acid	-	0.5	-	-
Riboflavin	-	0.5	-	-
Biotin	-	1.0	-	-
B_{12}	-	0.015	-	-
Growth regulators				
GK	1.0	2.0	-	2.0
Kynetin	0.01	0.5	0.04	0.5
Indoleacetic acid	2.0	-	1.0	-
Adenine	-	40.0	-	0.5
Ferulic acid	-	-	0.02	-
Agar	10,000	7,000	7,000	7,000
Activated charcoal	-	10,000	-	-

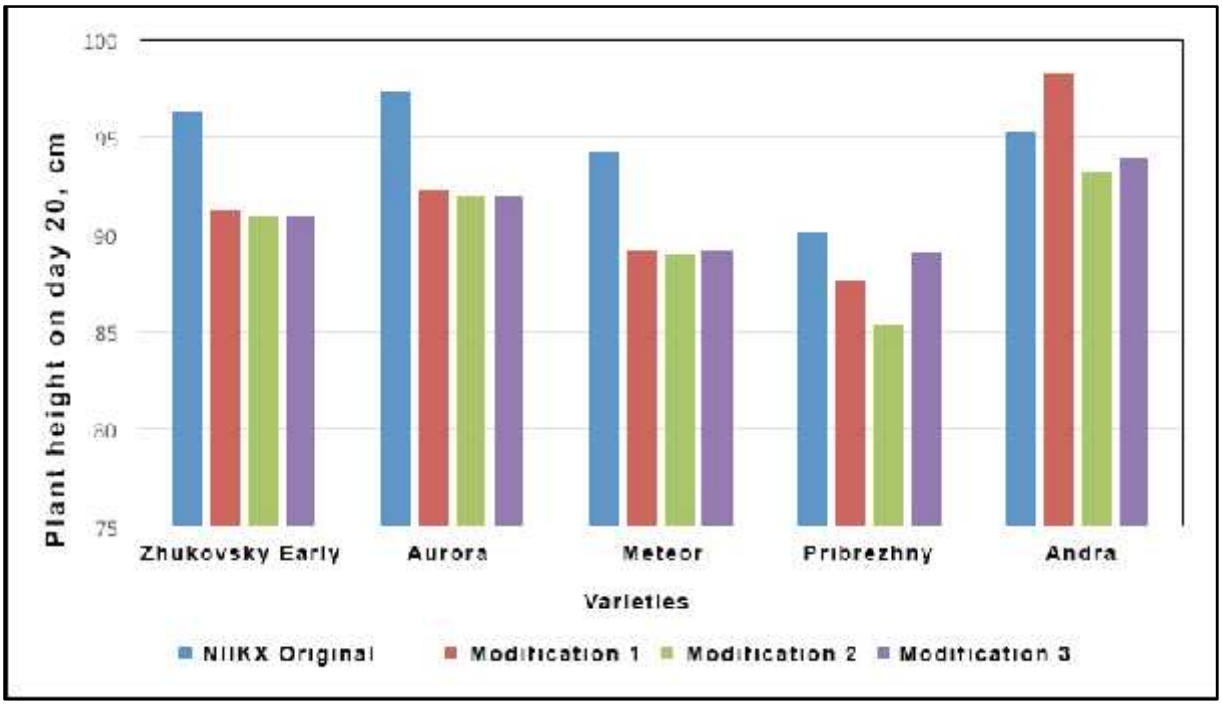

Fig. 1: Growth And Development Of Plants In Vitro On Substrates Of Various Modifications (Average For 2014-2016)

Table 2: Development Of *In Vitro* Plants' Root System On Substrates Of Various Modifications (Average For 2014-2016)

Medium	Varieties				
	Zhukovsky Early	Aurora	Meteor	Pribrezhny	Andra
R&D Institute of Potato Breeding original	+ + +	+ + +	+ + +	+ +	+ + +
Modification 1	+ + +	+ + +	+ + +	+ +	+ + +
Modification 2	+ +	+ +	+ +	+	+ + +
Modification 3	+ + +	+ +	+ + +	+	+ + +

One of the most important indicators in production of in vitro plants is the number of internodes and the yield of cuttings from each plant, i.e. the reproduction rate. The results of our research show that the number of internodes and the multiplication factor in most cases coincided, except for the periods when the plants were over or under-grown (Figure 2). Therefore, in terms of growth intensity and in vitro plant development, varieties Zhukovsky Early and Aurora grew with the interval of 1-2 days on the original nutrient medium developed at the all-Russian R&D Institute of Potato Breeding, on day 18 to 20. Varieties Meteor and Pribrezhny grew slower, reaching the shape required for grafting only on day 21-22. For variety Andra, the most favorable nutrient medium was modification 1 with addition of vitamins and growth regulators. Growth of plants of the studied varieties in other studied modifications (2, 3, 4) of the nutrient media was lower than in the original solution and modification 1.

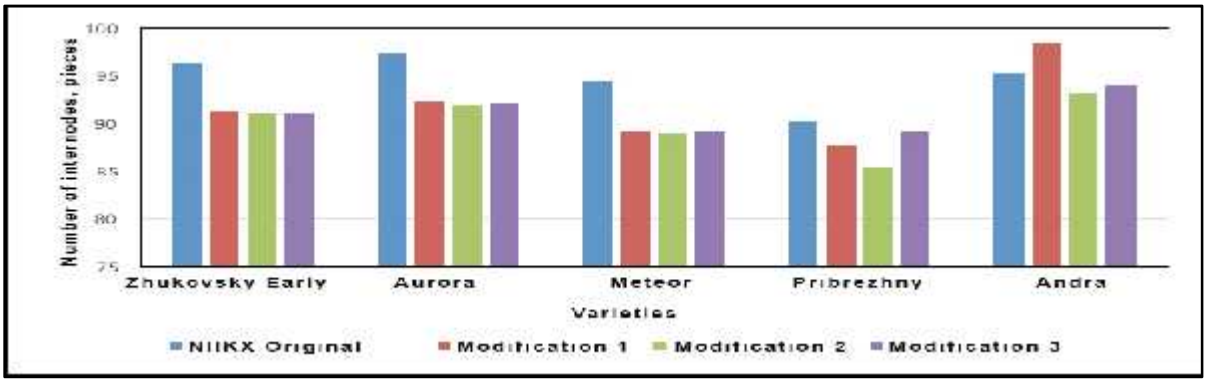

FIG. 2. Growth And Development Of *In Vitro* Plants' Internodes On Substrates Of Various Modifications (Average For 2014-2016)

Final evaluation of the ability of plants of potato varieties to regrow, according to our research, should be the 20th day of passage, since the intensity of the variety propagation in case of further grafting depends on the number of internodes with leaves, that is, on roots' strength and the container in which the plants are grown. Thus, it has been discovered that plant height and the number of internodes are not always directly proportional, as there have been changes in varieties and studied variants. Small adjustments were made during the years of study as well. The process of *in vitro* plant adaptation to the conditions of germination in various soil and climatic conditions when growing minitubers is quite difficult. It is compounded when the cheapest method of growing minitubers is used, i.e. when the in vitro plants are grown in open soil, rather than in the greenhouse. In our conditions, plants from test tubes were planted into pots in a stationary greenhouse of the Gorsky SAU. In the conditions of the greenhouse, deviations ranged from 1.7 % for variety Andra to 14.7% for vatietyPribrezhny, depending on the time of planting, varietal peculiarities, and years of research. Plants planted in cloudy weather, or during the evening hours, had better surviving ability than those planted in the morning, and did not require additional costs for sheltering from direct sunlight. There were deviations over years, but they were insignificant. The survival ability of plants in early spring and spring was much higher, due to the physiological and morphological peculiarities of *in vitro* plants. The survival ability of in vitro plants was high, and varied depending on the time of planting, temperature conditions during planting; there were insignificant deviations over the years of study (Figure 3).Mean annual data about *in vitro* plants' survival ability show that for varieties Zhukovsky Early, Aurora, Meteor, Pribrezhny and Andra, the maximum values were ensured by the original nutrient medium developed at the all-Russian R&D Institute of Potato Breeding. For variety Andra, high survival rate was noted in the nutrient medium of modification 1, which ensured the highest percentage of 98.3%. The minimum survival ability was observed for variety Pribrezhny in the variant with modification 2, which was 85.3%.

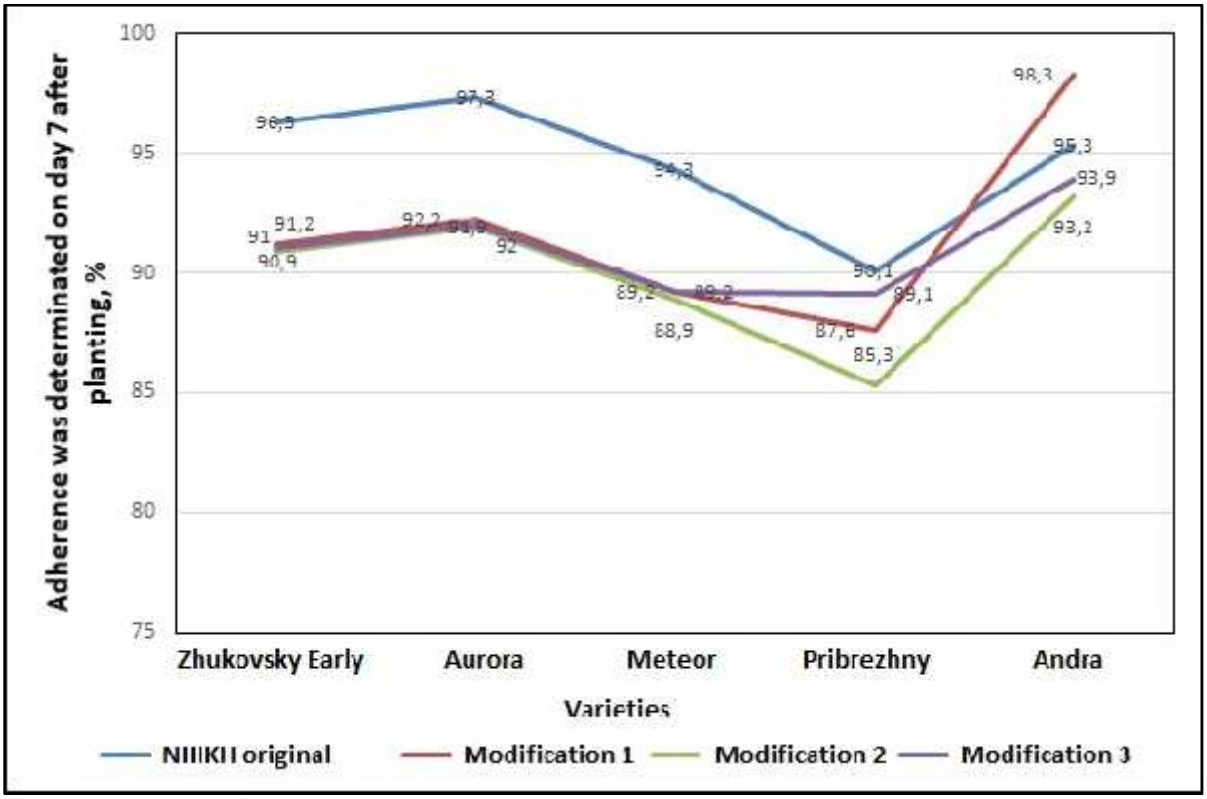

Fig. 3. The Survival Ability Of In Vitro Plants, Depending On The Nutritional Environment (Average For 2014-2016)

Conclusion

Assessment of the influence of various nutrient media on growth and development of plants of various varieties showed that the initial medium of the R&D Institute of Potato Breeding ensured the maximum growth and survival ability of plants of varieties Zhukovsky Early, Aurora, Meteor and Pribrezhny, and the nutritional medium modification 1 – of variety Andra. The maximum values were ensured by the original nutrient medium developed at the all-Russian R&D Institute of Potato Breeding. For variety Andra, the high survival rate was noted in the nutrient medium of modification 1, which ensured the highest percentage of 98.3%. The minimum

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