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Peculiarities of original potato seed breeding in high mountain conditions

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Abstract.
By the results of research it has been established that in the greenhouse conditions, different numbers of minitubers in pots were obtained from improved meristem material of in vitro plants of five varieties of Russian and foreign breeding. Variety Meteor provided the maximum number of tubers - 10.3 tubers/container on the original nutrient medium, which exceeded the yield of tubers in the remaining varieties by 0.6 to 3 tubers/container.
Keywords: primary seed breeding, adaptability of varieties and hybrids, in vitro, microtubers, potato diseases, productivity.

INTRODUCTION

Out of the studied nutrient media, the original medium was outstanding for most varieties and modifications of variety Andra. The highest survival rate was observed when they had been planted from test tubes into pots (98%) for variety Udacha, and the maximum survival rate - for variety Impala (100%). Plants' survival rate in the second and third variants ranged between 88% and 99%. The most productive were potatoes planted from pots into the ground, when the number of large and medium-size tubers was higher than in the other two variants. Starch, dry matter and vitamin C content was good for variety Udacha, but worse than for variety Impala.

The main goal of potato seeds' production is obtaining recovered tubers' material free of pathogens that ensures high growth energy and productivity. The main method of accelerated spread of healthy potato seed material in Russia is micrografting of plants in artificial nutrient media. With that, the biotechnological methods based on the *in vitro* apical meristem culture remain important, both in obtaining high-quality seed micro- and minitubers, and in rapid reproduction of promising and released potato varieties. The existing recommendations in the technology of growing revitalized material are largely contradictory. Therefore, improving the methods of shortening the cultivation period of new varieties and reducing the costs of selection, and development of new technologies for producing high quality original seed potatoes are urgent for improving efficiency of the breeding process.

The purpose of the study was identifying the features of potato genotypes' morphological development for obtaining healthy original material, and increasing high-quality yield of seeds in the original seed breeding in the North Caucasus region.

The research program included the following tasks:

- determining the optimum composition of the nutrient medium for increasing the number of microtubers;
- identifying the most efficient way of obtaining the source potato material for the conditions of protected soil;
- studying the effect of various nutritional medium compositions on formation of the morphological structures of microplants in the in vitro culture;
- assessing the quantitative and qualitative yield of standard seed material in original seed breeding nurseries, depending on the environmental factors;

It is known that in countries with developed potato breeding, including Russia, diagnostics of seed potato viral infections in the laboratory is mainly performed by means of EIA (Enzyme immunoassay). This method has high selectivity and sensitivity that allow to quantify the viruses in the extract in concentrations up to 1 ng/ml.

The most important advantage of this method is its high productivity, which makes it possible to analyze tens or hundreds of samples in a short time (2 days). Immune-specific reactants, diagnostic kits and instruments for EIA are made by many companies, and are widely available in the market [1].

Since the early 90s, a serious decline in potato yield has been observed in Russia, which had been caused by the wide spread of the potato spindle tuber viroid (PSTV), which affected most varieties. Currently in some regions of Russia, the disease caused by the viroid is considered to be the most commonly observed on potatoes and the most dangerous economically. PSTV is a small (about 360 nucleotides) circular RNA molecule with highly developed secondary structure. Viroids do not encode proteins; therefore they cannot be detected by EIA. Viroid infection of potato may be determined by the methods based on molecular hybridization of nucleic acids. Usually, the diagnostic forms for detecting viroids use the method of molecular hybridization with RNA probes marked with Digoxigenin or PCR [2].

MATERIALS AND METHODS

In the conditions of continental climate of the South of Russia, the most malicious is the PVY virus that causes potato degeneration, especially in mixed infections with PVX. Therefore, this work was mainly aimed at choosing and searching for sources of immunity to viruses PVY and PVX [3].

The symptoms of viral, viroid, and phytoplasmous (VVP) diseases on the foliage are grouped in 4 types:

- growth retardation;
- discoloration of various organs;
- changes in the shape and size of leaves, fruits, flowers; and
- necrotic infestations.

Visually, infestation of potato plants with viral diseases was determined according to Zykin A. G., with viruses in a latent form - by the improved method of buffer solution, and with the use of ammonium sulfate; EIA - virus-free potato seed breeding.

Resistance of potato varieties to late blight on foliage and tubers in the field was assessed by the 9-point scale.

The percentage content of starch and dry matter in the tubers was determined by the gravimetric method of Gantsyn and Makunin.

Browning of the pulp of raw and cooked tubers was assessed by the method of assessing suitability of the varieties for industrial processing and storage (guidelines for assessing potato varieties for their suitability for processing and storage, the all-Russian R&D Institute of Potato Breeding, 2008). In accordance with the guidelines, 70 varieties and interspecies hybrid of potatoes of domestic and foreign breeding of research institutes of the Russian Federation and 12 foreign countries were studied in 2010 and 2011 [4].

Starch content in tubers determines the consumer and technological quality, therefore, selection is performed in all

directions of breeding. In Russia, high starch content is traditionally associated with good taste tubers, their crispness and mealiness. Therefore, the best starch content in table varieties is considered to be 12-17%. However, unstable weather conditions and the short vegetation season, which are characteristic for the mountains and foothills of the North Caucasus, very often reduce these indicators by 2-3% on the average [1].

Our research was also performed by the combination of economically valuable attributes, such as assessing the biochemical composition of tubers of various varieties and hybrids of the source material, their productivity, resistance to viral and fungal diseases, the degree of raw and cooked tubers' darkening.

RESULTS AND DISCUSSION

The results of research have shown that in the greenhouse conditions, different numbers of minitubers in pots were obtained from improved meristem material of in vitro plants of five varieties of Russian and foreign breeding. The data in Table 1 show that variety Meteor provided the maximum number of tubers - 10.3 tubers/container on the original nutrient medium, which exceeded the yield of tubers in remaining varieties by 0.6 to 3 tubers/container. It has been shown that it is difficult to select any variety by this indicator. Therefore, no direct relationship had been detected between the nutritional environment and tubers' formation, except for variety Andra, which formed the maximum overall and standard yield of minitubers on the nutrient medium of modification 1 in all replications and during all years of research.

The minimum number of tubers was formed by variety Pribrezhny due to its varietal characteristics. Therefore, different nutritional environment should be searched for it, to which this variety will be adopted, which will contribute to a higher yield of grafts and tubers as the most important indicators of elite seed breeding.

The indicators of forming the overall number of seed minitubers on the average over the years of research may be considered satisfactory. Out of the studied nutrient media, one can outline the original medium for most varieties, and modification 1 for variety Andra, the other nutrient media may be studied for identifying their reaction to other varieties.

Reproduction of improved varieties with introduction of primary seed breeding into the scheme, and transferring them into production is rather slow. It is expedient to apply different methods of accelerated reproduction, which allow obtaining high rates of reproduction of the original tubers in covered soil. In this regard, further breeding and the reproduction of minitubers had been transferred to covered soil.

In our research, we also studied the following methods of growing seedlings: 1 - from in vitro plants planted directly into the soil; 2 - from in vitro plants planted as seedlings into the soil; and 3 - from in vitro plants planted into pots (Table 2).

It has been found that on the average among the varieties, the highest plants' survival rate was shown in the first variant (from test tubes into the pots) – 98% for variety Udacha, with the maximum for variety Impala (100%). Plants' survival rate in the second and third variants ranged on the average between 88% and 99%.

It has been found that in variant 2, where the plants were planted from pots into the soil (like seedlings), intensive growth of potatoes started 2-3 days earlier. Here the plants were planted into the soil with a clod of earth, which accelerated their survival rate, compared to variant 3 (from test tubes into the soil). The phase of flowering and tuber formation also occurred 4-5 days earlier, which improved the course of yield accumulation. The favorable temperature of air and soil (+18...+22^{o}C) in June and early July had positive influence on productivity in variant 2 (on the average for 2014-2017). With the relatively high growth of potato plants (Zhukovsky Early – 76.8 cm, Golubizna – 84.1 cm, Impala – 86.1 cm, Udacha - 96.8 cm), most foliage was formed in variant 1 - 355.6; 428.1; 438.1; and 555.6 g/bush, respectively (Table 3).

Table 1. The yield of minitubers depending on the method of *in vitro* plants' cultivation, 2014-2017

Indicators	Medium	Varieties				
		Zhukovsky Early	Aurora	Meteor	Pribrezhny	Andra
1. The overall yield of minitubers, tubers/container	NIIKH original	10.1	10.3	11.5	8.6	10.6
	Modification-1	8.2	9.7	10.5	8.1	12.4
	Modification-2	8.7	9.1	9.2	7.3	10.2
	Modification-3	9.1	9.4	10.2	7.9	10.5
2. The yield of standard tubers, tubers/container	NIIKH original	9.0	9.2	10.3	7.3	9.6
	Modification-1	7.1	8.6	8.4	7.0	11.2
	Modification-2	7.6	8.0	8.1	6.2	9.6
	Modification-3	8.0	8.3	9.0	6.8	8.9

Table 2. Plants' survival rate (%) from *in vitro* to *in vivo,* 2014-2017

Variety	Variants	Years of research				Average for 2014-2017
		2014	2015	2016	2017	
Zhukovsky Early	1	97	99	99	100	99
	2	96	100	98	90	96
	3	88	84	85	87	86
Golubizna	1	94	98	96	92	95
	2	93	98	92	96	95
	3	84	73	79	84	80
Impala	1	99	100	100	100	100
	2	97	100	99	96	98
	3	89	88	88	87	88
Udacha	1	98	100	97	98	98
	2	99	100	99	99	99
	3	88	87	88	89	88

Table 3. Elements of the structure of potato minitubers' yield obtained from revitalized material (on the average for 2014-2017)

Variety	Variants	Foliage weight, g/bush	Plant height, cm	Number of tubers, pcs.				Tubers' weight, g/bush
				small	medium	large	Total	
Zhukovsky Early	1	355.6	76.8	3.9	1.8	1.8	7.5	174.7
	2	300.6	61.8	1.3	6.6	5.3	13.2	470.6
	3	287.3	57.9	3.7	4.4	1.6	9.7	322.9
LSD_{05}								24.4
Golubizna	1	428.1	84,1	2.2	1.3	1.7	5.2	132.4
	2	402.6	63.2	1.2	4.6	3.9	9.7	372.5
	3	366.7	57.4	2.4	3.1	1.4	6.9	285.0
LSD_{05}								31.2
Impala	1	438.1	86.1	3.2	2.3	1.8	7.3	194.1
	2	412.6	65.2	4.3	7.6	5.6	17.5	490.3
	3	376.7	59.4	3.4	4.1	1.6	9.1	342.5
LSD_{05}								21.1
Udacha	1	555.6	96.8	3.4	2.8	1.9	8.1	184.5
	2	400.6	81.8	2.2	5.6	4.0	11.8	480.7
	3	387.3	77.9	3.7	4.4	1.6	9.7	332.2
LSD_{05}								28.3

Note: 1. Potato plants from test tubes into pots; 2. Plants from pots directly into the soil (like seedling); 3. Plants from test tubes into the soil.

Table 4. Quality indicators of the tubers obtained *in vitro* in the mountains of North Ossetia-Alania (on the average for 2014-2017)

Variety	**Variants**	**Yield, t/ha**	**Quality indicators**		
			starch, %	**dry matter, %**	**vitamin C, mg. %**
Zhukovsky Early	1	8.2	10.8	19.2	19.5
	2	22.1	11.1	18.6	19.1
	3	15.1	11.1	18.6	19.1
LSD_{05}		2,75			
Golubizna	1	6.3	14.1	22.0	20.9
	2	17.8	14.0	21.9	21.4
	3	13.4	13.8	22.2	20.8
LSD_{05}		1,45			
Impala	1	9.3	11.6	20.1	20.6
	2	23.5	12.0	19.9	20.9
	3	16.5	12.2	21.1	20.7
LSD_{05}		1.36			
Udacha	1	8.9	13.5	21.7	21.8
	2	23.1	13.9	21.3	22.5
	3	15.9	12.9	20.2	21.7
$HCP_{05\ t/ha}$		1.89			

Over the years of the research, virtually no plants affected by diseases were found. Only in variant 2 several plants were partially affected by late blight in 2017, which did not have any significant effect on tuber yield formation for variety Zhukovsky Early. Since potato plants had been obtained based on the apical meristem, not a single plant infected by viruses was found during the vegetation season in determining latent forms by the EIA method.

The research has shown that in variant 2, where the plants had been planted from pots into the soil (like seedlings), intensive growth of potatoes started 2-3 days earlier than in other variants. It should be outlined that the plants were planted into the soil with a clod of earth, which accelerated their survival rate, compared to variant 3 (from test tubes into the soil). The phase of flowering and tuber formation also occurred 4-5 days earlier, which improved the course of yield accumulation. The favorable temperature of air and soil (+18...+22^{o}C) in June and early July had positive influence on productivity in variant 2 (on the average for the year of research between 2014 and 2017).

Analysis of the obtained data shows that the most productive were potato plants from pots into the soil. In this variant, the maximum number of large and medium-sized tubers was noted, compared to other two variants (6.6 and 5.3 pcs. for variety Zhukovsky Early; 4,6 and 3,9 pcs. – for variety Golubizna; 7.6 and 5.6 pcs. – for variety Impala; 5.6 and 4.0 pcs. – for variety Udacha, respectively); the weight of tubers per bush was also higher on the average for the four years of research (470.6; 372.5; 490.3; 480.7 g/bush, respectively). Differences among the varieties in favor of variety Impala were found, including those for indicators such as the total number of tubers, the number of large tubers, and their weight per bush.

Tuber quality, its gustative advantages are judged by the content of starch and dry matter, which depend on a number of factors – primarily the varietal characteristics, the climatic conditions, the dosage of fertilizers introduced, maturity of tubers, etc. There is correlation between dry matter and starch content in tubers, which has been found by many authors - with starch content increasing, accumulation of dry matter increases, too.

Analyzing the productivity of potato tubers (Table.4), one can note that it was higher than in the second variant and

reached 23.5 t/ha for variety Impala. The lowest yield was observed in variant 1 (Zhukovsky Early – 8.2 t/ha, Golubizna – 6.3 t/ha, Impala - 9.3 t/ha, and Udacha – 8.9 t/ha, which was respectively 13.9; 11.5; 14.2; 14.2 t/ha below those in variant 2).

In terms of quality (content of starch, dry matter, and vitamin C), the differences among the variants were present, but they were insignificant. In general, these indicators were somewhat better for variety Udacha, but it was inferior to variety Impala in terms of the yield.

CONCLUSION

1. The optimum method of planting in vitro plants adapted to the environmental conditions that influence formation of morphological and structural parameters of potato microplants has been found.
2. In the mountain area of the North Caucasus, the seedling method of growing in vitro plants ensures the maximum tuber yield of varieties: Zhukovsky Early - 22.1 t/ha., Impala - 23.5 t/ha, Udacha - 23.1 t/ha.
3. The technology of increasing the weight of minitubers due to the seedling method of planting revitalized in vitro plants has been proposed.

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