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Efficiency of regenerating potato varieties by the apical meristem method

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Abstract.

This paper presents the results of scientific research in 2013-2016 on studying growth, development, yield and seed output of virus-free potato varieties with various ripening. In the zone of risky agriculture of the Western Siberia steppe, 40 varieties of the world's collection of potato gene pool were comprehensively studied. Potatoes regenerated by the method of apical meristem were used as planting material. In the conditions of field tests, parameters of the maximum leaf area of disease-free plants were determined; they were the highest in varieties Santé (Netherlands), Adretta (Germany) and Svitanok kievsky (Ukraine). The average leaf area was the lowest in varieties Moskovsky Rassvet (Russia), and the largest in varieties Adretta and Santé. Parameters of photosynthetic capacity (PSC) of regenerated potatoes were assessed. A number of varieties from the world's collection (Concord and Santé) had higher indicators than Svitanok kievsky. It was shown that in terms of productivity of virus-free plants, PSC and the average leaf area, varieties Santé and Rosary (Germany) were noted. In terms of the yield of regenerated varieties of potatoes, varieties Tuleevski, Rosara, Khoziaushka (Russia), Santé and Adretta stood out. The highest dry matter content was found in the tubers of varieties Santé, Svitanok kievsky and Adretta. In terms of total sugars, varieties Svitanok kievsky and Prominent were dominating. Vitamin C was contained in the highest amounts in varieties Svitanok kievsky and Rosimunda (the Netherlands). It was established that the content of nitrates in the yield was below the MPC for the studied crop. It was statistically shown that the greatest effect on the yield was made by the genotype, followed by the weather conditions and interaction of factors. Efficiency of regenerating potato varieties and the residual affection with viruses was shown. It was shown that potato planting material regenerated from viruses increased the yield of potato seeds by 42-58%. In the conditions of hydroponics, efficiency of adding macro- and microelements and a mixture of growth regulators Quartazine and Lime into the traditional nutrient medium was noted.

Key words: potato varieties, disease-free seed breeding, apical meristem, multiplication factor, viruses, PSC diagnostics, efficiency of regeneration, growth regulators.

INTRODUCTION

Potato is one of the most important agricultural crops. Every year, each Russian citizen should consume 126 kg of potatoes [1; 2]. However, in some regions of Russia, consumption is below this norm. According to Siberian scientists, the main objective of developing the potato industry of Siberia is the use of energy-saving technologies of potato cultivation. Despite the high productivity potential of modern potato varieties at the level of 8 to 9 t/ha, the actual yield in recent years in the conditions of Siberia has been only 1.2-1.8 t/ha. One of the reasons for the low yield is the extreme lack of high-quality regenerated planting material of new zoned and promising potato varieties. In this regard, accelerated multiplication of seed material regenerated by apical meristem method is required [3; 4; 5; 6; 7; 8; 9]. However, potatoes are highly susceptible to diseases of various nature: viroses, mycoses, bacterial diseases, and many other [10; 11].

Currently there are more than 100 pathogens known that affect potatoes, which differ in harmfulness and the amount of economic damage. [12; 13; 14]. Loss of potatoes from development of pests and diseases, according to the estimates of international experts of UNESCO, is 30 to 50% and more [15]. Potato infectious diseases are widespread, the trend of increasing their harm is observed in the areas of potato cultivation [16].

The severity of viral diseases is determined by deterioration of the processes of plants growth and development, and significant reduction of the yield perimeters and quality of tubers [15].

In this regard, particularly relevant is rapid reproduction of disease-free planting material of potato varieties with various ripeness group based on regular diagnostics of infestation with viruses.

METHOD

The research was performed in 2013-2016 at the leached black soil of experimental plots of educational-experimental farm (EEF) "Praktik" of the underlying economy of federal state educational institution of higher education (FSEI HE) "Novosibirsk State Agrarian University" located in the Novosibirsk district of the Novosibirsk region (Russia). The soil in the experimental plots was characterized by the average content of humus of 6.25%, gross nitrogen of 0.22%, phosphorus of 0.21%, and potassium of 0.05%. Easy hydrolysable nitrogen content was about 11.2 mg/100 g, mobile phosphorus - 16.7 mg/100 g, and exchangeable potassium - 14.9 mg per 100 g of soil at pH of salt extract of 5.82.

The meteorological conditions during the experiment varied, which allowed objective assessment of the obtained data. In terms of the temperature and humidity, the most favorable conditions were in 2016 (the sum of precipitation during the growing season was 376 mm). The accounting area of experimental plots was 25 m^2, the experiment was repeated four times, and the arrangement of plots was randomized.

Phenological phases of potatoes were determined by the method of Gosortoset (State variety testing of agricultural crops), the dynamics of leaf area growth were studied at the age of 20, 40, 50 days from the date of mass germination, and before harvesting, in 20 plants of each variant. The leaf area was calculated by the regression formulas based on methods of N.F. Konyaev [17]. Photosynthetic potential of plantings was determined according to A. A. Nichiporovich [18]. Plants infestation with diseases, preservation rate of tubers in the period of long-term storage was determined by the method of VNIIKH (All-Russian Research Institute of Potato Management) [19]. This study was focused on 40 varieties of potatoes, and the DNA extracted from the tissue of frozen leaves of experimental samples taken in the phase of new leaf formation (50-100 mg per sample).

The presence of the Y virus in potato (Potatovirus Y, 241 bp); X virus in potato (Potatovirus X 167 bp); S virus in potato (Potatovirus S, 278 bp); M virus in potato (Potatovirus MD 60 bp) was qualitatively determined with the use of diagnostic kits "Agro Diagnostica".

The reaction was performed three times for each sample, 4 samples of each variety.

Selection and homogenization, extraction of nucleic acid from the testes sample were performed in a box of biological safety class II.

The total DNA from the studied samples was isolated using reactants "PROBA-NK" (for samples of plant material during tests for viruses and viroids). Concentration and purification of DNA was checked at a spectrophotometer and by electrophoresis in 1% agar gel with the use of the standard DNA fragments' length tokens of 100-1000-3000 pairs of nucleotides.

25 µl of the reaction mixture volume contained 50 ng of total DNA; the PCR program was as follows:

1. Potato virus XD 67 bp
2. Potato virus S, 278 bp
3. Potato virus M, 160bp
4. Potato virus Y, 241 bp.

Denaturation lasted for 2 minutes at 94 °C, then 40 cycles 25 seconds each at 93 °C, 25 seconds at 58 °C, and 60 seconds at 72 °C; followed by final elongation for 5 minutes at 72 °C.

The products of implications were separated during electrophoresis in 2.5% agarose gel in tois-acetate buffer (TAE) at the voltage of 80 V for 30 min.

The chemical composition of marketable tubers was determined at the Common Use Center of analytical researches at the Novosibirsk State Agrarian University according to the following methods: dry matter - by drying, starch - polarographically by Evers, sugar - by Bertrand, vitamin C - by Murray, nitrates - by the ion-selective method.

During the growing season, in addition to PCR diagnostic, the plants were regularly checked for viruses using the EIA method with the use of the diagnostic kits of the Scientific Research Institute of Potato Growing. The results of the experiments were processed by the method of dispersion, correlation and regression according to B. A. Dospekhov [20].

RESULTS

In 2013-2016, on the leached black soil of the Northern forest-steppe of the Novosibirsk Priobye, comprehensive study of 40 varieties of world collection of the potato gene pool was performed. The planting material that had been regenerated by the apical meristem method was used. Growth characteristics and development of the regenerated varieties of potatoes were assessed.

It was shown that the varieties had various parameters of the photosynthetic apparatus (Table 1).

Table 1. Photosynthetic parameters and productivity of solutions for potato. Average over 2013-2016

Variant (variety)	Leaf area/ thousand m^2 /ha		PCP, thousand m^2 d./ha	Productivity		
				t/1 thousand m^2 of leaves	g/m^2 a day for	
	maximum	average			PCP	The average leaf area
1	2	3	4	5	6	7
Svitanok kievsky (st)	36.2	21.6	2,376	1.51	35.4	35.6
Fresco	34.6	18.5	1,782	1.84	34.2	34.2
Moskovsky Rassvet	29.8	15.6	1,654	1.83	32.6	31.2
Anosta	30.5	17.2	1,685	1.58	29.8	27.6
Udacha	32.4	20.0	1,910	1.52	33.5	32.4
Zhukov's anniversary	31.6	19.6	2,117	1.59	30.2	28.5
Zekura	30.8	18.4	1,822	1.60	29.5	27.3
Colorit	29.6	17.2	1,634	1.63	27.8	25.6
Vestnik	27.5	18.0	1,926	1.76	28.1	27.2
Filatovsky	29.8	19.3	2,093	1.44	29.3	27.0
Rosamunda	32.4	20.6	2,184	1.69	31.2	29.4
Lazarus	33.2	21.8	2,420	1.48	30.6	28.6
Prominent	34.0	22.3	2,140	0.96	27.4	25.3
Santé	37.6	24.8	2,778	1.46	39.5	36.5
Snegir	28.5	19.3	1,969	1.44	28.4	25.8
Atlantic	30.1	19.8	2,079	1.63	34.2	32.6
Latona	31.2	21.6	2,419	1.00	32.6	31.9
Van Gogh	28.6	19.3	1,936	0.69	30.1	28.5
I. Gold	26.5	16.5	1,815	1.64	26.2	25.3
Kuznechanka	29.4	17.6	1,814	1.68	31.6	29.6
Khozyaushka	32.7	20.1	2,332	1.91	34.8	32.4
Beloyarskiy ranniy	34.2	22.4	2,081	1.10	30.1	29.1
Timo	30.6	21.2	2,035	1.12	32.6	30.5
Autumn	29.8	19.6	1,960	1.41	30.2	28.7
Rozara	35.9	22.8	2,536	1.76	38.4	35.1
Tambovskiy	33.6	20.7	2,401	1.29	27.6	25.3
Karatop	34.2	22.7	2,247	1.38	30.5	27.4
Granola	28.6	19.6	2,254	1.40	28.2	26.2
Cardinal	33.4	21.3	2,548	1.64	34.6	31.8
Lasunok	34.9	22.1	2,666	1.34	32.8	30.2
Concord	35.6	23.8	2,875	1.27	31.2	29.6
Bor	28.1	20.1	2,271	1.18	27.6	25.1
Zhavoronok	26.7	18.6	1,879	1.37	25.2	23.8
Cordia	28.2	19.2	2,016	1.26	24.8	22.6
Lazurit	29.6	20.1	2,062	1.35	28.6	23.4
Tuleevsky	35.8	22.6	2,356	1.85	34.9	29.9
Mavrykovna	33.1	21.4	2,093	1.20	28.6	24.5
Adretta	36.5	24.8	2,505	1.49	35.8	32.6
Gatchinsky	32.6	22.6	2,622	1.39	32.4	30.7
Vytok	30.2	20.4	1,992	1.25	27.1	25.8
MSD_{05}	1.13	1.76	43.6	0.18	1.85	1.57

The maximum leaf area of disease-free plants was the highest in varieties Santé (37.6 thousand m /ha), Adretta (36.5) and in standard Svitanok kievsky (36.2 thousand m /ha). Fluctuations of the average leaf area ranged between 15.6 thousand m/ha for variety Moskovsky Rassvet and 24.8 thousand m^2/ha for varieties Adretta and Santé, with 21.6 thousand m^2/ha for the standard (V% = 18.7 with p = 800). The maximum parameters of the photosynthetic potential of regenerated potato were identified in varieties Concord - 2,875 thousand m days/ha, and Santé - 2,778 thousand m days/ha, which was 1.2 times higher than that of the standard (Svitanok kievsky). Economic productivity of leaves for the standard variety was 1.51 t/1 thousand m of leaves, and in variety Khozyaushka it increased by 29%, in variety Tuleevski – by 26 %, and in variety Fresco- by 25%. By the productivity of virus-free plants in terms of PCP and the average leaf area, varieties Santé and Rosary (veraciously 16% higher than the standard) were the best.

The yield indicators of the potato varieties regenerated with the use of the apical meristem method were significantly higher than those of the standard –Svitanok kievsky - for varieties Tuleevski by 26%, Rozara - by 24%, Khozyaushka - by 19%, Adretta - by 8% and Santé - by 12%. Other varieties had the yield equal to and significantly lower than the value of the standard (34.8 t/ha). The maximum tubers' marketability was in varieties Fresco, Svitanok kievsky (standard), Tuleevski, Santé and Adretta (all at the level of 90% and higher). By the content of dry matter, Santé (24%), Svitanok Kiev (23.8%) and Adretta (23.6%) were outstanding. Sugar content was higher in varieties Svitanok kievsky and Prominent. In terms of vitamin C content, the best were varieties Svitanok kievsky, Colorit and Rosamunda (above 7 mg/100 g). In all studied varieties, the content of nitrates in the product was 2.3 to 6 times lower than the MPC (maximum permissible concentration). The least amount of nitrates was in tubers of varieties Karatop - 37 mg/kg, and Adretta - 39 mg/kg. The maximum nitrate content was noted in varieties Mavrykovna and I. Gold - 138 mg/kg each (Table 2).

Table 2 - Productivity, quality and seed yield of potato varieties regenerated with the apical meristem method (the average over 2013 - 2016)

Variety	Productivity		Content (% in terms of raw substance)					seed yield,%	multiplication coefficient
	t/ha	increase to the standard,%	dry matter	starch	total sugars	vitamin C, mg/100g	nitrates, mg/kg		
Svitanok kievsky (st)	34.8	-	24.9	23.8	13.6	7.24	78	96	1:29
Fresco	37.2	+7	25.6	22.6	1.15	6.36	62	98	1:32
Moskovsky Rassvet	26.4	-24	24.1	14.3	1.20	5.24	102	79	1:8
Anosta	25.2	-27	23.8	12.6	1.30	6.12	114	78	1:12
Udacha	31.5	-8	24.0	16.2	1.14	5.86	70	85	1:19
Zhukov's anniversary	29.5	-14	24.2	15.7	1.23	5.14	96	76	1:15
Zekura	27.2	-22	24.5	14.9	0.90	6.72	104	80	1:13
Colorit	25.4	-27	24.0	13.6	0.96	7.12	78	75	1:9
Vestnik	30.0	-13	24.8	16.8	1.20	6.38	62	87	1:18
Filatovsky	23.6	-4	24.6	17.1	1.10	6.86	80	69	1:10
Rosamunda	32.8	-5	24.8	18.3	1.16	7.03	76	82	1:15
Lazarus	30.6	-12	24.5	15.6	1.08	6.92	62	80	1:17
Prominent	21.5	-38	24.3	12.7	1.32	6.58	112	82	1:7
Santé	38.9	+12	25.8	24.0	0.85	6.93	78	95	1:25
Snegir	26.4	-24	23.9	20.1	1.05	5.72	60	84	1:9
Atlantic	29.5	-15	24.5	18.6	0.90	6.16	12.6	85	1:12
Latona	20.1	-42	24.3	17.8	0.78	6.30	110	79	1:14
Van Gogh	18.6	-48	24.0	13.4	1.15	5.82	126	67	1:7
I. Gold	24.3	-31	23.8	12.6	1.10	5.74	138	70	1:8
Kuznechanka	33.8	-4	24.5	16.2	1.02	6.43	42	88	1:15
Khozyaushka	41.5	+19	24.8	19.6	1.12	6.27	43	90	1:20
Beloyarskiy ranniy	20.6	-40	24.2	14.2	1.23	6.10	87	75	1:9
Timo	30.2	-13	24.5	17.3	0.87	5.96	72	76	1:17
Autumn	23.4	-27	24.6	15.6	0.90	6.46	65	69	1:10
Rozara	43.2	+24	24.7	19.4	0.95	6.83	43	87	1:20
Tambovskiy	22.6	-35	24.3	14.6	0.86	5.78	78	70	1:9
Karatop	32.6	-6	24.4	16.2	1.03	6.26	37	78	1:12
Granola	25.1	-28	23.7	14.8	1.18	6.10	86	68	1:7
Cardinal	34.8	0	25.2	21.4	1.02	6.43	48	90	1:17
Lasunok	27.2	-22	25.1	20.9	0.95	6.32	54	83	1:15
Concord	29.6	-14	25.0	20.2	1.08	6.18	60	70	1:7
Bor	20.1	-32	23.6	14.3	1.23	5.36	95	68	1:5
Zhavoronok	22.4	-29	23.8	12.6	0.78	5.40	127	71	1:4
Cordia	21.6	-30	23.9	14.0	0.80	5.72	136	70	1:7
Lazurit	25.6	-26	24.2	15.3	1.12	6.10	116	84	1:9
Tuleevsky	43.7	+26	24.6	20.5	1.15	6.43	51	93	1:20
Mavrykovna	22.7	-34	24.1	15.2	0.86	5.78	138	68	1:6
Adretta	37.4	+8	25.4	23.6	1.03	6.29	39	90	1:26
Gatchinsky	30.2	-13	24.5	20.1	0.84	6.10	78	84	1:15
Vytok	22.1	-36	24.0	15.3	0.72	5.72	106	70	1:7
MSD_{05}	-	-	0.12	0.27	0.17	0.37	15.6	1.59	-

Note. The results of analysis of variance of the two-factor experiment (4.0 x 3) in terms of productivity of HCP_05 for private differences was 1.58 t, for factor A (variety) - 1,25 t, HCP_{05} for factor B (year) and for interaction AB was 1.52 t. The main effects and interactions: factor A (variety) – 46.9%, B (year) – 30.8%, AB – 19.6%.

It was shown that the seed yield was higher in varieties Fresko - 98%, Svitanok kievsky – 96%, Santé - 95%, and Adretta – 90%. Low seed yield was characteristic of varieties Filatovsky, Van Gogh, Granola and Concord. The rate of planting material reproduction varied from 1:6 for variety Bor to 1:32 for variety Fresco. This indicator was also high at the level of 1:25 in varieties Adretta, 1:23 - in Rosara, 1:18 - for the standard (variety Svitanok kievsky).

The use of analysis of variance of the two-factor experiment helped to identify the share of working factors on the overall yield. The most significant influence was that of the genotype - 47%, followed by the conditions of the year-31%, and the interaction of factors - 20%.

According to PCR diagnostics, a number of varieties - Fresco, Moskovsky Rassvet, Anosta, Van Gogh, Kuznechanka, Beloyarskiy ranniy, Colorit, Bor, Lazurit - had viruses Y, M, X, S. It was only in variety Adretta that these viruses were completely absent (Table 3).

Table 3 - Contamination of varieties of regenerated potato with viruses (elite) according to PCR diagnostics

Variety	Viruses			
	Y	M	X	S
Svitanok kievsky (st)	+	+	-	-
Fresco	+	+	+	+
Moskovsky Rassvet	+	+	+	+
Anosta	+	+	+	+
Udacha	+	+	+	-
Zhukov's anniversary	+	-	-	+
Zekura	+	-	+	+
Colorit	+	+	+	+
Vestnik	+	+	+	+
Filatovsky	+	-	+	+
Rosamunda	+	+	+	+
Lazarus	+	+	+	-
Prominent	+	-	+	-
Santé	+	+	+	+
Snegir	+	-	+	+
Atlantic	+	+	+	+
Latona	+	+	-	+
Van Gogh	+	+	+	+
I. Gold	+	-	+	+
Kuznechanka	+	+	+	+
Khozyaushka	+	-	+	+
Beloyarskiy ranniy	+	+	+	+
Timo	+	+	+	+
Autumn	+	+	+	+
Rozara	+	-	+	+
Tambovskiy	+	+	+	+
Karatop	+	+	+	+
Granola	+	-	+	+
Cardinal	+	-	+	+
Lasunok	+	+	+	-
Concord	+	+	+	-
Bor	+	+	+	+
Zhavoronok	+	-	+	+
Cordia	+	+	-	-
Lazurit	+	+	+	+
Tuleevsky	+	+	-	+
Mavrykovna	-	+	-	-
Adretta	-	-	-	-
Gatchinsky	+	+	+	-
Vytok	+	+	-	-

In the experiments of 2013-2016 performed at the industrial hydroponic installation "Potato tree 10" with the addition of micro - and macroelements and a mixture of growth regulators - 0.001%-nogovitzin and 0.001% of Lime into the traditional medium, up to 29 minitubers of early variety Udacha (24 minitubers in the reference without growth regulators), 25 minitubers of medium early variety Adretta and 34 minitubers of medium early variety Tuleevski (compared to 27 in the reference) were obtained from a single hole (with 17 in the reference).

DISCUSSION

The problem of increasing potato resistance to viruses is very urgent. In the extreme conditions of Siberia, this problem is particularly important. Against the background of low yields due to unfavorable environmental factors combined with insufficient product quality, special attention should be paid to choosing the varieties that are most adapted to the extreme local conditions along with development of an accelerated scheme of virus-free seed breeding. It has been found that such varieties are, in particular, German varieties Adretta and Rosary, Dutch varieties Santé and Fresco, and Siberian varieties Tuleevsky and Khozyaushka. Along with stress-resistance, these varieties have a set of economic-valuable characteristics such as high productivity, good quality of tubers, apparent resistance to diseases and certain pests, and preservation during long-term storage. By resistance to viral diseases (virus Y, M, X, S), variety Adretta stood out.

CONCLUSION

The planting material of potato varieties regenerated from viruses, when grown in open soil, ensures high rates of the leaf surface development, has high photosynthetic potential; its varieties have high yield capacity of 43.7 t/ha for Tuleevsky, 43.2 t/ha for Rozara, 37.4 t/ha for Adretta, combined with good quality of tubers and the nitrate content up to 6 times lower than MPC.

Introduction of growth regulators - Quartzite (0.001% solution) and 0.001% solution of Lime - into the nutrient medium when growing by the hydroponic method enhances the yield of virus-free minitubers of the varieties of various ripeness groups on the average of 32%.

The highest reproduction coefficient was identified for Dutch varieties: Fresko - 1:32 and Santé - 1:25; German varieties: Adretta - 1:25 and Rosara - 1:23; and Siberian varieties: Tuleevsky and Khozyaushka - 1:20. According to the PCR diagnostics, viruses Y M, X S were not found in variety Adretta.

The use of the hydroponic installation "Potato tree 10" has proved the efficiency of using the traditional mix of macro- and micronutrients with addition into the nutrient medium of growth regulators - 0.001% Lime preparation and their mixtures - which promote increased survival rate of meristem in vitro plants by 21%, formation of well-developed leaf surface to the level of photosynthetic capacity up to 2,400 thousand m^2 days/ha, and increase in the number of tubers per plant on the average by 32% up to 34 pcs in the mid-season Siberian variety Tuleevsky.

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