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ON USING DATA FROM MARKER-ASSISTED SELECTION OF SOURCE MATERIAL AND INTERVARIETAL HYBRIDS IN PRACTICAL POTATO BREEDING

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Abstract

The success of breeding research is in many ways determined by the successful selection of parental forms for hybridization. In recent years, along with traditional approaches, the results of marker-assisted selection (MAS) are actively used for the selection of parental lines, in order to combine valuable alleles of parental genotypes. Such programs are widely used for different crops in many countries, including Russia. The use of MAS is promising both at the initial stage in the selection of parental samples for crosses and at the stages of analysis of segregating hybrid populations. In this work, the selection of parental potato varieties for crosses was carried out based on the results of MAS of initial varieties with markers of *R*-genes conferring resistance to various harmful organisms as well as based on their economically valuable characters. To increase the efficiency of the selection of promising hybrid genotypes obtained in intervarietal crosses, we used an integrated approach that combines MAS with markers of *R*-genes for resistance to various diseases and pests with traditional methods for assessing economically valuable traits of hybrid populations. The resulting hybrids of three combinations (Gusar × Charoit), (Gusar × Aliy Parus), (Gusar × Sireneviy tuman) also participated in MAS with 8 markers of 6 *R*-genes conferring resistance to potato virus Y (*Ry$_{sto}$*) and potato virus X (*Rx1*), to golden (*H1*) and pale (*Gpa2*) potato cyst nematodes, and race-specific resistance to late blight (*R1*, *R3A*). Almost all the hybrids had different combinations of *R*-gene markers. To identify the allelic composition of the *R*-genes in the parental varieties, the segregation of DNA markers in each combination was analyzed, which allowed us to determine the level of heterozygosity of the marked loci in the parental varieties. Main economically valuable characters of the hybrids were also evaluated in the field trials. As a result, out of 144 hybrids, 31 genotypes were identified that have one or the other economically valuable traits (yield, marketability, shape of tubers, starch content, field resistance to late blight), and 113 hybrids were rejected. In 23 of the 31 selected hybrids, productivity varied from 600 to 1525 grams per plant. Twelve of these 23 hybrid genotypes combined relatively high productivity and marketability of tubers with various combinations of *Ry$_{sto}$*, *Rx1*, *H1*, *Gpa2*, *R1*, and *R3A* gene markers. Thus, the use of an integrated approach that combines traditional breeding methods and MAS increases the efficiency of the selection of promising genotypes with a given set of traits. The selected hybrid genotypes are of interest for further breeding aimed at creating competitive varieties with complex resistance to various pathogens and pests, including viral and nematode resistance, that will need fewer chemical treatments to protect the crop.

Keywords: potato, varieties, hybrids, valuable traits, disease resistance, *R*-genes, DNA

markers, MAS, PVY, PVX, potato viruses, *Globodera rostochiensis*, the golden potato cyst nematode, *Globodera pallida*, the pale potato cyst nematode, late blight, *Phytophthora infestans*

Plant breeders use various approaches and methods to choose pairs for hybridization which are based on ecological and geographical features, differences in productivity parameters, duration of growing phases or unequal resistance to diseases and pests, and the combining ability of parents [1, 2]. However, despite the long history of investigations aimed to improve these methods, successful hybrid combinations remain largely unpredictable [3]. In recent years, along with conventional approaches, marker assisted selection (MAS) are used to combine valuable alleles of parental genotypes. In potato breeding, their complementarity is mostly used in combination with MAS for DNA markers of different genes of plant resistance to diseases and pests [4, 5]. The combination of MAS with markers of resistance *R*-genes with common selection of hybrid genotypes by yield and other agronomical traits increases the breeding efficiency, which was shown by programs of creating nematode-resistant [6] and virus-resistant [7, 8] breeding material and selection of new promising potato clones with complex resistance to various pathogens [9-13]. Similar programs are being implemented in Russia [14-16].

Since potato cultivars are highly heterozygous tetraploid genotypes, intervarietal hybrids segregate, including by the presence/absence of DNA markers of a certain locus. Data on the inheritance of DNA markers of the dominant alleles of the resistance *R*-genes facilitate selection of promising intervarietal hybrid genotypes in the offspring. In addition, characterization of the allelic composition and heterozygosity of the marked loci in parental varieties makes it possible to predict appearance of the offspring resistant to a specific pathogen and the siaze of analyzed segregating populations [17-21]. In hybrid populations, depending on the degree of heterozygosity of the marked *R*-locus in the initial cultivar, the frequency of resistant genotypes carrying at least one dominant allele will be 100% for quadriplex (*RRRR*) or triplex (*RRRr*), 83.4% for duplex (*RRrr*), and 50% for simplex (*Rrrr*) [17].

Potato varieties with economically valuable traits registered for use in the North-West region of Russia have derived from the original multispecies hybrids [22-24]. Our earlier MAS results for these varieties [25, 26] detected different DNA markers associated with genes for resistance to the causative agent of potato wart, the most common and harmful Y and X potato viruses (PVY and PVX), markers of genes for race-specific resistance to the late blight (*Phytophthora infestans* Mont. de Bary), and markers of genes for resistance to various types of cyst nematodes, i.e., the golden potato nematode (GPN) *Globodera rostochiensis* (Wollenweber) Behrens and pale potato nematode (PPN) *G. pallida* (Stone) Behrens. The selection of genotypes resistant to these nematodes using methods of plant pathology is very difficult because of internal and external quarantine for GPN and PPN, respectively. Markers of genes for resistance to *G. pallida* revealed in breeding material is of particular value, since this nematode species has not yet been found in the Russian Federation, although there is a great danger of its introduction from the outside [27].

In this work, based on the MAS data [26], we selected parental pairs and performed a series of intervrietal crosses in order to combine SCAR markers of *R*-genes conferring resistance to various diseases and pests in one genotype. The maternal form was the cv. Gusar possessing markers of genes for PVY and GPN resistance. This variety is unsuitable as a pollinator due to the inherent cytoplasmic male sterility [26, 28]. The paternal forms in three combinations were cv. Charoit, Alyi Parus, and Sirenevyi tuman in which DNA markers of genes for resistance to PVX and to PPN were identified. In this study, segregating hybrid populations derived from these

combinations were involved in MAS to revealed promising genotypes with various combinations of markers of dominant alleles of *R*-genes conferring resistance to various diseases and pests and to search among them for clones with a complex agronomically valuable traits (productivity, marketability, flattened surface of tubers, and starchiness).

The aim of the work was to increase the efficiency of breeding with the use of an integrated approach that combines MAS and common breeding methodology to select promising intervarietal hybrids.

Materials and methods. Experiments were performed in 2017-2019 (Belogorka Leningrad Research Institute of Agriculture, Gatchinsky District, Leningrad Province).

Parental pairs used in intervarietal crosses possessed economically valuable traits (productivity, quality of tubers, and resistance to the most common diseases). The varieties were created in different years and adapted to the conditions of the North-West region of Russia (the originators are Belogorka Leningrad Research Institute of Agriculture and LLC Breeding firm LiGa, Leningrad Province) [29-31]. Data on molecular screening of potato varieties [26] performed by us earlier with DNA markers associated with *R*-genes for resistance to PVY, PVX, GPN, BKN and genes for race-specific resistance to late blight were also used to involve varieties with markers of different *R* genes in hybridization. Crossings were performed in 2017 in three combinations: Gusar × Charoit, Gusar × Alyi Parus, and Gusar × Sirenevyi tuman.

In 2018, seeds of hybrid offsprings were sown in a greenhouse followed by open-field planting of the seedlings. In July 2018, leaves of each hybrid genotype plants were collected to extract DNA, and in September 2018, tubers were collected individually from each hybrid. In the spring of 2019, based on the results of storing tubers, 144 hybrids from three combinations were selected and planted (a 2.3 m^2 8-tuber plot for each hybrid genotype). The best hybrid genotypes were selected in 2019 (an experimental field, Belogorka Leningrad Research Institute of Agriculture, Leningrad Provinceregion) by late blight resistance and resistance to viral diseases during the growing season and by a complex of economically valuable traits (productivity, presentation of tubers, uniformity of nests and tubers in the nest, content of dry matter and starch in tubers) in lab test and after harvesting.

MAS was performed for 112 out of 144 hybrid genotypes, including 27 hybrids for Gusar × Charoite combination, 30 hybrids for Gusar × Alyi Parus, and 55 hybrids for Gusar × Sirenevyi tuman hybrids.

The leaves collected from individual hybrid plants in the summer of 2018 (an experimental field, Belogorka Leningrad Research Institute of Agriculture, Leningrad Province), were fixed in liquid nitrogen and delivered to the laboratory in dry ice. Total DNA was CTAB-extracted in our proposed modification [32].

MAS of intervarietal hybrids was performed with 8 SCAR (sequence characterized amplyfied region) markers associated with six *R*-genes for resistance to viruses PVY (*Ry*$_{sto}$), PVX (*Rx1*), to cyst potato nematodes *G. rostochiensis* of pathotype Ro1 (gene *H1*), *G. pallida* of pathotypes Pa2/Pa3 (gene *Gpa2*), and with genes for race-specific resistance to late blight (*R1*, *R3a*).

PCR was run in a 20 μl reaction mixture containing 40 ng of total DNA, 1× reaction buffer (Dialat Ltd, Moscow), 2.5 mM $MgCl_2$, 0.6 mM each dNTPs, 0.25 μM forward and reverse primers and 1 unit of BioTaq-DNA polymerase (Dialat Ltd, Moscow) (a Mastercycler® nexus gradient DNA amplifier, Eppendorf, Germany). The PCR protocols and annealing temperatures generally corresponded to those given in the literature for each of the markers

used [10, 33-38]; the TouchDown function was added to the protocols as indicated below to increase the specificity.

The PCR protocols for the markers were as follows. For YES3-3A: 3 min 30 s at 94 °C; 45 s at 94 °C, 1 min at 60 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min at 72 °C (5 cycles); 40 s at 94 °C, 40 s at 55 °C, 1 min at 72 °C (35 cycles); 10 min at 72 °C (TouchDown) [34]. For YES3-3B: 3 min 30 s at 94 °C; 45 s at 94 °C, 45 s at 58 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min at 72 °C (5 cycles); 40 s at 94 °C, 40 s at 53 °C, 1 min at 72 °C (35 cycles); 10 min at 72 °C (TouchDown) [34]. For 5Rx1: 3 min 30 s at 94 °C; 45 s at 94 °C, 45 s at 55 °C, 1 min at 72 °C (35 cycles); 10 min at 72 °C (with an increased time for denaturation and annealing) [33]. For 57R: 3 min 30 s at 94 °C; 45 s at 94 °C, 1 min at 65 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min at 72 °C (5 cycles); 45 s at 94 °C, 45 s at 60 °C, 45 s at 72 °C (35 cycles); 10 min at 72 °C (TouchDown) [35]. For N195: 3 min 30 s at 94 °C; 45 s at 94 °C, 45 s at 66 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min 30 s at 72 °C (8 cycles); 30 s at 94 °C, 30 s at 58 °C, 1 min 30 s at 72 °C (35 cycles); and then 10 min at 72 °C (TouchDown) [10]. For Gpa2-2: 4 min 30 s at 94 °C; 30 s at 94 °C, 30 s at 60 °C, 1 min at 72 °C (35 cycles); 10 min at 72 °C [36]. For R1: 3 min 30 s at 94 °C; 45 s at °C, 1 min at 65 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min 30 s at 72 °C (10 cycles); 45 s at 94 °C, 45 s at 55 °C, 1 min 30 s at 72 °C (30 cycles); 10 min at 72 °C (TouchDown) [37]. For R3a: 3 min 30 s at 94 °C; 45 s 94 °C, 45 s at 68 °C with a decrease in the annealing temperature by 1 °C per cycle, 1 min 30 s at 72 °C (10 cycles); 45 s at 94 °C, 45 s at 58 °C, 1 min 30 s at 72 °C (35 cycles); 10 min at 72 °C (TouchDown) [38]. DNA of parental cultivars were positive controls and distilled water was a negative control.

Amplified DNA fragments were separated in 2% agarose gels in TBE buffer. The gels were stained with ethidium bromide, followed by visualization in transmitted UV light.

Assessed economically valuable traits of intervarietal hybrids were their productivity, marketable type of tubers, and content of dry substances and starch in tubers. The study involved 144 hybrid genotypes, for each of them the indicators for 8 plants were determined. Productivity was assessed gravimetrically, the average tuber weight, the number of tubers per plant, and the average tuber weight per nest were calculated. The number of commercial and non-commercial tubers, their weight per plant, the evenness of the nests and the number of tubers per nest were recorded. The marketability was assessed by Banadisev wet al. [39]. The contents of dry matter and starch were determined by the specific weight of tubers converted into the percentage of dry matter and starch using a special table [40]. The taste of tubers was scored according to a 9-point scale where 9 points are excellent, 1 point is bad (bitter, unpleasant) [41]. The culinary type was assigned to A (table potatoes, not boiled soft), B (poorly boiled soft), C (boiled soft), and D (highly boiled soft) as per the international classification of potato tuber table qualities [41]. The plant resistance to late blight was assessed under strong natural infection in 2019 (an experimental field, Belogorka Leningrad Research Institute of Agriculture, Leningrad Province) using a scale from 1 (whole plant affected) to 9 points (no disease symptoms).

Statistical processing of agronomic trait characteristics was performed by conventional methods [42]. Chi-squared (χ^2) test was used to estimate deviations from the theoretically expected segregation in hybrid populations (1:1 or 5:1) at different levels of heterozygosity of marked *R*-loci in the carriers of dominant alleles.

1. Characterization of parental potato varieties involved in intervarietal hybridization in 2017 (the originators are Belogorka Leningrad Research Institute of Agriculture and LLC Breeding firm LiGa, Leningrad Province)

Trait	Gusar	Alyi Parus	Sirenevyi tuman	Charoit
Markers of *R*-genes [26] for resistance:				
to viruses				
YES3-3A/YES3-3B (PVY, Ry_{sto})	+/+	–/–	–/–	–/–
5Rx1 (PVX, *Rx1*)	–	+	+	+
to late blight				
R1 (*R1*)	+	-	–	–
RT-R3a (*R3a*)	–	+	+	+
to potato cyst nematodes				
57R/N195 (golden, *H1*)	+/+	+/+	–/–	–/–
Gpa2-2 (pale, *Gpa2*)	–	+	+	+
Commercially valuable traits and diseases resistance ([lant disease estimates) [29-31]	Mid-season, the yield up to 60 t/ha, 15-19% starch, long period of tuber dormancy; resistant to golden potato nematode, potato wart, late blight, common scab, and rhizoctonia	Mid-season, the yield up to 50 t/ha, 18-23% starch, resistant to golden potato nematode, viruses, and potato wart	Mid-season, the yield up to 60 t/ha, 14-17% starch, resistant to potato wart, late blight, and viruses	Super-early potato, the yield up to 55 t/ha, resistant to potato wart, rhizoctonia, and common scab

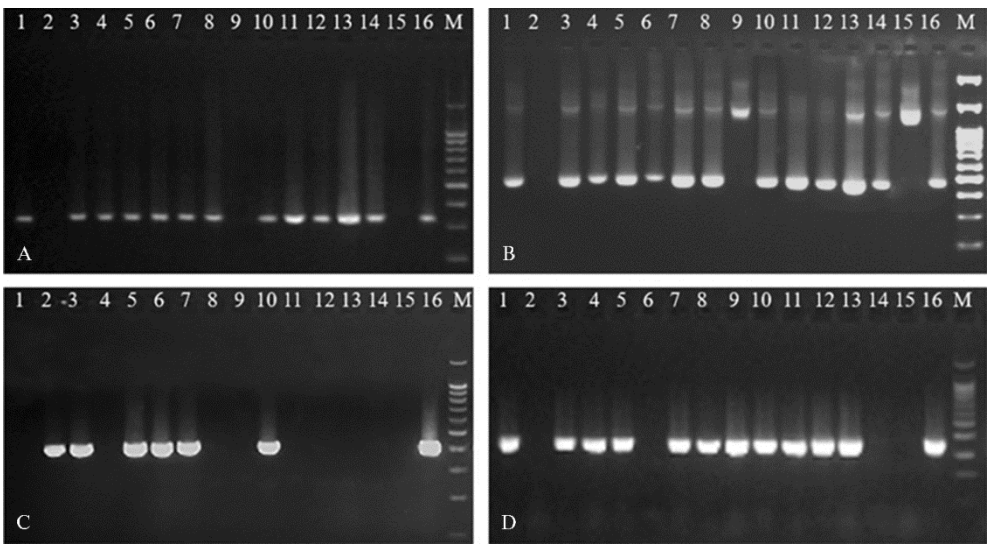
N o t e. Marker RT-R3a was detected in cv. Alyi Parus in biomaterial received directly from the originators (N.M. Gadzhiev, V.A. Lebedeva). In a plant of cv. Alyi Parus cultivar that we obtained from other sources, this marker was not previously detected [26].

Results. Table 1 shows varieties with SCAR markers of different *R*-genes involved in hybridization.

Molecular screening of intervarietal hybrids for markers of *R*-genes conferring plant resistance to diseases and pests. Three combinations of crosses produced 144 intervarietal hybrids of which 112were screened 8 SCAR markers associated with six *R*-genes, the Ry_{sto} and *Rx1* (PVY and PVX resistance), *H1* and *Gpa2* (resistance to golden and pale potato nematodes), and *R1* and *R3a* (race-specific resistance to late blight).

One or another *R*-resistance gene marker combination was characteristic of almost all 112 studied hybrid genotypes (Fig.). The only exception was 7.18-7 (Hussar × Sirenevyi tuman) in which we did not find any of the 8 markers used in MAS. Of the 112 hybrid genotypes, there were 58 hybrids with markers of two genes, the *H1* and *Gpa2*, and 42 hybrids with markers of Ry_{sto} и *Rx1*. In four of the 112 studied hybrid seedlings (5.18-9 and 5.18-17 from Gusar × Charoite and 6.18-12 and 6.18-37 from Gusar × Alyi Parus), we detected all eight markers of six resistance genes used in MAS.

Intervarietal hybrids derived from all three combinations segregated by the presence or absence of DNA markers of the six *R*-loci (Table 2), therefore, no quadriplexes or triplexes for the marked *R*-genes were identified among the initial varieties. Segregation could be attributed to two variants of crosses with different levels of heterozygosity of dominant alleles, the first one is a simplex *Rrrr* × nulliplex *rrrr* with theoretical segregation of 1 *R–*:1 *rrrr*, the second one is a duplex *RRrr* × a nulliplex with theoretical segregation of 5 *R–*:1 *rrrr*.



Screening of the intervarietal potato hybrid Gusar × Syrenevyi tuman for ДНК markers of *R*-genes: N195 (A) and 57R (B) for resistance to golden cyst potato nematode, Gpa2-2 (C) to pale potato nematode, and YES3-3a (D) to PVY; 1 — Gusar, 2 — Sirenevyi tumanман, 3 — hybryd 7.18-1, 4 — hybryd 7.18-8, 5 — hybryd 7.18-2, 6 — hybryd 7.18-9, 7 — hybryd 7.18-3, 8 — hybryd 7.18-10, 9 — hybryd 7.18-4, 10 — hybryd 7.18-11, 11 — hybryd 7.18-5, 12 — hybryd 7.18-12, 13 — hybryd 7.18-6, 14 — hybryd 7.18-13, 15 — hybryd 7.18-7, 16 — hybryd 7.18-14; M — molecular weight marker 100 bp + 1500 + 3000 (NPO SibEnzyme, Russia).

Analysis of the inheritance pattern of DNA markers in each combination (see Table 2) revealed the allelic composition of *R*-genes and heterozygosity of the marked loci in parental forms. For example, Gusar has one dominant allele (simplex) of Ry_{sto} and *R1* genes and two dominant alleles (duplex) of *H1* gene, Charoite was a simplex for *R3a* gene and duplexes for *Rx1* and *Gpa2-2* genes, Alyi

Parus had *R3a* and *H1* simplexes and duplexes for of *Gpa2* and *Rx1* genes, and Syrenevyi tuman had one dominant allele (simplixes) of *R3a*, *Rx1* and *Gpa2-2*.

2. Segregation of potato inrervarietal hybrid offspring derived from three combination of cv. Gusar crossing by DNA marker of *R*-genes conferring resistance to diseases and pests (ratio of hybrids with and without marker of a corresponding gene) (Belogorka Leningrad Research Institute of Agriculture, Leningrad Province, 2018)

Crossing	YES3-3A/YES3-3B (gene *Ry*$_{sto}$)	5Rx1 (gene *Rx1*)	57R/N195 (gene *H1*)	Gpa2-2 (gene *Gpa2*)	R1 (gene *R1*)	RT-R3a (gene *R3a*)
Gusar × Charoit	16:11[a]	22:5[b]	23:4[b]	22:5[b]	11:16[a]	10:17[a]
Gusar × Alyi Parus	15:15[a]	22:8[b]	27:3[c]	21:9[b]	15:15[a]	14:16[a]
Gusar × Syrenevyi tuman	33:22[a]	28:27[a]	46:9[b]	26:29[a]	18:37	22:33[a]

N o t e. DNA markers YES3-3A/YES3-3B (*Ry*$_{sto}$, PVY resistance), 5Rx1 (*Rx1*, PVX resistance), 57R (*H1*, resistance to pathotype Ro1 of golden potato nematode), N195 (*H1*, resistance to pathotype Ro1 of golden potato nematode), R1 (*R1*, resistance to *Phytophthora infestans*), and RT-R3a (*R3a*, resistance to *Ph. infestans*). Superscripts: a — segregation for the specified *R*-locus in the combination at p_{05} corresponds to the theoretical 1:1 for simplex × nulliplex (*Rrrr* × *rrrr*); b — segregation at p_{05} corresponds to the theoretical 5:1 for duplex × nulliplex (*RRrr* × *rrrr*); c — segregation at p_{05} corresponds to the theoretical 11:1 for duplex × simplex (*RRrr* × *Rrrr*).

Agronimical traits of the intervarietal hybrids. The best hybrid genotypes were selected in 2019 by tuber productivity, shape and marketability, field resistance to late blight and viruses, and MAS. Economically valuable traits were measured for 8 plants for each of 144 hybrid genotypes. As a result, 31 promising hybrids were detected which possess certain economically valuable traits and various combinations of *R*-gene markers. Of these, 23 hybrid genotypes showed relatively high productivity (Table 3), in the remaining 8 hybrids, productivity was low but they had high field resistance to late blight and should be involved in further crosses. The reason for the rejection of the remaining 113 hybrids was excessively long stolons or damage to plants by late blight and viruses.

In 23 selected hybrids, total tuber weigh per plant varied from 600 to 1525 g which corresponds to a yield of 24.5-62.2 t/ha; some of these hybrids were distinguished by other economically valuable traits (high marketability of tubers, good taste, starchiness) (see Table 3). For example, we note the hybrid 7.18-3 (Gusar × Syrenevyi tuman) with gene markers for resistance to PVY, PVX, and both nematodes, with high marketability of tubers and a productivity of 1350 g per plant which corresponds to a 55.1 t/ha yield (see Table 3).

The highest productivity (1525 g per plant or 62.2 t/ha) was characteristic of the hybrid 5.18-7 which also carried markers YES3-3A/3B, 5Rx1, N195, 57R, and Gpa2-2. That is, the 5.18-7 potentially has complex resistance to PVY and PVX viruses and to two types of nematodes, GPN and PPN (see Table 3).

Approximately half of hybrid genotypes with high productivity, 12 of 23, show evenness of nests and tubers in the nest, even and regular shape of tubers (Table 4).

DNA markers facilitate selection of target genotypes with dominant alleles of various *R*-genes in breeding, which is especially important when identifying clones with markers of resistance genes to quarantine objects, since assessments of plant resistance to such agents using methods of plant pathology is very complicated and laborious [27, 43]. In this work, we identified more than 40% of hybrids with markers of two genes (*H1* and *Gpa2-2*) for resistance to Ro1 pathotype of golden potato nematode and Pa2/Pa3 pathotypes of pale potato nematode. The high frequency of such hybrids is explained by the presence of duplexes of these genes in the parental varieties, the *H1* gene in the cv. Gusar and the *Gpa2-2* gene in the Alyi Parus and Charoit cultivars. Note that Ermishin et al. [20], when analyzed segregation for DNA markers in 11 intervarietal potato populations, came

3. Selection of 23 potato hybrid genotypes derived from three intervarietal for DNA markers of *R*-genes for resistance to diseases, pests and by rconomically valuable traits устойчивости к болезням и вредителям и по хозяйственно ценным признакам (an experimental field of Belogorka Leningrad Research Institute of Agriculture, Leningrad Province, 2019, 8 plants of each genotype tested)

Variety, hybrid	Productivity, g/plant (min-max)	Marketability, %	Content in tubers, %		Taste, points	Cooking type	DNA markers of genes for resistance to PVY, PVX, late blight, golden and pale potato nematodes
			starch	dry matter			
Невский (st)	700 (560-810)	92	14.2	20.0	5.5	A	RT-R3a
Ten hybrids from crossing Gusar × Charoit							
5.18-3	787 (690-855)	97	15.4	21.1	7.5	B	5Rx1, N195, 57R, Gpa2-2, RT-R3a
5.18-7^{hp}	1525 (1300-1740)	97	10.0	15.0	7.0	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2
5.18-15	643 (590-735)	98	12.9	18.6	8.0	AB	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, R1
5.18-17	600 (430-810)	95	14.2	20.0	7.0	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, R1, RT-R3a
5.18-19^{hp}	925 (780-1060)	97	11.7	17.4	5.0	A	YES3-3A/3B, 5Rx1, Gpa2-2, R1
5.18-24^{hp}	1253 (1010-1470)	94	14.7	20.4	6.0	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, RT-R3a
5.18-25	860 (620-910)	99	11.7	17.4	5.5	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, R1
5.18-27	800 (680-865)	91	13.4	19.1	6.7	A	Нет данных
5.18-32	630 (520-700)	86	14.4	20.1	6.0	A	YES3-3A/3B, 5Rx1, Gpa2-2, R1
5.18-34^{hp}	1340 (1100-1480)	94	15.7	21.5	5.5	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2,
Eight hybrids from crossing Gusar × Alyi Parus							
6.18-2	685 (583-770)	99	14.2	20.0	7.5	A	5Rx1, N195, 57R, Gpa2-2, R1, RT-R3a
6.18-9	735 (615-842)	95	15.2	21.0	6.5	A	YES3-3A/3B, N195, 57R
6.18-13^{hp}	1340 (1200-1420)	94	12.9	18.6	5.5	A	5Rx1, Gpa2-2
6.18-17	770 (630-865)	97	11.0	16.7	6.5	A	5Rx1, N195, 57R, Gpa2-2
6.18-24^{hp}	1015 (810-1360)	94	11.0	16.7	6.0	A	YES3-3A/3B, N195, 57R, RT-R3a
6.18-26^{hp}	1155 (900-1320)	88	14.2	20.0	6.0	A	YES3-3A/3B, N195, 57R, R1
6.18-36^{hp}	870 (820-945)	93	13.4	19.1	7.5	AB	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2,
6.18-42	765 (630-880)	96	14.7	20.5	6.0	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, RT-R3a
Five hybrids from crossing Gusar × Sirenevyi tuman							
7.18-3^{hp}	1350 (1210-1545)	96	11.0	16.8	5.5	A	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, RT-R3a
7.18-4	780 (660-863)	97	12.9	18.6	6.5	AB	YES3-3A/3B, R1, RT-R3a
7.18-42^{hp}	1010 (820-1220)	92	12.2	18.0	6.0	A	5Rx1, N195, 57R
7.18-55^{hp}	1255 (960-1320)	96	13.4	19.1	8.0	B	N195, 57R, R1, RT-R3a
7.18-56^{hp}	1280 (1000-1380)	91	12.2	18.0	7.0	AB	YES3-3A/3B, 5Rx1, Gpa2-2, R1, RT-R3a

Note. Cv. Nevskii (st) is a standard in assessing agronomic characteristics in the Belogorka Leningrad Research Institute of Agriculture. All markers of resistance genes were detected in hybrid 5.18-17; hp — 12 high-productive hybrid genotypes (for their additional characteristics, see Table 4).

4. Economically valuable traits of 12 high-productive hybrid genotypes (n = 8, (an experimental field of Belogorka Leningrad Research Institute of Agriculture, Leningrad Province, 2019)

Variety, hybrid	DNA markers of *R*-genes	Tuber skin color	Tuber shape	Tuber eye depth and color	Evenness, points from 9 to 1)	
					nests	tubers in nest
Невский (st)	RT-R3a	White	Ovate	Small, малиновые	8	8
5.18-7	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2	White	Ovate	Small	7	8
5.18-19	YES3-3A/3B, 5Rx1, Gpa2-2, R1	White	Elongated	Small	7	7
5.18-24	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, RT-R3a	Yellow	Ovate	Small	8	8
5.18-34	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2,	Yellow	Ovate	Small	8	8
6.18-13	5Rx1, Gpa2-2	Pink	Elongated oval	Small, crimson	8	8
6.18-24	YES3-3A/3B, N195, 57R, RT-R3a	White	Elongated oval	Small	8	8
6.18-26	YES3-3A/3B, N195, 57R, R1	Pink	Ovate	Small, crimson	8	8
6.18-36	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2,	White	Ovate	Small	7	8
7.18-3	YES3-3A/3B, 5Rx1, N195, 57R, Gpa2-2, RT-R3a	White	Rounded oval	Small	8	8
7.18-42	5Rx1, N195, 57R	Pink	Ovate	Small, crimson	8	8
7.18-55	N195, 57R, R1, RT-R3a	Pink	Ovate	Small	8	8
7.18-56	YES3-3A/3B, 5Rx1, Gpa2-2, R1, RT-R3a	Yellow	Elongated	Small	8	7

N o t e. Cv. Nevskii (st) is a standard in assessing agronomic characteristics in the Belogorka Leningrad Research Institute of Agriculture.

to the conclusion that duplexes of genes for resistance to quarantine objects are most often detected. In addition, in the cultivars Alyi Parus and Charoit, duplexes were found for the *Rx1* gene which is closely linked to the *Gpa2-2* gene [44]. Therefore, in the hybrid combinations with the participation of these parental cultivars, there was a joint inheritance of the DNA markers of genes for resistance to PPN and to PVX.

Thus, traditional methods of breeding hybrids for economically valuable traits coupled with DNA marking allow us to reveal 12 high-productive hybrid genotypes with tubers even and regular in shape and markers of genes for resistance to cyst forming nematodes. Seven of these hybrids additionally have SCAR markers of genes for resistance to potato viruses Y and X (Ry_{sto} and *Rx1*). These hybrids are of interest for creating competitive varieties resistant to different groups of pests and pathogens, including group resistance to cyst nematodes and group resistance to potato viruses.

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