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MULTIPLEX PCR-BASED IDENTIFICATION OF POTATO GENOTYPES AS DONORS IN BREEDING FOR RESISTANCE TO DISEASES AND PESTS

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Abstract

The breeding of potatoes with the traditional technology of hybridization and selection of individual plants is a time-consuming process. The use of DNA markers linked to genes underlying resistance to diseases and pests can significantly improve the efficiency of the selection of valuable genotypes in the early stages of breeding process. The aims of the work were i) screening of potato genetic resources from the VIR collection (Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg) for the presence of genes encoding resistance to cyst-forming nematodes, potato wart, potato viruses X and Y (PVX and PVY) by the multiplex PCR method; ii) evaluation of the effectiveness of molecular markers for the identification of potato genotypes resistant to the golden nematode, potato wart and PVY. A total of 90 accessions from the VIR collection were studied, among them the cultivated potatoes from two subspecies, the *S. tuberosum* subsp. *chiloense* (native varieties of Chile) and *S. tuberosum* subsp. *tuberosum* (breeding varieties), as well as hybrid clones have been distinguished as sources and donors of potato resistance to pathogens of the economically significant or quarantine diseases. In this work, several molecular markers that were early recommended for the identification of potato genes responsible for the resistance to cyst nematodes, Y and X viruses, and potato wart were first used for the multiplex PCR analysis of genetically diverse material. Ten markers used were TG 689, 57 R, N 195 of *H1* gene and Gro1-4-1 of *Gro1-4* gene (resistance to the golden nematode *Globodera rostochiensis* pathotypes Ro1, Ro4), marker Gpa 2-2 of *Gpa2* gene (resistance to the pale nematode *G. pallida* pathotype Pa2), RYSC3 marker of Ry_{adg} gene, Ry 186 marker of Ry_{chc} gene and YES3-3A marker of Ry_{sto} gene (all genes provide immunity to the potato virus Y), the PVX marker of the *Rx* gene (immunity to potato virus X) and the NL 25 marker of the *Sen1* for resistance to potato wart caused by *Synchytrium endobioticum* (Schilb.) Percival. The PCR screening results were matched with the phenotypic characteristics of the test potato genotypes for resistance to the golden nematode, wart and potato virus Y. Multiplex PCR analysis allowed us to identify potato genotypes with several (up to five) resistance genes, including those providing resistance to the nematode *G. rostochiensis* patotype Ro1, *S. endobioticum* patotype 1 and potato virus Y. A significant association was established between the molecular markers linked to the *H1* gene and the resistance of potato genotypes to the nematode *G. rostochiensis* Ro1 (r_A = 0.59, r_s = 0.72-0.79), and between the marker N L25 of *Sen1* gene and potato resistance to wart (r_A = 0.62). No association was detected between Ry_{adg} and Ry_{sto} molecular markers and plant resistance to potato virus Y due to a large number of tested resistant potato genotypes which possibly carry unknown immunity genes.

Keywords: potato, *Solanum* ssp., interspecific hybrids, DNA markers, marker assisted selection, potato wart, *Synchytrium endobioticum*, nematodes, *Globodera rostochiensis* Ro1, potato virus Y

Potato varieties are created by hybridization of pre-selected parental forms the progeny of which has a high probability of the genotypes with an optimal combination of the necessary traits [1, 2]. High heterozygosity and tetraploid nature of the forms involved in crossing (varieties and interspecific hybrids) causes a phenotypic diversity of F_1 hybrids. Segregants (potentially new varieties of potato) are evaluated according to 40-50 characters of production and quality, as well as resistance to adverse abiotic and biotic factors [3-5]. The conventional potato breeding scheme is based on an annual phenotypic assessment and selection of the best genotypes. Individual genotypes are retained by vegetative propagation in the form of clones the number of which gradually decreases with a simultaneous increase in the number of laboratory and field tests. The duration of selection from the first stage, which is related to visual assessment and selection in the F_1, until the transfer of promising clones to the state variety testing is at least 10 years [3, 4, 6]. Improvement of this process via advanced molecular techniques and the creation of new breeding technologies is one of the current priorities.

Many valuable breeding traits of potato, including resistance to pathogens and pests, e.g. buckeye rot (pathogen *Phytophthora infestans* Mont. de Bary), Potato Virus X (PVX), Potato Virus Y (PVY), Potato Virus S (PVS), Potato Leaf Roll Virus (PLRV), cyst nematodes, potato wart caused by *Synchytrium endobioticum* (Schilb.) Perc., have monogenic nature [1]. Molecular markers linked to *Rpi*-resistance to buckeye rot, the Ry_{sto}, Ry_{adg} and Ry_{chc} genes, which control immunity to PVY, markers of the *Rx1* gene, which control immunity to PVX, the *H1* and *Gro1-4* genes for resistance to the golden nematode *Globodera rostochiensis* (Wollenweber) Behrens and the *Sen1* gene for resistance to the potato wart, can be an effective tool for breeding intensification. Their use to identify valuable genotypes, including forms with several resistance genes, can significantly improve the efficiency of selection in the early steps of breeding [7-9]. The list of DNA markers that are linked to resistance genes or are fragments of potato genes for resistance to pests is constantly expanding [10-12]. A new approach to the use of DNA markers is the development of multiplex PCR analysis technology for the simultaneous testing of varieties and breeding lines for several genes that control resistance to viruses, nematodes, and buckeye rot [9, 13, 14].

Screening of varieties and parental forms for the presence of disease and pest resistance genes is important for the development of potato breeding [15-17]. A permanent source of valuable germplasm for breeders from Russia and neighboring countries is the potatoes collection of Vavilov All-Russian Institute of Plant Genetic Resources (VIR) [18-20]. Molecular genetic characterization of potatoes interspecific hybrids, which are provided to breeders as sources and donors of pest resistance traits, will allow more reasonable selection of parental pairs for crossing.

In the present paper, Chilean aboriginal potato varieties were first characterized using DNA markers of *H1*, *Gro1-4*, *Gpa2*, Ry_{sto}, Ry_{adg}, Ry_{chc}, *Rx1*, and *Sen1* genes. The results of simultaneous testing of clones of interspecific hybrids and potato varieties for several genes were obtained for the first time. The effectiveness of multiplex PCR was evaluated by comparing the results of DNA analysis of collection samples with their phenotypic characteristics for resistance to the golden nematode, potato wart, and Potato Virus Y.

The goal of the study was the molecular screening of varieties and potato breeding material from the collection of Vavilov All-Russian Institute of Plant Genetic Resources (VIR) to identify forms with genes for resistance to cyst

nematodes, potato wart, potato viruses X and Y using multiplex PCR analysis technology, as well as an assessment of effectiveness of the PCR test.

Techniques. A total of 90 accessions from the VIR potatoes collection were studied, which represent the species *Solanum tuberosum* L. and hybrid clones isolated according to a complex of breeding and economically valuable traits in the progeny resulting from interspecific crosses with wild-growing and cultivated species of the *Petota* Dumort. section of the *Solanum* L. genus. The sample studied included 9 forms of the subspecies *S. tuberosum* subsp. *chiloense* (A.DC.) Kostina (indigenous varieties from Chile) and 14 forms of the subspecies *S. tuberosum* subsp. *tuberosum* (5 domestic and 9 foreign varieties). The remaining 67 analyzed forms were clones selected in the progeny of various combinations resulting from cross-breeding or self-pollination of hybrids created on the basis of wild-growing and cultivated species, varieties or breeding lines of potatoes. They included 12 clones created in 1990-1997 by K.Z. Budin at VIR, 3 clones obtained in 2008 by V.A. Kolobayev at the All-Russian Research Institute of Plant Protection, and 52 clones selected in 1999-2011 at VIR. Among the hybrid clones, 22 were two-species hybrids, including 16 selected in combinations of *S. tuberosum* with wild-growing relatives: the endemic Bolivian species *S. alandiae* Card. (12 clones), Mexican species *S. stoloniferum* Schlecht. (2 clones) or widespread in South America *S. chacoense* Bitt. (2 clones). Another 6 clones represented progeny resulting from crossing wild-growing species *S. okadae* Hawkes et Hjerting and *S. chacoense*. The three-species hybrids included 21 clones obtained by crossing samples of cultivated potato species *S. tuberosum*, *S. andigenum* Juz. et Buk. and *S. rybinii* Juz. et Buk. or *S. phureja* Juz. et Buk. The complex multi-species hybrids included 24 clones; in their creation, varieties, breeding lines, and species of cultivated and wild potatoes (*S. acaule* Bitt., *S. stoloniferum*, *S. bulbocastanum* Dun., *S. microdontum* Bitt., *S. polytrichon* Rydb., *S. spegazzinii* Bitt. and *S. vernei* Bitt. et Wittm) were crossed.

Multiplex PCR analysis was performed to identify eight genes that control potato resistance to the most dangerous pathogens, including cyst nematodes golden nematode *G. rostochiensis* pathotype Ro1, Ro4 (*H1*, *Gro1-4* genes) and pale nematode *Globodera pallida* (Stone) Behrens pathotype Pa2 (*Gpa2* gene); PVY (Ry_{sto}, Ry_{adg} and Ry_{chc} genes) and PVX (*Rx1* gene), and potato wart pathogen *S. endobioticum* pathotype 1 (*Sen1* gene). DNA was isolated from leaves of the collection potato plants (the experimental field of the VIR Pushkin laboratories, St. Petersburg—Pushkin).

To improve the analysis efficiency, eight markers with similar amplicons sizes (RYSC3, Ry 186, YES3-3A, TG 689, 57 R, N 195, Gro 1-4-1 and Gpa2-2) were combined into one multiplex reaction. For the internal positive multiplex control, the length of the analyzed fragments in the Golubizna, Kolobok, Uladar, and Belosnezhka varieties were used as standards. Two more markers with larger amplicon sizes (NL 25 and PVX) were combined into another reaction using the length of the analyzed Meteor fragments for their control. Amplification products were sequenced (a Nanofor 05 sequencer, Institute of Analytical Instrumentation RAS, Russia). Direct primers were marked using 6FAM or 5R6G fluorescent dyes (Sintol LLC, Russia).

The amplification reaction of 8 multiplex PCR markers was performed (Applied Biosystems 2720 Thermal Cycler, ThermoFisher Scientific, USA) according to the following program: 10 min at 94 °C (1 cycle); 30 s at 94 °C, 30 s at 68 °C, 1 min at 72 °C (5 cycles); 30 s at 94 °C, 30 s at 58 °C, 1 min at 72 °C (35 cycles); 30 s at 94 °C, 5 min at 72 °C (1 cycle). The amplification reaction of two markers with larger sizes was performed using the same instrument according to the same program, but with a 30 s increase in the elongation time on

all cycles.

The resistance of *S. tuberosum* and hybrid clones to PVY was assessed by the method of artificial infection with subsequent ELISA diagnosis of viral infection. Information about the resistance of indigenous varieties from Chile was found in the paper by Kostina [22]. Data on the resistance of breeding varieties to the nematode *G. rostochiensis* (pathotype Ro1) and potato wart *S. endobioticum* (pathotype 1) were based on the characteristics of domestic varieties included in the State Register of Breeding Achievements and foreign varieties presented in the database (http://www.europotato.org). The resistance of breeding clones to the nematode was assessed in laboratory experiments at All-Russian Research Institute of Plant Protection (VIZR, St. Petersburg—Pushkin), and resistance to the potato wart was assessed during laboratory experiments at Lorch All-Russian Research Institute of Potato Farming (VNIIKH, Moscow Province) [23].

The relationship between the identified resistance of varieties and hybrid clones to PVY, nematode or potato wart, and detected DNA markers of R-genes was determined using the χ^2 test. The H_0-hypothesis was rejected if $\chi^2_F \geq \chi^2_{st} = 10.83$ for the significance level $\alpha = 0.1\%$. The strength of the relationship between the resistance of the studied potato samples to PVY, nematode or potato wart, and the detected DNA markers of R-genes was evaluated using the association coefficient r_A. The significance of the association coefficient was determined by the Student's *t*-test. The H_0-hypothesis was rejected if $t_F \geq t_{st}$ for significance level $\alpha = 0.1\%$ [24].

Results. We screened 90 potato samples using 10 DNA markers recommended for the detection of R-genes that control the resistance of varieties and selection clones to different types of cyst nematodes, potato viruses X and Y and potato wart (Table 1).

1. *R*-genes and DNA markers used for molecular screening of potato samples (*Solanum* L.) from the collection of the Vavilov All-Russian Institute of Plant Genetic Resources (VIR)

Gene	Chromosome	Trait	DNA marker (specific fragment)	Reference
Ry adg	11	Immunity to Potato Virus Y (PVY)	RYSC3 (321 bp)	[25]
Ry sto	12	Immunity to PVY	YES3-3A (341 bp)	[26]
Ry chc	7	Immunity to PVY	Ry 186 (587 bp)	[13]
H1	5	Resistance to *Globodera rostochiensis* pathotypes Ro1, Ro4	TG 689 (141 bp)	[27]
H1	5	Resistance to *G. rostochiensis* pathotypes Ro1, Ro4	57 R (452 bp)	[28]
H1	5	Resistance to *G. rostochiensis* pathotypes Ro1, Ro4	N 195 (337 bp)	[29]
Gro1-4	7	Resistance to *G. rostochiensis* pathotypes Ro1, Ro4	Gro 1-4-1 (602 bp)	[29]
Gpa2	12	Resistance to *G. pallida* pathotype Pa2	Gpa 2-2 (452 bp)	[29]
Rx	12	Immunity to potato virus X	PVX (1230 bp)	[13]
Sen 1	11	Resistance to potato wart (*Synchytrium endobioticum*)	NL 25 (1400 bp)	[30]

Screening using multiplex PCR identified genotypes with markers of the *H1* and *Gpa2* genes that control resistance to different types of cyst nematodes, the *Sen1* gene, which provides resistance to potato wart, and the *Rx* and Ry_{adg}, Ry_{sto} genes that provide immunity to Potato Viruses X and Y (Table 2). The Gro1-4-1 markers of the *Gro1-4* gene (another gene for resistance to the golden nematode *G. rostochiensis* pathotypes Ro1, Ro4) and Ry 186 of the Ry_{chc} gene (resistance to Potato Virus Y) were not detected in the studied sample.

The frequency of detecting DNA markers of genes for resistance to harmful organisms was different (see Table 2). The largest number of such markers in one genotype was detected in commercial potato varieties. Markers of five genes, the Ry_{sto}, *Rx*, *Sen1*, *Gpa2,* and *H1*, were detected in the varieties Meteor (Lorch VNIIKH) and Nur-Alem (Kazakhstan). Markers of *Sen1* and *H1* genes were detected in Nayada and Nevsky varieties, *Sen1* and Ry_{adg} in Valeriy and

2. Frequency of genotypes with DNA markers of *R*-genes for resistance to pathogens in potato samples (*Solanum* L.) of different origin

Pedigree-based group	Number of genotypes (total $n = 90$)	Frequency of gene markers							
		H1			*Gpa2*	*Sen 1*	*Ry adg*	*Ry sto*	*Rx*
		TG 689	57 R	N 195	Gpa 2-2	NL 25	RYSC3	YES3-3A	PVX
Indigenous Chilean varieties (*S. tuberosum* subsp. *chiloense*)	9	0	0.22	0	0.11	0.44	0.11	0	0
Bred varieties (*S. tuberosum* subsp. *tuberosum*)	14	0.14	0.36	0.14	0.14	0.64	0.21	0.14	0.14
Hybrids (*S. tuberosum*, *S. alandiae*)	12	0.25	0.58	0.58	0	0.42	0	0	0
Hybrids (*S. tuberosum*, *S. stoloniferum*)	2	0	0	0	0	0	0	0	0
Hybrids (*S. tuberosum, S. chacoense*)	2	1.0	1.0	1.0	0	0.50	0	0.50	0
Hybrids (*S. okadae*, *S. chacoense*)	6	0	1.0	1.0	0	0	1.0	0	0
Hybrids (*S. tuberosum*, *S. andigenum*, *S. rybinii* or *S. phureja*)	21	0.33	0.33	0.33	0	0.57	0	0.09	0
Complex multispecies hybrids	24	0.25	0.17	0.17	0.08	0.21	0.04	0	0

Udacha varieties. Among the 9 studied Chilean indigenous varieties, Frutilla had markers of Ry_{adg} and *Gpa2* genes, Amarilla redonda of *Sen1* and *H1* (57 R) genes, Magellanes and Negra of *Sen1* gene, and Negra var. infectum had the marker 57 R of *H1* gene. It should be noted that not only commercial potatoes varieties but also Chilean indigenous varieties had markers of *H1*, Ry_{adg}, and *Gpa2* genes. The source of these genes in breeding varieties is the highland Andean potato which is considered by Russian botanists as an independent species *Solanum andigenum* Juz. et Buk., and by foreign researchers as subspecies *S. tuberosum* [22]. Isolated area and a clear difference in morphological and biological characteristics from Chilean potatoes are the main arguments for determining the species status of *S. andigenum*. A possible reason for the detection of DNA markers of *S. andigenum* genes in Chilean potato samples in the VIR collection may be reproduction of some samples of this collection through seed reproduction. In this case, the progeny resulting from free (uncontrolled) self-pollination may have some genotypes of hybrid origin.

Markers of the *H1* gene were detected with a high frequency in hybrid clones selected in the first generation of *S. tuberosum* hybrids or backcrossings with an endemic Bolivian species *S. alandiae*. The marker *Sen1* was also detected in two genotypes (24-2 and 135-2-2006) of this group. Markers of the *H1* and *Sen1* genes were detected in four clones the pedigree of which contained the cultivated species *S. tuberosum*, *S. andigenum,* and *S. rybinii* or *S. phureja*, and clone 94-5 (two-species hybrid resulting from crossing Bobr × *S. chacoense*). Markers of the *H1* and Ry_{sto} genes were detected in the genotype of clone-sibling 99-10-1, *Sen1* and Ry_{sto} in the genotype of three species hybrids, *Sen1, H1* and *Gpa2* in 167-1-2008, *Sen1* and Ry_{adg} in all six two-species hybrids resulting from crossing *S. okadae* × *S. chacoense*.

3. The number of potato samples (*Solanum* L.) with identified DNA markers of resistance *R*-genes in different phenotypic classes

Marker (gene)	Phenotype		χ^2_Φ	r_A
	resistant	susceptible		
Resistance to *Synchytrium endobioticum*				
NL 25 (*Sen1*) (+)	13	7		
Nor found (–)	1	26		
Total	14	33	17.81*	0.62*
Resistance to *Globodera rostochiensis*				
TG 689 (*H1*) (+)	0	4		
57 R (*H1*) (+)	0	2		
57 R + N 195(*H1*) (+)	8	0		
TG 689+57 R + N 195 (+)	12	2		
Nor found (–)	4	27		
Total	24	35	18.53*	0.59*
Resistance to Potato Virus Y				
RYSC3 (Ry_{adg}) (+)	6	5		
YES3-3A (Ry_{sto}) (+)	3	0		
Nor found (–)	12	35		
Total	21	40	5.56	–

Note. "+" and "–" mean the presence and absence of a marker; χ^2_Φ is sample-based criterion χ^2, r_A is association coefficient. A dash means that the coefficient was not determined, since the marker-trait relationship was not established.

* The value is statistically significant at $\alpha = 0.1\%$.

Among the 47 genotypes evaluated for the resistance to *S. endobioticum* pathotype 1, 14 were resistant (Table 3). Among them, *S. tuberosum* prevailed (8 modern varieties and 3 Chilean indigenous varieties). Two clones which were derivatives of *S. alandiae* and one selected in the progeny resulting from the crossing of *S. okadae* × *S. chacoense*, were stable in two-year laboratory tests. Among the 59 genotypes evaluated for the resistance to *G. rostochiensis* pathotype Ro1, 24 were resistant (see Table 3). This group included two varieties (Meteor and Nayada) and 22 hybrid clones selected in the progeny resulting

from different combinations of two, three or more cultivated and wild *Solanum* species. Among them, 4 clones were derivatives of *S. alandiae*, 9 clones were two-species hybrids, derivatives of *S. okadae*, *S. chacoense* or *S. stoloniferum*, as well as previously created nematode resistance donors (clones 190-4, 90-7-7, 90-6-2) [21] and the progeny resulting from crossing 90-6-2 with varieties.

In studying the response of 61 potato genotypes to the PVY infection, we identified 21 samples resistant to PVY (see Table 3). The studied set contained only one PVY-resistant variety, the Meteor; the remaining breeding and indigenous samples were affected by the virus during artificial infection. Among 20 PVY-resistant hybrid clones, 9 were three-species hybrids the pedigree of which included *S. tuberosum*, *S. andigenum*, and *S. rybinii*. These are clones 90-6-2, 97-155-1, the donors of resistance to virus Y previously created at VIR [23] and the progeny resulting from their crossing with varieties. All two-species hybrids with *S. chacoense* were resistant to PVY: six clones were selected in progeny resulting from crossing with *S. okadae,* and two clones were derivatives of *S. tuberosum* × *S. chacoense*. Three complex multi-species hybrids were also resistant to the virus.

To evaluate the relationship between the presence of DNA markers and the resistance of potato varieties and hybrid clones to the nematode, potato wart and PVY, we used analyses of fourfold tables that presented the number of two phenotypic classes (resistant and susceptible samples) and groups with identified and not detected DNA markers of the corresponding resistance genes (see Table 3). The results of detecting different markers of the genes for resistance to the nematode and PVY took into account the total number (sum) of all detected markers. Assessment of the distribution of samples according to the phenotypic classes and groups using the χ^2 leads to the conclusion that there was a statistically proven relationship between the potato response to the nematode *G. rostochiensis* pathotype Ro1 and markers of the *H1* gene, as well as between the response of plants to the *S. endobioticum* pathotype 1 and marker of the *Sen1* gene. Sample χ^2 values significantly exceeded the critical ones (see Table 3), and the H_0-hypothesis was refuted at a high level of significance ($p < 0.001$). We also established a relationship between resistance to the nematode and potato wart and the detected markers of the corresponding R-genes, as evidenced by the significant association coefficients r_A (see Table 3). Among DNA markers of the *H1* gene used for molecular genetic screening, markers 57 R and N 195 had the strongest marker-trait relationship (Spearman's correlation coefficients $r_s = 0.72$ and $r_s = 0.79$).

Comparison of data on marker detection and assessment for resistance to the nematode *G. rostochiensis* Ro1 revealed false-positive results in Atzimba and Nevsky samples (marker 57 R detected), clones 160-1, 160-17, 159-31, 97-152-8 (marker TG 689) and clones 90-7-2, 39-1-2005 (all three markers of the *H1* gene). The probable reason for the presence of marker fragments amplified with primers used in potato samples affected by the nematode can be the complex structure of the *H1* locus which is characterized by the high-abundance of homologous fragments of other R-genes or genes encoding structural protein in the plant cell wall [28]. The fact that the markers of the *H1* gene were not detected in the nematode resistant clone 99-6-6 is consistent with the results of the molecular screening of the VNIIKH collection in which the markers of the *H1* gene were also not detected in four clones, including 99-6-6 [31]. Segregation in the progeny resulting from the self-pollination crossing of the clone 99-6-6 with the susceptible varieties Zagadka Pitera and Peterburgskii indicates the polygenic nature of the clone 99-6-6 resistance to the nematode [32] which explains the absence of DNA markers of the *H1* gene in PCR analysis.

In comparing data on the detection of the marker NL 25 of the *Sen1* gene and the assessment for resistance to *S. endobioticum,* false-positive results were obtained in one clone which is derivative of *S. alandiae*, four clones the pedigree of which included cultivated types of potatoes, and two multi-species hybrids. To explain the genetic nature of potato resistance to *S. endobioticum*, two models are proposed according to which the protective effect is manifested as the result of the expression of one dominant gene *Sen1* or the combined action of two dominant genes *Sen1* and *Sen1-4* localized respectively on the 11th and 4th chromosomes [30, 33]. It is possible that the differences in the efficiency of using the NL 25 marker of the *Sen1* gene for the screening of hybrid clones were associated with differences in the genetic control of the forms used as donors of resistance. False negative results of the samples resistant to *G. rostochiensis* Ro1 or to *S. endobioticum* could be obtained due to insufficient accuracy of phenotypic evaluation in laboratory tests or as a result of recombination at sites located between the marker fragment and the gene. It is also possible that other genes [7] provide resistance.

In this study, the χ^2 significance of the potato samples' distribution on the resistance to PVY and the presence of the markers of Ry_{adg} and Ry_{sto} genes was not proved due to the large number of resistant genotypes in which markers of known potato genes for PVY resistance were not detected (see Table 3). The RYSC3 marker of Ry_{adg} gene was detected in four samples susceptible to the PVY (the Chilean Frutilla form, the varieties Valeriy, Bintje, and clone 97-162-5). The recommendation to use the SCAR marker RYSC3 to identify samples with the Ry_{adg} gene was based on the results of studying 103 varieties and breeding clones, including the Bintje variety, in which the indicated marker was not initially detected [25]. Perhaps the differences in the PCR protocols led to false-positive results in the experiment. In the case of the YES3-3A marker of the Ry_{sto} gene, no false positive results were detected. This marker was detected in clone 97-155-1 resistant to virus Y and was not detected in clones 160-1 and 160-17 isolated in its progeny from self-pollination. In the experiment, we studied another donor of resistance to virus Y, the clone 90-6-2 and five clones isolated in the progeny of the 90-6-2 combination with variety Hertha or subsequent crosses. All the studied hybrid genotypes inherited the virus Y resistance, but markers recommended for identifying the *Ry* genes were not detected.

The RYSC 3 and YES 3A markers are DNA segments flanking *Ry* genes, the complete sequence of which is not yet known. Many researchers evaluated the diagnostic value of DNA markers of the Ry_{sto} and Ry_{adg} genes in PCR using a number of primers. In all experiments on screening varieties and breeding clones from different collections, as well as segregating populations, an incomplete concordance between the data of phenotypic evaluation and marker analysis was detected [34-36]. It is obvious that additional studies on the genetic nature of resistance to the virus Y in various potato forms are required, as well as the development of more advanced DNA markers of resistance genes.

For the first time in world practice, we carried out a multiplex PCR analysis of genetically diverse variety samples and selection clones of potatoes using DNA markers of eight R-genes. In the studied sample of 90 potato genotypes, the maximum number (five resistance genes) was detected in Meteor and Nur-Alem varieties. The results obtained are consistent with data from other researchers who, when studying varieties and breeding material of different origin, found no more than 5% of unique genotypes which were the potential sources of five to six resistance genes simultaneously [13, 17]. It is obvious that genotypes with a pyramid of potato genes for resistance to different pathogens are not widely spread, but their creation with purposeful breeding work seems to be an

achievable task. This is confirmed by the successful production of interspecific potato hybrids in which four to five *R*-genes for resistance to buckeye rot have been identified [37]. Molecular screening, of course, does not mean abandoning the phenotypic evaluation of breeding material, including in terms of resistance to diseases and pests. However, the use of multiplex PCR analysis to identify genotypes with several resistance genes of different specificity will accelerate the creation of potato varieties resistant to the complex of dangerous diseases and pests. The cost of multiplex PCR analysis for five markers of a single potato sample is 28 times less than the cost of assessing resistance to a single pest (cyst nematode or virus), and the economic benefit will be especially noticeable during mass analysis at the initial stages of breeding [9]. The apical meristem cultures and microclonal propagation are included in modern seed farming for healing seed potatoes from viruses. Similarly, multiplex PCR analysis should be integrated into potato breeding programs.

Thus, multiplex PCR analysis allows identification of genotypes with several (up to five) resistance genes of different specificity, including those providing resistance to the nematode *Globodera rostochiensis* Ro1, potato wart *Synchytrium endobioticum* (pathotype 1), and Potato Virus Y, among genetically diverse potato forms. A strong relationship exists between the 57 R and N 195 markers of the *H1* gene and the resistance to nematode *G. rostochiensis* Ro1 (association coefficient $r_A = 0.59$, Spearman's correlation coefficient $r_s = 0.72$-0.79) and between the NL 25 marker 5 of the *Sen1* gene and the resistance to potato wart ($r_A = 0.62$). To carry out marker-assisted selection (MAS) for Potato Virus Y resistance genes, it is necessary to study the genetic nature of the resistance of different potato forms and develop new effective DNA markers of resistance genes.

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