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Evaluation of interspecific potato breeding material with a complex of genes of immunity to Potato virus Y using molecular markers

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Abstract. The article presents the results of research on potato culture, the presence of Ry_{adg}, Ry_{sto}, and Ry_{chc} genes in complex interspecific potato hybrids, and based on the use of the DNA markers for selection of resistant forms to Potato virus Y (PVY). These genes are derived from different genetic sources of the species *Solanum andigenum, S. stoloniferum and S. chacoense,* respectively. The selected potato formswith a complex of agronomic characters are recommended for inclusion in the selection process, for creation of new varieties, as well as as a valuable source material for interspecific hybridization.

Key words: variety, source material, R-genes, assisted selection (MAS), stability.

INTRODUCTION

Development of potato varieties with extreme genetic resistance to pathogens, especially the Potato virus Y (PVY), is associated with a number of difficulties. In particular, during the hybridization of tetraploid forms in hybrid populations, a wide variation and a complex nature of economically valuable characteristics splitting is observed, which ultimately complicates the selection of genotypes with the desired characteristics due to the need to develop sufficiently large amount of hybrid potato seedlings.

Many methods are labor-intensive enough, long (they take up the entire growing season), and not accurate enough. The solution of this problem in improving the potato breeding efficiency is the use of the method of molecular marker of genes (*R*-genes) of resistance to pests and diseases (Barone, 2004; Kilchevsky & Khotyleva, 2014; Ermishin et al., 2016).

The study of potato DNA markers is associated with the application and introduction into the breeding process of a wide range of genetic material of wild and cultivated forms of potatoes, with genes of resistance to pathogens (Hawkes & Jackson, 1992; Jansky, 2000; Gebhardt & Valkonen, 2001).

An important area of molecular markers application is marker assisted selection (MAS), which is widely introduced into breeding practice. DNA markers are successfully used both at the stage of initial sources selection for hybridization, and at the subsequent analysis of the hybrid material and the resulting variety. DNA-markerof economically valuable characteristics allows them to be involved in MAS, which is designed to provide higher efficiency, lower cost and shorter duration of obtaining new varieties and hybrids compared to traditional methods of selection (Watanabe & Peloquin, 1991; Shamshin & Porotnikova, 2014).

The availability of specific information about certain genes present in potato allows optimizing the selection of parental forms and predicting the probability of seedling selection in hybrid populations with economically valuable characteristics (Ermishin et al., 2016). The use of molecular technologies makes it possible to significantly simplify the pyramiding of genes for resistance to one pathogen, but originating from different sources (Solomon-Blackburn & Barker, 2001; Vales, 2010; Szajko et al., 2014). Currently, there are more than 10 known genes that provide resistance to PVY, some of which are cloned or mapped (Solomon-Blackburn & Barker, 2001; Barone, 2004; Flis, 2005; Sato, 2006; Szajko et al., 2014; Grech-Baran et al., 2020). Some of them are hypersensitive resistance (HR) genes involving programmed cell death (HR), which are isolated in some wild species and in various varieties of *Solanum tuberosum*. These genes provide resistance to only some of the currently known PVY strains (Tian & Vakonen, 2013). The genes of extreme resistance (ER) - are of the greatest interest and value for breeding. It is known that the ER genes - Ry_{adg}, and Ry_{chc} were mapped on chromosomes XI, XII, respectively. The recent study found that Ry_{sto} is associated with the TIR-NLR immune receptor (Grech-Baran, 2020).

Potato is one of the crops with the most fully mapped genome (350 markers that characterize about 90% of the genome) (Gebhardt & Valkonen, 2001; Gebchardt, & Vreugdenhil, 2007). According to a number of authors, it is possible to increase the potato resistance to PVY and ensure its resistance to a wide range of strains by using ER genes in breeding and combining them in a single genotype (Solomon-Blackburn & Barker, 2001; Vales, 2010; Tian & Vakonen, 2013).

Thus, the use of DNA markers makes it possible to simplify the selection of valuable samples. The efficiency of selection increases due to a decrease in the sample size (based on the presence of economically valuable characteristic), therefore, only samples with identified loci that are characteristic of this species and are of interest for selection.

The purpose of this research was to identify the genes originating from different types of potatoes in complex interspecific potato hybrids, to isolate genotypes with a complex of different genes that provide resistance to PVY with a complex of agronomic characters.

CONDITIONS, MATERIALS AND METHODS

Experimental work was carried out in the Selection and Seed Center of Potato Growing of the Ural SRIA− Branch of the FSBSU UrFARC UrB RAS, city of Yekaterinburg, within the framework of the scientific and technical program on the assignment of Exploratory research carried out by the contractor-organizations within the framework of the CPSR Development of potato breeding and seed growing. The

object of the study was a complex interspecific hybrids of collection nursery potato and collection nurseries variety samples, created in the Selection and Seed Center of Potato Growing of the Ural SRIA-Branch of the FSBSU UrFARC UrB RAS, in total 189 samples were analyzed. Field experiments were laid in the second half of May on the experimental field of selective crop rotation. The predecessor is a cropped fallow. The soil is soddy-medium podzolic with the following agrochemical characteristics: humus content (GOST 26213-91) - 4.86%; salt pH (GOST 26483-85) - 5.68; total absorbed bases −20.0 m–eq. 100 g^{-1}; easily hydrolyzed nitrogen– 124 mg kg^{-1}; mobile phosphorus (GOST 26207-91) - 360 mg kg^{-1} and potassium exchange (GOST 26207-91) - 136 mg kg^{-1} of soil (Vasiliev & Gorbunov, 2020) nutrient status - $N_{60}P_{60}K_{90}$.

The evaluation of selection and source material for the presence of DNA markers were carried out in the PCR laboratory of the Selection and Seed Center of Potato Growing of the Ural SRIA - Branch of the FSBSU UrFARC UrB RAS. In our research, we used genetic material from potato species: *Solanum tuberosum* sbsp. *andigenum*, *S. stoloniferum and S. chacoense.* Based on reference data (Kasai et al., 2000; Flis, 2005; Song & Schwarzfischer, 2008; Mori et al., 2011), to evaluate the source material of potato, intended for creating varieties with ER to PVY, DNA markers of resistance genes were selected, which are most reliable and technological in use: $RYSC3_{321}$, $YES3\text{-}3A_{341}$, Ry186 (Table 1).

Table 1. Markers characteristics of *R*-genes of potato resistance to PVY used in the work

Gene	Character	Marker	Primers (5' – 3')	Source
Ry_{adg}	Resistance to PVY	$RYSC3_{321}$	F – ATACACTCATCTAAATTTGATGG R – AGGATATACGGCATCATTTTTCCGA	Kasai, 2000
Ry_{sto}	ER to PVY	$YES3\text{-}3A_{341}$	F – TAACTCAAGCGGAATAACCC R – AATTCACCTGTTTACATGCTTCTTGTG	Song & Schwarzfischer, 2008
Ry_{chc}	Resistance to PVY	Ry186	F –TGGTAGGGATATTTTCCTTAGA R –GCAAATCCTAGGTTATCAACTCA	Mori, 2011

F – forward primer; R – rewers primer.

A set of Synthol reagents was used to isolate plant DNA. DNA extraction protocol is standard for Syntols set. The crushed potato leaves are transferred into microcentrifuge 2 mL tubes. Added 800 µl of lysis solution and 15 µl of proteinase-K to each tube and then mixed the solution. The tubes were incubated for 30 min. at 60 °C, occasionally mixed on a shaker. After that, the tubes were cooled for 5 min. at room temperature (RT) and centrifuged for 5 min. at 13,000 rpm. During that, a precipitation reagent was prepared in new tubes, 200 µl of sorbent buffer and 40 µl of sorbent. 200 µl of supernatant was gently transferred to new tubes with precipitation reagent. Incubated for 10 min. at RT. Centrifuged for 1 min. at 7,000 rpm. The supernatant was removed. Added 300 µl of washing solution A to the precipitate and mixed. Centrifuged for 30 sec. at 7,000 rpm. The supernatant was removed. Added 500 µl of wash solution B to the precipitate, mixed and centrifuged for 30 sec. at 7,000 rpm. The supernatant was removed. This step was repeated two times. Opened tubes were placed in a thermostat at a 60 °C until the liquid had completely evaporated. Added 200 µl of TE-buffer to the dry precipitate, mixed on a shaker. Incubated for 5 min. at 60 °C. The suspension was centrifuged for 2 min. at 13,000 rpm. 150 µl supernatant with DNA was transferred into new 0.2 µl tubes.

The protocol for DNA isolation is standard. Under the same amplification conditions, the reaction was set up separately for each marker. The reaction mixture for PCR with 12 mL primers contained: 2 µl of DNA, 1 µl of standard buffer for Taq-polymerase, 0.3 µl of dNTP, 0.4 µl of $MgCl_2$, 0.25 µl of each primer, 0.1 µl of Taq-polymerase, and 8.3 µl of H_2O. Amplification was performed in the mode: 94 °C - 3 min.; 35 cycles: 92 °C - 45 sec., 60 °C - 45 sec., 72 °C - 1 min; 72 °C - 10 min.

Based on the results of amplification and subsequent electrophoresis in 1.5% agarose gel, all results are displayed on PC screen.

RESEARCH RESULT

In the field conditions of 2018–2019, an assessment of complex interspecific genotypes by a complex of characteristics was made. During the growing season, interspecific potato hybrids were evaluated for resistance to viral diseases, in particular to PVY against a natural infectious background. During the budding-flowering phase, leaf samples were collected for testing for the presence of viruses and for the presence of DNA markers of resistance to PVY. When diagnosing the disease pathogen by PCR analysis, it was found that in the studied samples, PVY was present in only nine hybrids, this is 4.8%. A high occurrence was noted for the spread of the Potato virus M (PVM) - 32.1%. The number of samples with Potato virus X (PVX), Potato virus A (PVA), and Potato Leafroll virus (PLRV) viruses was low - 0.2–1.3%.

The screening of the potato gene pool and breeding material was performed using three (RYSC3321, YES3-3A341, Ry186) practically applicable markers of PVY resistance genes. The selective value of hybrids and varieties with resistance to PVY increases if several resistance genes from different potato species are present. Dominant alleles of the Ryadg, Rysto, and Rychc genes in complex interspecific hybrids were identified by DNA markers. According to research data, 32% of hybrids with DNA markers for three PVY resistance genes were isolated from the total number of studied samples (Table 2).

Table 2. Characteristic of the best potato samples with a complex of agronomic characters, in the genotype of which percense all three molecular markers of resistance to Potato virus Y, 2018–2019

Variety sample	Yield (t ha^{-1})	Starch content (%)	Resistance to leaf blight, points
05-15-15	32.2	16.5	8.0
05-15-40	34.7	15.2	7.0
05-15-7	42.9	18.7	8.5
06-11-1	32.9	11.2	5.0
08-10-1	31.2	16.0	5.0
08-20-2	29.3	15.8	7.0
08-41-5	35.5	11.0	7.0
08-41-7	32.0	10.9	5.0
10-11-24	34.4	14.8	7.0
10-22-21	32.1	14.2	8.0
10-22-7	36.7	10.0	7.0
10-54-5	29.8	14.7	8.0
10-54-9	30.0	14.8	8.0
10-9-3	33.7	15.4	5.0
12-12-8	31.7	13.2	5.0
12-22-47	31.2	12.2	5.0
12-22-66	30.2	12.0	5.0
13-41-13	36.9	16.5	8.5
12-29-12	32.6	12.8	7.5
12-24-14	32.8	13.5	7.0
12-47-13	35.7	12.8	8.5
15-2-4	30.8	14.4	5.0
15-22-4	40.5	10.9	7.0
12-32-8	38.9	17.8	7.0

High-yielding varieties were identified: 05-15-7 (42.9 t ha^{-1}), 15-22-4 (40.5 t ha^{-1}); with increased starch content in tubers: 05-15-7 (18.7%), 12-32-8 (17.8%), 05-15-5 (16.5%), 13-41-13 (16.5%); with better relative resistance to leaf blight: 05-15-7 (8.5 points), 13-41-13 (8.5 points), 12-47-13 (8.5 points). According to the results of the analysis, as the most valuable for inclusion in hybridization, in order to pyramid resistance genes to PVY from various sources, varieties are recommended that simultaneously carry markers to three resistance genes that are listed in Table 2.

A group of potato varieties from this list has been selected, which have now passed the state test and are included in the Register of Selection Achievements of the Russian Federation and are allowed to be used: 05-15-7 - Alaska; 05-15-40 - Gornyak; 08-10-1 - Mishka; 08-41-7 - Terra; 10-9-3 - Carmen; 10-22-7 - Prime; 12-32-8 - Flamingo.

The Alaska variety. Mid-ripening variety of table potato, tubers are oblong-oval, red, white color of flesh. Starch content is 13–20%. Average weight of commercial tuber is 110–140 g. Number of tubers in the bush is 12–16 pcs. Potential yield is 82 t ha^{-1}. Consumer qualities: good taste, culinary type BC. The variety is resistant to potato wart disease, potato cyst nematode, wrinkled and streak mosaic, curlytop virus. Preservation capacity when stored is good. Note: intensive type of variety, characterized by a consistently high yield, resistance to late blight. Included in the State Register of the Russian Federation and approved for use since 2020 for 4 and 12 regions.

The Gornyak variety. Early-ripening table variety. Tuber skin color is yellow, flesh color is light yellow, tuber shape is rounded. Potential yield is 60.0 t ha^{-1}, number of tubers per bush is 12–18 pcs., average weight of commercial tuber is 90–140 g., starch content is 14.0–19.0%. Medium-resistant to heat and drought. The variety is resistant to potato wart disease and late blight, poorly susceptible to potato cyst nematode. Preservation capacity when stored is good. It is recommended to cultivate on light and medium types of soil, it is flexible, highly resistant to scab. It is included in the State Register of the Russian Federation since 2015 for 4 and 9 regions.

The Mishka variety. Early, for table purposes. Tubers are round-oval, skin color is red, flesh is white. Potential yield is 50.0 t ha^{-1}, number of tubers per bush is 10–14 pcs., average weight of commercial tuber is 100–130 g., starch content is 13.0–17.7%. Resistance to disease: variety is resistant to potato wart disease, potato cyst nematode, medium-resistant to late blight and scab. Preservation capacity when stored is good. A distinctive feature is the early accumulation of commercial yield. It is included in the State Register of the Russian Federation since 2018 for 4 and 10 regions.

The Terra variety. Very early variety, for table purposes. Tuber skin color is yellow, flesh color is light yellow, tuber shape is oval. Potential yield is 55.0 t ha^{-1}, number of tubers per bush is 10–12 pcs., average weight of commercial tuber is 150–180 g., starch content is 10.0–14.0%. Taste is good, overcooking is weak (type B). Resistant to potato wart disease, potato cyst nematode; medium-resistant to late blight. Keeping capacity when stored is good. Note: early accumulation of commercial yield, high marketability. It is included in the State Register of the Russian Federation since 2020 for 4 and 10 cultivation regions.

A group of potato varieties with two resistance genes was identified - 39% of the total analyzed number, which also deserve close attention as genetic sources of resistance to PVY. The absence of all three studied genes was recorded in 8% of the studied genotypes. It is indicative that these samples also lack economically valuable

characteristics - they are characterized by low yield, starchiness and low resistance to late blight.

As a result of a comprehensive assessment of the studied material by economically valuable characteristics (field experiments), evaluation by PCR analysis for the presence of pathogens and molecular genetic labeling, a number of promising samples were selected for use in selection programs for the creation of virus-resistant potato varieties. The value of these samples as genetic sources is complex resistance to PVY, although they belong to different groups of ripeness, but the breeding significance is higher in the samples of the early-ripening group: 06-11-1 and 08-41-5. Characteristics of samples that are of interest in terms of saturation with various PVY resistance genes from several sources, with a complex of economic and useful features, are presented in Table 3.

Table 3. Characteristic of valuable potato genotypes that combine a comprehensive assessment of field resistance and the presence of PVY resistance markers, 2018–2019

Variety sample	Ripeness	Field resistance to Potato virus Y, point	Identified presence of markers of Potato virus Y resistance genes
05-15-15	mid	8.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry$_{chc}$*
05-15-7	mid	8.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
05-22-35	mid-early	7.5	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*
06-11-1	early	9.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
08-41-5	early	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
10-11-24	mid	7.5	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry$_{chc}$*
10-22-7	mid	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry$_{chc}$*
10-22-23	mid	7.5	RYSC3$_{321}$ - gene *Ry$_{adg}$*; Ry186 - gene *Ry$_{chc}$*
10-54-5	mid-early	8.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
10-54-9	mid-early	8.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto;}$* Ry186 - gene *Ry $_{chc}$*
12-32-8	mid-early	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
12-47-10	mid-early	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*
12-47-13	mid-early	9.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
13-41-13	mid-early	8.5	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*
13-41-53	mid-early	8.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; Ry186 - gene *Ry $_{chc}$*
14-9-24	mid-early	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; Ry186 - gene *Ry $_{chc}$*
15-22-4	mid-early	7.0	RYSC3$_{321}$ - gene *Ry$_{adg}$*; YES3-3A$_{341}$ - gene *Ry$_{sto}$*; Ry186 - gene *Ry $_{chc}$*

The sample 06-11-1 (the Start variety) presented in the table deserves special attention, it has a maximum value of the characteristic of the field resistance to PVY (9 points), according to the results of PCR analysis on average over the years, the pathogen of the potato virus Ywas not detected in any sample. This variety is included in the Register of Selection Achievements, but is not allowed to be used because of the average indicators of economic valuable characteristics. With the presence of three studied markers in the genotype $RYSC3_{321}$, $YES3\text{-}3A_{341}$, Ry186, field resistance to PVY, this variety is recommended as a genetic source for targeted selection, the fertility of this variety is 52.0%, which allows to include both the maternal and paternal forms in hybridization.

CONCLUSION

As a result of a comprehensive assessment of potato selection material in 2018–2019 for agronomic characteristics, field resistance and genotyping for the presence of DNA markers of the corresponding PVY resistance genes, 17 promising selective samples with a complex of markers of ER to PVY were identified, which are recommended for use as initial parent forms for hybridization in the direction of creating virus-resistant forms, as well as a valuable material in breeding: 05-15-15, 05-15-7, 05-22-35, 06-11-1, 08-41-5, 10-11-24, 10-22-7, 10-22-23, 10-54-5, 10-54-9, 12-32-8, 12-47-10, 12-47-13, 13-41-13, 13-41-53, 14-9-24, 15-22-4.

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