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FACILITATION OF INTROGRESSIVE HYBRIDIZATION OF WILD POLYPLOID MEXICAN POTATO SPECIES USING DNA MARKERS OF *R* GENES AND OF DIFFERENT CYTOPLASMIC TYPES

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Abstract

Nowadays potato breeding is targeting to develop genetically divers high yielding varieties with multiple pathogen resistance traits. Interspecific hybridization jointed with marker-assistant-selection (MAS) can effectively combine the *R* genes from different resistance sources. Additionally to effective pyramiding the target genes, MAS allows to restrict introgression of genetic factors conferring the undesirable traits, for example, male sterility of interspecific hybrids associated with *Solanum stoloniferum*-derived W/gamma cytoplasm that complicate the traditional breeding. Current study is targeting to search for the opportunities to improve the efficiency of introgressive hybridization between common potato and Mexican polyploid species *Solanum. neoantipoviczii* (=*S. stoloniferum*) and *S. guerreroense* using MAS with DNA markers for different cytoplasmic types and markers associated with major *R*-genes to the most harmful potato pathogens. DNA-based markers of genes for late blight resistance (*R2 like*, *R3a*, *Rpi-blb1*, *Rpi-sto1*), for extreme resistance to Potato virus Y (PVY) (*Ryadg*, *Rysto*, *Ry-fsto*) and for *H1* gene for resistance to the root cyst nematode (*Globodera rostochiensis*, pathotype Ro1) were used in this study. Based on the MAS, hybrid genotypes with different combinations of these markers were selected. Among them, there were the clones with high field resistance to late blight and to PVY. Of 29 hybrid clones from different combinations of crossing with polyploid Mexican species used as the maternal forms, 15 had a W/γ cytoplasmic type and were male sterile; both these traits were maternally inherited. The remaining hybrids with W/α cytoplasm produced fertile pollen and were used in interspecific crosses as pollinators. Selection of resistant clones with W/alpha cytoplasm and elimination of genotypes with sterile W/γ cytoplasm among wild species germplasm could increase the probability of obtaining male fertile introgressive lines. This approach allows to obtain the multi-species hybrid genotypes that combine *R* genes for resistance to pathogens from different Mexican species and to avoid various types of male sterility in breeding. The joint use of two systems of DNA markers, i.e. nuclear markers associated to *R* genes, and cytoplasmic markers for male sterility factors, could reduce costs and increase efficiency of target gene pyramiding programs.

Keywords: *Solanum* spp., potato, DNA markers, *R* genes, cytoplasmic types, interspecific hybridization

Based on studying wild and cultivated potato accessions from the VIR col-

lection (All-Russian Institute of Plant Genetic Resources), the formation of which began during expeditions of 1926 to 1933 [1-3], Russian scientists had developed a theory on the centers of origin and diversity of potatoes [1, 2] and for the first time proved and realized introgressive interspecific hybridization as a new trend in the world potato selection [2-4]. By the end of 20th century, interspecific hybridization became the main potato selection technique owing to the developed methods for overcoming pre- and post-zygotic interspecific incompatibility, such as planning crosses based on EBN (endosperm balance number) of parent species, variation of the ploidy of crossing samples, search for bridge species, reciprocal crossbreeding for leveling nuclear-cytoplasmic conflicts, which allowed involvement of wild species from secondary and tertiary genetic pools in breeding [5-9].

Nowadays, potato selection is focused on broadening the genetic variety of the genetic sources and donors of commercially valuable traits to produce cultivars which combine high productivity with complex and group pathogens resistance. These problems are solved be means of interspecific hybridization, marker-assisted selection (MAS) [10-12] and developed biotechnological methods [13]. MAS drastically increases the efficiency of stacking target genes/QTLs for pathogen resistance, and facilitates control of spreading unwanted genetic determinants. Thus, the hybrids with *R* genes introgressed from Mexican polyploid *Solanum stoloniferum* and *S. demissum* often inherit features which complicate traditional selection [14, 15]. Many hybrids and varieties possessing immunity to the potato virus Y (PVY) are, at the same time, male-sterile, that is, forming fully sterile pollen grains with anomalous morphology [16, 17]. This feature hindering the selection of crossings pairs is transmitted via the maternal line from wild species *S. stoloniferum* which is the sources of gene Ry_{sto} for the extreme YBK resistance [16, 17]. Markers associated with α, β and γ mitotypes have been developed, and a statistically significant relationship between the γ mitotype (W/γ cytoplasm type) and cytoplasmic male sterility has been established in the varieties and hybrids having *S. stoloniferum* in their pedigree [18]. These results found their confirmation in the works of other researchers [16, 17]. At the same time, PVY resistant cultivars with the W/α cytoplasm type are mostly fertile [16]. The hybrids which conferred *R* genes for race-specific resistance to late blight and cytoplasmic determinants from *S. demissum* Mexican species are another examples of joint transfer of target genes and male sterility [17, 19]. The hybrids and cultivars with D cytoplasm (W/α) from *S. demissum* can form morphologically normal pollen grains which, however, are functionally sterile [17, 20]. It is obvious that, while transfer of sterilizing cytoplasm types to various introgressive forms, the *R* gene stacking may have serious limitations.

In the present paper, we report on improving efficiency of introgressive hybridization based on screening parental forms for DNA markers of cytoplasm types and *R* genes encoding potato plant resistance to the most harmful pathogens. Hybrid clones selected within the segregating populations of two- or multi-species hybrids derived from polyploid Mexican species *S. neoantipoviczii* (=*S. stoloniferum*) and *S. guerreroense* due to their resistance to pathogens and tuber characteristics were involved in MAS for the first time. For the *S. guerreroense* species, the relationship of male sterility and W/γ cytoplasm type has been shown for the first time as well.

The aims of this study were i) to breed the progenies of the multi-species hybrids derived from wild polyploid Mexican potato species, and ii) to screen obtained progenies for the type of cytoplasm and resistance to the most dangerous potato plant pathogens, *Phytophthora infestans,* potato virus Y (PVY) and golden potato nematode (GPN), using DNA markers.

Techniques. The initial parental forms of the original interspecific hybrids

were the accessions of Mexican and South American species, breeding clones and varieties from VIR and SLU (Swedish University of Agricultural Sciences) collections, which were selected due to high foliar and/or tuber resistance to late blight and PVY resistance [21-23]. The VIR accessions were the clones of the Mexican wild species *S. neoantipoviczii* (=*S. stoloniferum*) (K-8505) (nan) and *S. guerreroense* (K-18407) (grr). These clones possess high foliar resistance to late blight and also are PVY resistant: *S. neoantipoviczii* is resistant to three strains, PVY^0, PVY^{N-Wi}, PVY^{NTN}, and *S. guerreroense* is resistant to two strains, PVY^0, PVY^{N-Wi} [22]. Most likely, the Ry_{sto} gene of *S. neoantipoviczii* K-8505 is in the homozygous state, because PCR analysis revealed Ry_{sto} gene marker YES3-3A in each of 20 individually tested seedlings [24]. The South American wild species were the clones of accessions with high (*S. microdontum*, K-20320) (mcd) and partial (*S. tarijense*, K-10712) (tar) foliar resistance [22, 25] or tuber resistance (*S. kurtzianum*, K-12488) (ktz) to late blight [22]. The clones of *S. kurtzianum* (K-12488) and *S. tarijense* (K-10712) at long day conditions are capable of forming tubers with good morphologic characteristics. A part of the plants from population of cultivated Andian species *S. tuberosum* subsp. *andigenum* (selected from K-8077) (adg) showed tuber late blight resistance. The fertile and productive *S. tuberosum* breeding clones from SLU collection, with good tuber characteristics, were involved in hybridization. These clones were SW-0906512 which shows field resistance to PVY, SW93-1015 of unknown origin, with resistance to PVY [23] and high field late blight resistance of constitutive type [26] conferred by *R2 like* gene [27], and SW93-1015 having an increased content of α-chaconine in tubers [23]. In this evaluation, the breeding clone NZ2010-10nb with foliar late blight and PVY field resistance derived from *S. stoloniferum* and the potato varieties Campina (nematode resistance), Desirée, Sarpo Mira (high late blight resistance) and Superb from SLU collection were also used in crossing in order to improve the agronomic traits of developing forms. All interspecific hybrids were developed by Dr. N.M. Zoteeva. Earlier some hybrids derived from two-species crossing were evaluated in the field and laboratory phytopathological tests [28-30]. In order to develop multi-species hybrids, the genotypes selected for PVY and late blight resistance were involved in further crossings. The current study examined a total of 35 hybrid clones (11 cross-combinations) which were split into three groups having different initial maternal forms (the cytoplasmic determinant donors).

The DNA was isolated by modified CTAB method from leaves of field grown plants [31].

The primers used in molecular screening for PVY [16, 32-33], foot rot [27, 34-37] and golden potato nematode [38] resistance markers were chosen based on the analysis of the parental forms of wild Mexican species.

The cytoplasm types of hybrids was determined as per K. Hosaka and R. Sanetomo [17] using a kit they proposed for four markers to detect various plastid DNA sites (*ndhC/trnV*, *rpl32/trnL-UAG*, *cemA*, *rps16/trnQ* loci) and two mitochondrial genome sites (*rps10* and *rps19* loci).

The PCR was carried out in 20 μl reaction mixture containing 10 ng total DNA, 1× buffer (Dialat Ltd., Russia), 2.5 mM $MgCl_2$, mixt of dNTPs at a final concentration of 0,4 mM each, forward and reverse primers (0.2 μM each), 1 unit of Taq DNA polymerase (Dialat Ltd., Russia). For ALM4/ALM5 primers, the dNTPs concentration was 0.6 mM. PCR was carried out in a Mastercycler® Nexus Gradient (Eppendorf, Germany) at annealing temperatures reported earlier (see the *Results* section). PCR was repeated at least 3 times for all markers except CAPS, and at least 5 times for ALM4/ALM5 primers.

The restriction was carried out in 30 μl reaction mixture according to the manufacturer's protocol (NPO SibEnzyme, Russia; http://russia.siben-zyme.com).

The electrophoresis was carried out in the TBE buffer and 2 % agarose gel followed by ethidium bromide staining and UV visualization. The molecular weight marker 100 bp + 1500 bp + 3000 bp (NPO SibEnzyme, Russia) was the standard.

Fertility of the hybrids was determined by staining the pollen with acetocarmine and by crossing method.

Foliar field resistance to late blight was assessed in 2014-2015 under the strong infection pressure (SLU, Sweden) as well as in the epiphytic season of 2016 in the experimental field of VIR Pushkin labs (Leningrad Province). Each year, the assessment was carried out from the first infection symptom manifestation on susceptible varieties (Desire and Bintje, SLU collection) until the end of vegetation. The nine-grade estimation scale was used, with 1 for totally diseased plant and 9 for the absence of disease symptoms.

The field resistance of the hybrids to PVY was assessed in 2012-2014 (SLU) when high infection spreading had been initiated by strong aphid invasion on untreated plantations. In the same field, the susceptible variety Magnum Bonum (SLU collection) annually exhibited strong disease symptoms. The plants without visible viral disease symptoms were studied using ELISA kit (BIOREBA AG, Switzerland) according to the manufacturer's instruction. The absorption was measured on a Multiskan™ GO Microplate spectrophotometer (Thermo Fisher Scientific, USA). The absorption above 0.1 means a positive reaction (plant sensitivity to viral infection). The tests were carried out twice a year (at the end of June and at the very beginning of August) according to the age-specific requirements for plants of different maturity groups (the International Potato Center, http://www.cipotato.org).

Results. Earlier it has been shown that *S. guerreroense* accession (K-18407) had a marker of the *R3a* gene [24], and *S. neoantipoviczii* (=*S. stoloniferum*) (K-8505) had a marker of the Ry_{sto} gene [24]. Since the aborigine and currently used potato varieties resistant to PVY and GPN (pathotype Ro1) have been involved in hybridization, the markers of the *Ryadg* and *H1* genes were also used in the molecular screening.

The PCR conditions and primers used in the molecular screening of the obtained potato hybrids are given in Table 1.

1. DNA markers associated with *R* genes

1	2	3	4	5	6	7
			Resistant to potato virus Y			
Ry_{sto}	XII	GP122-406/EcoRV	F: CAATTGGCTCCCGACTATCTACAG R: ACAATTGCACCACCTTCTCTTCAG	52	406	[32]
$Ry\text{-}f_{sto}$		YES3-3A	F: TAACTCAAGCGGAATAACCC R: AATTCACCTGTTTACATGCTTCTTGTG	55	341	[16]
Ry_{adg}	XI	RYSC3	F: ATACACTCATCTAAATTTGATGG R: AGGATATACGGCATCATTTTTCCGA	60	321	[33]
			Late blight resistance			
Rpi-blb1	VIII	blb 1	F: AACCTGTATGGCAGTGGCATG R: GTCAGAAAAGGGCACTCGTG	58	821	[34]
Rpi-sto1	VIII	Rpi-sto1	F: ACCAAGGCCACAAGATTCTC R: CCTGCGGTTCGGTTAATACA	65	890	[35]
R 1	V	R1-1250	F: CACTCGTGACATATCCTCACTA R: GTAGTACCTATCTTATTTCTGCAA-GAATTCTTATTTCTGCAAGAAT	65	1205	[36]
R2-like	IV	R2area 1/2	F: AAGATCAAGTGGTAAAGGCTGATG R: ATCTTTCTAGCTTCCAAAGATCACG	60	1137	[27]
R3a	XI	RT-R3a	F: ATCGTTGTCATGCTATGAGATTGTT R: CTTCAAGGTAGTGGGCAGTATGCTT	56	982	[37]
			Golden potato nematode resistance			
H1	V	57 R	F: TGCCTGCCTCTCCGATTTCT R: GGTTCAGCAAAAGCAAGGACGTG	60	452	[38]

Note. 1 — gene, 2 — chromosome, 3 — marker, 4 — primer sequence (5′→3′), 5 — primer annealing temperature, T_m, °C, 6 — diagnostic fragment size, bp, 7 — references.

The cross-combinations many of which are reported for the first time are

described in Table 2.

2. Molecular screening and phenotyping of potato hybrids derived from crossings with polyploid wild Mexican species (in groups depending on the initial maternal form)

Group, variant	Combination	Cytoplasm type (number of studied seedlings)	PF, %	Diagnostic fragments of *R* gene markers									Late blight resistance in 2014-2016, points
				R1	*R2 like*	*R3a*	*blb1*	*Rpi-sto 1*	Ry_{vadg}	Ry_{sto}	$Ry\text{-}f_{sto}$	***H1***	
I	SW93-1015 (♀)		0	–	+	–	–	–	–	+	+	+	8.0; 8.0; nd
1	(SW93-1015 × adg) × Desirée	W/γ (3)	0-1.0	–	+	–	–	–	–	+	+	–	nd; 7.0; 8.0
2	(SW93-1015 × adg) × {[nan × × (mcd × trj)] × (grr × adg)}	W/γ (3)	0-0.3	–	+	–	–	–	–	+	+	+	7.0; 6.0; 6.0
II	*S. neoantipoviczii* (♀)												
3.1	[nan × (mcd × trj)] × (grr × adg)	W/α (1)	69.8	–	+	–	+	+	+	+	–	–	7.0; 8.0;7.0
3.2	[nan × (mcd × trj)] × (grr × adg)	W/α (1)	66.7	–	+	–	+	+	+	+	–	–	8.0; 8.0; 6.0
4	[{nan × (mcd ×tar)} × (grr × × adg)] × SW-0906512	W/α (1)	26.7	–	–	–	–	–	+	+	+	–	6.0; 6.0; 5.5
III	*S. guerreroense* (♀)												
5	grr × adg	W/α (6)	18.0-24.1	–	+	+	–	–	+	–	–	–	8.0; 8.0; 7.0
5.1	grr × Superb	W/α (4)	38.0-65.0	–	+	+			–	–	–	–	8.0; 7.0; 8.0
6	(grr × Superb) × Sarpo Mira	W/α (3)	16.0-16.8	–	+	+	–	–	+	–	–	–	8.0; 7.0; 8.0
7	(grr × Superb) × Desirée	W/α (1)	14.5	–	–	–	–	–	+	–	–	–	6.5; 6.5; 6.9
8.1	(grr × Superb) × NZ2010-10nb	W/γ (1)	0.2	–	–	–	+	+	–	+	+	–	6.5; 7.0; 6.0
8.2	(grr × Superb) × NZ2010-10nb	W/γ (1)	0	–	–	–	+	+	–	+	+	–	7.0; 7.0; 5.5
9	[(grr × Superb) × NZ2010-10nb] × ktz	W/γ (1)	1.0	–	+	+	–	–	+	–	–	–	nd; 6.5; 7.0
10	[(grr × Superb) × NZ2010-10nb] × {[nan × (mcd × tar) × × (grr × adg)] × SW-0906512}	W/γ (4)	0-0.7	–	–	+	+	+	+	+	+	–	nd; 7.0; 6.5
11.1	[(grr × Superb) × NZ2010-10nb] × cv. Campina	W/γ (1)	0	–	+	+	+	+	–	+	+	–	nd; 6.0; 5.0
11.2	[(grr × Superb) × NZ2010-10nb] × cv. Campina	W/γ (1)	0	–	+	+	–	–	–	+	+	+	nd; 7.0; 7.0
11.3	[(grr × Superb) × NZ2010-10nb] × cv. Campina	W/γ (1)	AF	–	–	–	–	–	–	+	+	–	nd; 4.0; 1.0

N o t e. Three crossing series were carried out (the samples were grouped according to the initial maternal forms used). *R* gene marker data is given for individual hybrid genotypes selected from the segregating populations for resistance to pathogens and/or tuber morphology. PF — pollen fertility in the analyzed genotypes (min-max), AF — absence of flowering in the 11.3 hybrid; "+" — marker found, "– " — marker not found, nd — no data (parameter was not studied). Potato species: grr — *Solanum guerreroense*; ktz — *S. kurtzianum;* mcd — *S. microdontum*; trj — *S. tarijense*; nan — *S. neoantipoviczii* (=*S. stoloniferum*); adg — *S. tuberosum* subsp. *andigenum*. The cytoplasm types are given for all analyzed genotypes, of 1 to 6 per combination.

It is known that the potato plants of the same effective ploidy (EBN) can relatively easy cross with each other producing viable hybrid seeds [39]. Crossings of tetraploid potatoes (EBN = 4) with wild Mexican hexaploid *S. guerreroense* (EBN = 4) species closely related to *S. demissum* (both are the members of series Demissa Buk. in classic taxonomy) are good examples of such combinations. Analogously, all the forms involved in the combination nan × (mcd × trj), i.e. *S. neoantipoviczii* (=*S. stoloniferum*), *S. microdontum* and *S. tarijense*, have EBN = 2 (see Table 2). The EBN rule does not apply to more complex combinations, which often occurs in the production of multi-species hybrids [9].

Screening of cytoplasm types and *R* gene using molecular markers. *Hybrids from crossings of the breeding clone SW93-1015.* All studied hybrid clones from the two crossings combinations, derived from the breeding clone SW93-1015 (maternal form), had the W/γ cytoplasm type and fully sterile pollen of anomalous morphology (see Table 2), which indicate on the presence of *S. stoloniferum* in the SW93-1015 pedigree. The presence of *S. stoloniferum* characteristic markers YES3-$3A_{341}$ and GP122-406/$EcoRV_{406}$ linked to genes Ry_{sto} and $Ry\text{-}f_{sto}$, respectively, which are located on chromosome XII is in favor of this assumption. These markers are absent in other parental forms, i.e. *S. tuberosum* subsp. *andigenum* K-8077 and Desirée variety, used in the combination No. 1 [16, 40].

Moreover, the selected hybrid clones had the markers of *R2-like* gene for resistance to late blight and the *H1* gene for GPN resistance (see Table 2). Compared to the breeding clone SW93-1015, the hybrids from the combination No. 1 (SW93-1015 × adg) × Desirée produced more tubers per plant which were more uniform in size.

Hybrids derived from crosses with S. neoantipoviczii (=*S. stoloniferum*). It is shown that of two cytoplasm types, W/α and W/γ, characteristic of *S. stoloniferum* [41], only W/γ is associated with male sterility [16-18, 41]. *S. neoantipoviczii* (=*S. stoloniferum*) K-8505 having W/α cytoplasm was a maternal form of these hybrids. All hybrids of K-8505 also had the W/α cytoplasm and varied in fertility levels (26.7-69.8 %). In further crossing, the hybrid form [nan × (mcd × trj) × (grr × adg)] was a successful pollinator, which indicates functional fertility of its pollen (see Table 2).

All hybrids of group II had the markers of *R* genes for extreme resistance to PVY, and multi-species hybrid No. 4 had markers of three genes, Ry_{adg}, Ry_{sto}, $Ry\text{-}f_{sto}$ (see Table 2). The genotypes with markers of late blight resistance genes *R2 like*, *Rpi-blb1, Rpi-sto1* were selected from multi-species hybrids of this group.

Hybrids derived from S. guerreroense. S. guerreroense species was involved in breeding for the first time. Hybrid plants derived from the combinations (grr × adg) and (grr × Superb) often had violet corolla color typical for *S. guerreroense*, but unlike the wild parent, could form tubers under long light day conditions. The plants of hybrid combination (grr × Superb) had long stolons and an irregular tuber shape. Progenies of (grr × Superb) × Desirée hybrid showed high polymorphism by tuber shape and skin color. The plants of *S. guerreroense* hybrids formed a large number of berries via self-pollination. The percentage of fertile pollen in these hybrids reached 24 % and the plants of the (grr × adg) hybrid were successfully used as effective pollinators for production of multi-species hybrids, which indicated functional fertility of their pollen (see Table 2). Two-species hybrids of three combinations, (grr × adg), (grr × Superb), and [(grr × Superb) × Desirée], had the W/α cytoplasm type. However, the γ mitotype was detected in multi-species hybrids of the group III with *S. guerreroense* as an initial maternal form, at that, all of the multi-species hybrids of the combinations Nos. 8-11 were sterile (see Table 2). The absence of amplification in the intragene spacer *rps10 cob* (mtDNA cytoplasm type W/y) in these multi-species hybrids could be associated with the rearrangement of mtDNA sequences which occurred due to multiple hybridization.

The hybrid clones from group III derived from *S. guerreroense* displayed in various combinations up to 4 markers for late blight resistance genes and 1 to 3 markers for PVY resistance genes (see Table 2). The variety Superb and *S. tuberosum* subsp. *andigenum* K-8077 could be donors of the Ry_{adg} gene in the selected hybrid clones. In multi-species hybrids combinations Nos. 8-11, the donors of the Ry_{sto} and $Ry\text{-}f_{sto}$ genes were either *S. neoantipoviczii* (=*S. stoloniferum*) (combination No. 10), or the PVY-resistant breeding clone NZ2010-10nb, derived from *S. stoloniferum* (combinations Nos. 8, 9, 11) (see Table 2).

We did not revealed genotypes with R1-1250 marker for *R1* gene in any of the combinations studied (see Table 2).

In each of the hybrids of groups II and III, the markers for genes *Rpi-sto1* and *Rpi-blb1* were found simultaneously (see Table 2). It is known that the *Rpi-blb1* and *Rpi-sto1* genes are orthologues related to the same *Rpi-blb1* family which is located on chromosome VIII close to CT88 marker, and their sequences possess high homology [34, 42, 43]. Functional homology of these genes has also been reported [43]. Intragenic markers developed for *Rpi-blb1* gene of diploid Mexican *S. bulbocastanum* species were found in Mexican polyploid species

S. stoloniferum and *S. papita* (=*S. stoloniferum*) [34] and also in hybrids derived from *S. stoloniferum* [12]. Therefore, in our research, both markers, Rpi-sto1 and Rpi-blb1, detect the sequences of the same *Rpi-sto1* gene in the hybrids derived from *S. stoloniferum*.

Assessment of hybrid resistance to late blight and PVY. In 2014-2015, the plants of the Bintje and Desirée varieties were significantly affected by *Ph. infestans* 2.5 weeks after the first symptom manifestation, and in the epiphytotic season of 2016, plants of the same varieties in the same dates were affected completely. Simultaneously, the hybrid clones which were selected earlier from segregating populations were studied. The study in epidemic 2016 season confirmed resistance to late blight of the number of clones derived from crosses with SW93-1015, *S. neoantipoviczii* K-8505 and *S. guerreroense* K-18407 (see Table 2). High field resistance (7-8 point score) was observed in two-species hybrid combinations Nos. 1, 5, 6 (see Table 2). Previously, the hybrids combination (grr × adg) expressed the same hypersensitivity reaction in the lab leaflet test as the parental *S. guerreroense* plants [24]. The leaflet test, in which the inoculums of three *Ph. infestans* isolates, SW058 [26], 88069 [44] and H7, were used at the concentration three times higher than that of the standard [30], allows us to select the genotypes with extremely high late blight resistance from segregating population of the grr × adg hybrid. These data indicate that *S. guerreroense* K-18407 is a valuable source of late blight resistance which effectively transmits this trait to hybrid progenies. Clones of the multi-species hybrid [nan × (mcd × trj) × (grr × adg)] showed high resistance to late blight (see Table 2). In 2017 season with strong *Ph. infestans* invasion, high resistance of hybrid clones expressed in the season of 2016 was confirmed completely (data are not shown).

In some cases, sequential crosses of hybrids with varieties and/or breeding clones led to a decrease in late blight resistance degree though other agronomic characteristics improved. Thus, late blight resistance scores in some clones from the combinations Nos. 7, 8, 9, 10, and 11 were 6.0 to 7.0 points, and in hybrids No. 4 and No. 11.1 the scores averaged 5.5 to 6.0 points (see Table 2). The clone No. 11.3 was selected for good agronomic characteristics (cultivated type tubers) but its plants were totally affected by late blight in 2016. In this clone, none of the markers of genes *R2 like*, *R3a, Rpi-blb1, Rpi-sto1* conferring late blight resistance were detected (see Table 2).

Among plants of groups I, II and III, the molecular screening revealed late blight resistant genotypes with various combinations of markers for broad-spectrum resistance genes *R2 like*, *Rpi-blb1*, *Rpi-sto1*, and the race-specific resistance gene *R3a*. However, the presence of these markers did not always determine phenotypic resistance. E.g. hybrid No. 11.1 had all four markers for late blight resistance genes (see Table 2) bur showed moderate pathogen resistance. Apparently, the markers which we used in the analysis detected in these genotypes the non-functional homologues of *R* genes. At the same time, high field late blight resistance (7-8 points) in the absence of some markers may be due to an introgression of not yet identified genes/QTLs from *S. guerreroense* (e.e. combinations Nos. 5 and 7, see Table 2) or resulted from functioning other *R* genes in these hybrids (e.g. hybrid No. 6 could confer the dominant alleles of *R4*, *R8*, *Rpi-Smira1* genes from the Sarpo Mira variety) [46, 47].

The field PVY resistance was assessed only in two hybrids combinations, No. 3 and No. 5, and in their progeny (10 seedlings per combination). During the entire study, the plants of multi-species hybrid [nan × (mcd × trj)] × (grr × adg)] showed no susceptibility to PVY under high infection pressure and, by ELISA test, were free of the viral infection (absorption value from 0 to 0.001). This hybrid had the markers for two genes, Ry_{sto} and Ry_{adg}, which determine extremely

high resistance to PVY (see Table 2). All studied clones in the hybrids progeny of the combination No. 3 also showed field resistance to PVY during the entire study period. The plants of PVY susceptible variety Magnum Bonum grown in the same field developed strong disease symptoms (the absorption value in ELISA test for PVY infection was from 2.19 to 2.37). RYSC3 marker for Ry_{adg} gene determining extreme PVY resistance was detected in the combination No. 5 (grr × adg). About $^3/_4$ of this hybrid population consisted of plants free from viral infection.

Hence, the results of our study indicate that 17 of 35 hybrid clones evaluated had W/α cytoplasm, and 18 had W/γ cytoplasm. Of 18 hybrid genotypes with the W/γ cytoplasm type, 17 formed fully sterile pollen grains of anomalous morphology and one genotype did not flower. The male sterility and the W/γ cytoplasm type were transferred via the maternal line to multi-species hybrids of various crossing combinations. In 17 hybrids with the W/α cytoplasm the amount of fertile pollen grains varied. Some of these hybrids were used as pollinators. This pattern was characteristic of both the plants of groups I and II which were based on *S. stoloniferum* cytoplasm and the hybrids of group III derived from *S. guerreroense* as maternal forms. In the latter, the γ mitotype might have appeared in multi-species hybridization. Such a linkage between the male sterility and W/γ cytoplasm type in breeding clones and varieties derived from *S. stoloniferum* has been reported earlier [16, 17]. Note, for *S. guerreroense*, this phenomenon was shown for the first time. Our data and the findings obtained by other researchers allows us to assume that searching for the pathogen resistance sources within the populations of *S. stoloniferum*, *S. guerreroense* (and possibly other Mexican species) can be done simultaneously with the selection against the donors of sterilizing cytoplasm type W/γ which is undesirable for the traditional breeding. On the contrary, clones with the W/α cytoplasm would allow for an increase in availability of introgressive male fertile forms. It is highly likely that in the future the selection vector can change. In particular, fixation of genotypes with sterilizing cytoplasm in breeding material may be promising for the development of heterotic hybrid selection as a new direction of potato breeding [48].

To conclude, the combined use of nuclear and cytoplasmic DNA markers for identification of R resistance genes and cytoplasm types will facilitate parental pair selection to reduce the time and the cost for hybridization, and to control crossing when combining genes of desirable traits in one genotype.

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