IDENTIFICATION OF INTERSPECIFIC POTATO HYBRIDS WITH COMBINED RESISTANCE TO LATE BLIGHT (*PHYTOPHTHORA INFESTANS*) AND NEMATODE (*GLOBODERA ROSTOCHIENSIS*)

Nadezhda Zoteyeva^{1,#}, Guna Sprūde², Natalia Klimenko¹, and Ieva Mežaka²

¹ N. I. Vavilov Institute of Plant Genetic Resources, 42 B. Morskaya Str., St. Petersburg, RUSSIA; nzoteyeva@gmail.com

² Institute for Agricultural Resources and Economics, 2 Zinātnes iela Str., Priekuļu pag., Priekuļu nov., LV-4126, LATVIA

Corresponding author, nzoteyeva@gmail.com

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Late blight (agent Phytophthora infestans) and potato cyst nematode (PCN) caused by Globodera rostochiensis are economically important pathogens, which may significantly reduce potato yields. In this study interspecific potato hybrids were used as a source of resistance for combined resistance to economically important potato pathogens: late blight and cyst nematode. The aim of our study was to identify hybrid progenies with combined resistance to both pathogens and to verify the applicability of several molecular markers associated with resistance to G. rostochiensis pathotype Ro1 to identify resistant plants. Ninety-two clones of eleven original interspecific potato hybrids obtained in crosses with the cultivated S. tuberosum group tuberosum, S. tuberosum group Andigena, S. tuberosum group Phureja and wild S. guerreroense, S. microdontum, S. kurtzianum, S. neoantipoviczii and S. tarijense potato species were screened in bioassays and by molecular markers. PCN resistant or moderately resistant clones were found among the progenies of nine hybrids. Results were highly correlated with resistance status detected by molecular markers linked to the H1 (marker 57R) and Gro1-4 (marker Gro1) genes. Marker CP113 (linked to the H1 gene) was not polymorphic and failed to detect resistance status. Combination of foliar late blight resistance and resistance to PCN was identified in hybrids obtained in crosses with plants of species S. microdontum, S. tarijense and S. phureja and in the hybrid between S. guerreroense and Black's P. infestans race differential carrying gene R-5.

Key words: Solanum L., marker assisted selection, host plant resistance, late blight, nematode.

INTRODUCTION

Improvement of resistance to economically important diseases and pests in current potato cultivars is one of the main goals of breeding programmes worldwide. Late blight (agent *Phytophthora infestans*) and potato cyst nematode (*Globodera rostochiensis*) can both reduce yields by up to 50% (Marks and Brodie, 1998; Fry, 2008; Guha and Grünwald, 2014). The International Potato Centre has demonstrated that potato farmers in Peru benefit USD 130 per ha and have a 26% return of investment rate by increasing tuber yield by 9% and by the reduction of fungicide application due to growing late blight resistant cultivars (Sevilla and Ortiz, 2010). Resistance to nematodes is one of the mostly desirable traits in newly developed potato cultivars. Currently, potato breeding requires basic material with re-

sistance to two and more pathogens. *Solanum* L. species are intensively utilised in potato breeding as sources of resistance to many diseases and pests. Resistance genes from wild relative species have been incorporated into the genotypes of a number of potato varieties (Glendinning, 1983; Ross, 1986; Flis *et al.*, 2005; Aversano *et al.*, 2007; Chimote *et al.*, 2008).

Infection with the potato cyst nematode (PCN) can also lead to severe decline in potato yield wherever potato is grown. The most common pathotype of *G. rostochiensis* in the North-Western zone of Europe where Russian Federation and Latvia are situated is Ro1 (Holgado and Magnusson, 2010). In the United Kingdom, Ro1 also essentially predominates (Bradshaw and Ramsay, 2005).

The necessity to grow nematode resistant varieties limits variety choice. Resistance to *G. rostochiensis* has been found in *Solanum* species (Dalamu *et al.*, 2012) and it is obligatory for registrations of new potato varieties (Galek *et al.*, 2011). Several loci conferring resistance to PCN have been identified with either partial (*Gro1.2*, *Gro1.3*, *Gro1.4*, *Grp1*) or nearly-full (*H1*, *Gro1*, *GroIV*) resistance (Finkers-Tomczak *et al.* 2011). Breeders are working to develop nematode resistant cultivars, but the number of cultivars available is still limited. A large, genetically diverse gene pool has to be used in hybridisation to achieve success, as *S. tuberosum* has a narrow source of resistance genes (Sudha *et al.* 2016). In most of the recently released potato varieties, resistance against *G. rostochiensis* pathotype Ro1 is ensured by the *H1* gene, which is localised on potato chromosome V (Gebhardt *et al.*, 1993; Bakker *et al.*, 2004; Paal *et al.*, 2004; Park *et al.* 2018). Use of species related to cultivated potato offers the possibility to enlarge the gene pool for pest and disease resistance. Worldwide breeders achieved success in producing potato varieties resistant to *G. rostochiensis* by introducing the *H1* gene. However, widespread and extensive use of varieties with *H1* has caused a rapid worldwide increase in *G. pallida* (Bradshaw *et al.*, 1998; Bryan *et al.*, 2002). Currently, breeding is focused on development of varieties resistant to a broad spectrum of PCN populations. The DNA marker *Gro1* can identify the *Gro1-4* gene (Gebhardt *et al.*, 2006). *Gro1-4* confers nearly absolute resistance to all *G. rostochiensis* pathotypes and is considered as a useful resistance gene (Baron *et al.*, 1990; Paal *et al.*, 2004).

Marker assisted selection has become an important tool for crop improvement utilising hybrid breeding or pyramiding of genes for resistance to different harmful organisms. Nevertheless, bioassays are still the basic approach to select resistant plants (Yu *et al.*, 2000; Park *et al.* 2018). The crucial point in identification of resistant hybrids by MAS is the concordance of the resistance status detected by DNA markers and bioassays. Reliability of marker 57R has been

validated in several studies — Schultz *et al.* (2012) found almost perfect concurrence with only one exception in Australian and Scottish breeding material, Milzarek *et al.* (2014) has found 93.7% agreement between both tests in Młochów Research Centre material, but Park *et al.* (2018) found 91.5% match in the New York breeding programme. Recently, hybrid progenies derived from *S. tuberosum* L., *S. tuberosum* Group Andigena and *S. tuberosum* Group Phureja have provided insight on the evolution of the *H1* locus Finkers-Tomczak *et al.* (2011).

In this study, interspecific potato hybrids of different genetic backgrounds were assessed for resistance to cyst nematode pathotype Ro1 and to late blight. Late blight resistance was assessed by bioassays, and PCN was screened both by bioassays and available molecular markers- CP113 and 57R linked to the gene *H1* conferring resistance to *G. rostochiensis* pathotype Ro1 and marker *Gro1* linked to resistance to *Gro1-4*.

The aim of the present study was to validate the molecular markers CP113, 57R and *Gro1-4* for identification of PCN resistant hybrids and to identify those with combined resistance to late blight and to *Globodera rostochiensis*.

MATERIAL AND METHODS

Plant material. Interspecific hybrids evaluated were obtained in crosses using the parental accessions mentioned in Table 1.

Evaluation was performed on a set of the following potato hybrids: grr × B.d.R5, grr × adg, (grr × adg) × cv. Omega, nan × phu-1, nan × (tub × phu-2), nan × AU, mcd × tar, (mcd × tar) × ktz, AU × rzc, tub × phu-2 and (AU × tub) × phu-2. Some hybrids and the parental accessions were previously evaluated for foliar or tuber resistance to late blight. Hybrid populations included in the current research were newly obtained from seeds.

Table 1. Parental accessions of the evaluated potato interspecific hybrids

Species	Abbreviation of the species*	No. in VIR catalogue	Inventory No in other collections	Resistance to late blight		References
				foliar	tuber	
<i>S. guerreroense</i> Corr.	grr	18407	USA, PI 161727	R	n.t.**	Zoteyeva <i>et al.</i> , 2012
<i>S. neoantipovicii</i> Buk.	nan	8505	Germany, GLKS93.1(67.93.1)	R	PR	Zoteyeva <i>et al.</i> , 2012
<i>S. kurtzianum</i> Bitt. et Wittm.	ktz	12488	Argentina, OKA 4964; ÑUJA, PI 472938	S	R	Zoteyeva, 2000
<i>S. microdontum</i> Bitt.	mcd	20320	USA, PI 458358	R	n.t.	Zoteyeva <i>et al.</i> , 2012
<i>S. tarijense</i> Hawkes	trj	10712	USA, PI 473336	PR	n.t.	Zoteyeva <i>et al.</i> , 2012
<i>S. Ruiz-ceballosii</i> Card.	rzc	7370	Bolivia, Horlahon, 8664	R	PR	Zoteyeva <i>et al.</i> , 2012
Selection from <i>S. tuberosum</i> Group. Andigena Hawkes	adg	8077	—	S	R	Zoteyeva, 2014
<i>S. phureja</i> Juz. et Buk.-2	phu	unknown	—	R	PR	Zoteyeva, no published data
<i>S. phureja</i> - Juz. et Buk.-1	phu	—	USA PI 320358	S	n.t.	Zoteyeva, no published data
Selection from cv. Aurora	AU	—	—	MR	S	

* species abbreviation acc. to the Centre for Genetic Resources, Wageningen, The Netherlands.

** n.t., not tested.

Phenotyping. Foliar late blight resistance assay. Resistance was assessed in leaflet tests using the isolate (virulence genes 1.2.3.4.6.7.11.12.) sampled from local populations of *P. infestans*. The genotypes of Black's differential set offered by IHAR-Mlochow (Poland) each carrying a single R-gene (*RI-R11*) were applied to identify *P. infestans* genes for virulence.

Detached leaflets collected from plants grown in a greenhouse were sampled from the mid part of plants. Three leaflets detached from each plant in two replications were drop inoculated. Inoculum concentration comprised 20 000 sporangia/ml. Leaflets of susceptible var. *Brasla* were used as a susceptible and of an accession of *S. guerreroense* (*grr*) as resistant controls. Disease was scored using a 1–9 grade scale, where 9 indicated lack of disease symptoms and grade 1 reflected 90–100% of diseased area (Zarzycka, 2001).

Pot-plant nematode resistance assay. Tubers were planted in 10 cm diameter pots in a greenhouse with soil infected with cysts of *Globodera rostochiensis* of pathotype Ro1 according to Stelter (1971). Inoculation was carried out in May and after 8–11 weeks the infection rate was determined and the susceptible clones were identified. Genotypes were designated as resistant if there were no cysts found in any of the replicates. If one to three cysts were found in one to two replicates, the clone was considered to be moderately resistant, clones with 4–5 cysts were rated as moderately susceptible and clones with more than 5 cysts as susceptible. The revealed symptoms were compared with those of the resistant cultivar 'Brasla' and susceptible cultivar 'Mutagenagrie'.

PCR marker assay. Extraction of genomic DNA. Genomic DNA of parents and F1 hybrids was extracted from leaf material following the protocol of Wulff *et al.* (2002). DNA was extracted from fresh leaves of pot-grown plants. Collected leaves were stored at –20 °C until DNA extraction. Approximately 80 mg of leaf tissue per genotype were used for DNA extraction. PCR amplification. The *H1* gene for resistance to *Globodera rostochiensis* was tested with flanking molecular markers CP113 (Skupinová *et al.* (2002) and 57 R (Finkers-Tomczak, 2011) and the *Gro1-4* gene was accessed by locus *Gro1* (Gebhardt *et al.*, 2006). The resistant allele for resistance to root cyst nematode *Ro1* pathotype was detected by marker CP113 according to a protocol adapted from Skupinová *et al.* (2002). The forward primer was 5'- GCG TTA CAG TCG CCG TAT-3' and reverse primer was 5'-GTT GAA GAA ATA TGG AAT CAA A-3'. The marker CP113 was amplified in 20 µl total volume of 2.5 mM MgCl₂, 0.2 mM dNTPs, primers at concentration 0.3 mM each and 0.65 U Taq polymerase. Cycling conditions were 93 °C for 180 s followed by 35 cycles of 93 °C for 30 s, 51 °C for 45 s, 72 °C for 90 s and finally 70 °C for 90 s). The *Gro1-4* resistance gene was screened by the SCAR marker *Gro1* as described by Gebhardt *et al.* (2006). The forward primer was 5'-TCT TTG GAG ATA CTG ATT CTC A-3' and the reverse primer was 5'-CGA CCT AAA ATG AAA AGC ATC T-3'. PCR was per-

formed in 20 µl of 2.5 mM MgCl₂, 0.2 mM dNTPs, primers at concentration 0.4 mM each and 1U of Taq polymerase. The PCR parameters for amplification were: 94 °C for 180s, 35 cycles of 92 °C for 20s, 48 °C for 20 s, 72 °C for 50 s and final extension of 72 °C for 10 min. Amplification products were resolved on 1% agarose 1x TBE buffer gels (w·v⁻¹) and were visualised by ethidium bromide staining. Individuals were declared resistant, if an amplification product was present and was 760 bp for marker CP113 and 602 bp for marker *Gro1*. Marker 57R was amplified as described by (Finkers-Tomczak, 2011) with primer sequences 57R_F: TGCCTGCCTCTCCGATTCT and 57R_R: GGTTTCAGCAAAGCAAGGACGTG. The PCR parameters for amplification were: 95 °C for 10 min, 30 cycles of 95 °C for 45s, 63 °C for 45 s, 72 °C for 45 s and final extension of 72 °C for 10 min. In resistant genotypes 450 bp product was amplified.

RESULTS

Phenotypic evaluation. Clones obtained from individual seedlings were assayed phenotypically and in molecular tests. The results obtained by PCR and biological tests are presented in Table 2. Hybrid progenies derived from the accessions of Mexican (*S. guerreroense*, *S. neoantipoviczii*) and Bolivian (*S. ruiz-ceballosii*, *S. microdontum*) wild potato species with high foliar late blight resistance (Table 1) were screened in leaflet tests using the aggressive isolate of *P. infestans* and in pot-plant bioassay for resistance to nematode.

The majority of tested plants expressed resistance to late blight and to a smaller extent to pathotype Ro1 nematodes. Resistance levels to both pathogens are shown for the progeny of each hybrid in Table 2. In populations completely resistant to late blight *grr* × *adg*, *nan* × *phu-1* and *nan* × (*tub* × *phu-2*), only one plant of hybrid *nan* × (*tub* × *phu-2*) expressed nematode resistance. In the population of hybrid *grr* × B.d. R5, half of the plants resistant to late blight were resistant or moderately resistant to nematode (Table 2). Hybrids of population Aurora × (*tub* × *phu-2*) were segregating for resistance to both pathogens — five of 15 clones were resistant and six moderately resistant to late blight and two clones were resistant and four moderately resistant to nematode. Resistance to both pathogens in this population was expressed in only three plants (Table 2). Among five tested progenies of hybrid *nan* × cv. Aurora, two combined resistance to late blight and nematode. One late blight resistant clone was moderately susceptible to nematode. Two late blight susceptible clones included nematode susceptible and moderately susceptible phenotypes.

Both tested clones of hybrid (*grr* × *adg*) × cv. Omega were late blight resistant and one of them was resistant to nematode. Combined resistance to both pathogens was found in all three tested clones of *mcd* × *tar*. Among three tested clones of (*mcd* × *tar*) × *ktz*, two with resistance to late blight were also resistant to nematode and one was susceptible to both pathogens. Among the two tested clones of

Table 2. Resistance to late blight and response to *Globodera rostochiensis* of pathotype *ro1* of interspecific potato hybrids with /without markers cp113, gro1-4 and 57r

Accession	Number of plants									
	late blight phenotype resistance rating:			nematode phenotype resistance rating:				with haplotype:		
	Ro1	MR	S	R	MR	MS	S	CP113	57R	Gro1-4
<i>grr</i> × <i>adg</i>	24	0	0	0	0	0	24	24	0	0
<i>grr</i> × B.d. <i>R5</i>	6	-	-	6	-	-	-	6	n.t.	6
	2	-	-	-	2	-	-	2	n.t.	2
	3	-	-	-	-	3	-	3	n.t.	3
	6	-	-	-	-	-	6	6	n.t.	3
Aurora × (tub × phu-2)	1	1	-	2	-	-	-	2	n.t.	2
	2	1	1	-	4	-	-	4	n.t.	4
	2	4	3	-	-	-	9	9	0	0
(grr × <i>adg</i>) × cv. Omega	1	-	-	1	-	-	-	1	n.t.	1
	1	-	-	-	-	-	1	1	n.t.	0
nan × (tub × phu-2)	1	-	-	1	-	-	-	1	n.t.	1
	6	-	3	-	-	-	9	9	n.t.	0
nan × Aurora	2	-	-	2	-	-	-	2	2	n.t.
	1	-	1	-	-	2	-	2	2	n.t.
	-	-	1	-	-	-	1	1	0	n.t.
tub × phu-2	1	0	0	0	1	0	0	1	n.t.	1
mcd × tar	3	-	-	3	-	-	-	3	0	3
(mcd × tar) × ktz	2	-	-	2	-	-	-	n.t.	0	2
	-	-	1	-	-	-	1	n.t.	0	0
nan × phu-1	7	-	-	-	-	-	7	7	0	n.t.
Aurora × rzc	-	1	0	-	1	-	0	1	0	n.t.
	1	-	-	-	-	1	-	1	0	n.t.
Controls in biological tests										
<i>grr</i>	5	-	-							
Brasla	-	-	5							
Brasla				5	-	-	-			
Mutagenagrie				-	-	-	5			

¹⁾ R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible;

²⁾ n.t., not tested

Aurora × rzc, one was late blight resistant but infected by nematode and the other expressed moderate resistance to both late blight and nematode.

Molecular tests. The marker CP113 was not polymorphic and in all plants amplified an allele linked to the gene *H1* providing resistance to *G. rostochiensis* of pathotype Ro1, including all phenotype susceptible plants of *grr* × *adg*. All plants of hybrid *grr* × *adg* were also tested with marker of gene *Gro1-4* and none were positive. DNA marker tags for gene *H1* were found in all 20 plants of the hybrid nan × phu population. All progenies of hybrids *grr* × B.d.*R5*, (*grr* × *adg*) × Omega, nan × (tbr × phu), and Aurora × (tbr × phu) also amplified the gene *H1* resistance allele (Table 2). PCR tests detected marker Gro1 of gene *Gro1-4* in resistant plant material with an exception of three clones of hybrid *grr* × B.d. *R5*, where three susceptible clones were positive for this marker. Marker Gro1 was amplified in 11 resistant clones of *grr* × B.d. *R5*, in one of two clones of (*grr* × *adg*)

× Omega, in one of 10 clones of hybrid nan × (tbr × phu-2), in the one tested clone of hybrid tbr × phu-2, in each of three tested clones of mcd × tar and in two of three clones of hybrid (mcd × tar) × ktz (Table 2). Molecular screening of ktz parental accessions showed presence of marker Gro1 in three of four clones (data are not shown in table).

In PCR tests using the marker 57R linked to the *H1* gene locus confirmed the presence of the *H1* gene allele only in resistant clones of hybrid nan × cv. Aurora. This marker (adopted from Skupinova (2002)) did not amplify gene *H1* fragments in hybrids *grr* × *adg*, in susceptible clones of Aurora × (tub × phu-2), in plants of nan × phu-1, mcd × tar and (mcd × tar) × ktz.

The results revealed a contradiction between the data of biological and molecular tests. Marker CP113 was detected in all clones from 24 plant populations of hybrid *grr* × *adg* that was susceptible as determined by biological tests. None of the plants of this population amplified the resistant allele of

marker 57R. The resistance allele of marker CP113 was detected in all 15 tested clones of hybrid AU × (tbr × phu-2) while only six expressed phenotypic resistance. In susceptible plants of hybrids (grr × adg) × cv. Omega, nan × (tub × phu-2), nan × Aurora and nan × phu-1, the resistance allele of marker CP113 was amplified. In nematode moderately resistant and moderately susceptible clones of hybrid AU × rcz, the *H1* gene resistant allele was detected only using marker C113 and there was no amplification of marker 57R. Resistance allele of gene *Gro1-4* was detected only in cases where clones expressed resistance in bioassays. It was not amplified in susceptible clones with the exception of three plants of hybrid grr × B.d. R5 (Table 2). Resistance conferred by *Gro1-4* gene was frequently detected in hybrids derived from the South-American potato species *S. phureja*, *S. kurtzianum*, *S. microdontum* and *S. tarijense*.

Combined resistance to late blight and nematode in the current evaluation was found in individual clones of hybrids: nan × cv. Aurora, nan × (tub × phu2), tub × phu-2, cv. Aurora × (tbr × phu-2), Aurora × rzc, grr × B.d. R5, (grr × adg) × cv. Omega, mcd × tar and (mcd × tar) × ktz. The results showed a contradiction between the data of biological and molecular tests. Resistance allele of marker CP113 was detected in all tested individuals of 10 hybrids while only a part of clones expressed resistance under inoculation.

DISCUSSION

Wild potato species can be considered as promising plant material to increase durable resistance due to their high polymorphism (Nunziata *et al.*, 2010). Current potato breeding requires the combination of several useful traits into one genotype. Due to high economic losses caused by crop diseases, breeders need to develop varieties combining resistance to two or more pathogens. The search for combined resistance to *G. rostochiensis* and potato late blight is essential due to high yield losses caused by these pathogens.

Marker-assisted selection could represent a useful tool for genetic studies and breeding for resistance to *G. rostochiensis* (Ortega and Lopez-Vizcon, 2012). Its application for identification of disease resistance in potato breeding programmes can reduce the time required for the selection of resistant parental accessions for crosses. Markers linked to resistance genes of economically important diseases and nematodes are useful tools for potato breeding (Asano and Tamiya, 2016). In recent breeding for combined resistance to nematodes, potato virus Y and late blight has employed marker assisted selection (López *et al.*, 2015, Milczarek *et al.*, 2017). Asano *et al.* (2012) evaluated potato varieties and lines by the multiplex PCR method, and 264 of 812 were positive for the *H1* marker. Of these, 117 varieties and lines were positive for two markers — *H1* and *Gpa2*. In contrast to these markers, the authors could not find varieties and lines having *Gro1-4*, although evaluated samples included the PCN-resistant *S. spegazzinii*. This fact emphasises the importance of developing resistant plant material containing *Gro1-4* (Asano *et al.*, 2012). The resistance

genes contained in the narrow genetic background of many potato breeding programmes are already unsuitable to breed novel PCN resistant varieties. The utilisation in breeding of other resistance sources, including those possessing the gene *Gro 1-4*, should be promoted to broaden the genetic background of newly developed cultivars.

The locus *H1* conferring resistance to *G. rostochiensis* pathotypes *Ro1* has been mapped to chromosome V (Gebhardt *et al.*, 1993). Skupinova *et al.* (2002) found a high correlation between the occurrence of the *H1* gene and phenotypic resistance to *G. rostochiensis*. According to Skupinová *et al.*, (2002), the 0.76 kb PCR marker detects only the dominant *H1* allele in potato genotypes. Schultz *et al.*, (2012) and Park *et al.*, (2018) reported that the presence of the 57R marker linked to the *H1* locus is predictive for PCN resistant phenotypes. Use of MAS in screening for resistance to *G. rostochiensis* permits early selection of potato clones carrying the *H1* gene (Milczarek *et al.*, 2014). Identification of potato cultivars resistant to *G. rostochiensis* by molecular and biological assessments to validate the suitability of marker TG689 has been recently reported (Galek *et al.*, 2011, Milczarek *et al.*, 2011, Felcher *et al.*, 2012).

Resistance to *G. rostochiensis* has been already introgressed into *S. tuberosum* from some wild *Solanum* L. species including *S. vernei* and *S. spegazzinii*, as well as the South American cultivated species *S. tuberosum* Group Andigena (Ross, 1986; Dale and de Scurrah, 1998). Potato cultivars resistant to the golden cyst nematode (pathotype *Ro1*) were developed by incorporating the *H1* gene from *S. tuberosum* Group Andigena (Ross, 1979; Gebhardt *et al.*, 1993). Despite Andigena being a source of resistance to nematode, Stelter (1971) reported its susceptibility to some *G. rostochiensis* pathotypes. Several cultivars were developed in the Netherlands using germplasm of the nematode resistance species *S. vernei* (Mulder and van der Wal, 1997). Interspecific hybrids included in this study were obtained from crosses with wild *Solanum* species, which were not evaluated in previous studies. Results of our study showed the low percentage of nematode resistant clones among hybrids obtained in crosses with late blight resistant Central-American species.

The habitats of potato species influence plant potential to resist diseases and pests due to long term selection under climatic conditions favourable for the pathogen (Hawkes and Hierting, 1989). Initial evaluation of *Solanum* germplasm showed *S. tuberosum* Group Andigena and *S. vernei* as the sources of resistance to *G. rostochiensis* (Ellenby, 1948, 1952, 1954). Dunnett (1960) was also one of the first to evaluate the large gene pool of tuber-bearing *Solanum* species, and determined useful traits for breeding purposes, including resistance to *G. rostochiensis* among the Bolivian species *S. microdontum* and *S. megistacrobolum*. Castelli *et al.* (2003) reported resistance to nematode found in Bolivian species *S. berthaultii*, *S. microdontum* as well as in *S. bukasovii* — a species partially distributed in Bolivia. Gene *H1* conferring resistance to pathotype *Ro1* was found in the accessions of *S. kurtzianum* (Dalamu *et al.*, 2012). These data

correspond to the results obtained in the current evaluation regarding resistance expressed by hybrids obtained in crosses with Bolivian *S. microdontum* and *S. tarijense* and Argentinian species *S. kurtzianum* with partial tuber resistance to late blight (Zoteyeva *et al.*, 2012). In hybridisation of mcd × tar with ktz we applied the bulk pollen of the accession ktz kVIR-12488. In the current study three of four tested parental ktz kVIR-12488 showed presence of the *Gro1-4* gene resistance allele.

Mexican *Solanum* L. species have also been evaluated for resistance traits. Castelli *et al.* (2003) reported screening results that showed the susceptibility in *S. guerreroense* and *S. stoloniferum* accessions and partial resistance in only 20% of tested *S. demissum* accessions. Resistance to PCN was found in accessions of *S. semidemissum* and *S. schenkii*, which is closely related to *S. demissum* (Castelli *et al.*, 2003). The current study identified resistance in clones of hybrids between *S. guerreroense* and clone R5 from the set of Black's late blight race differentials, which is a hybrid of *S. demissum*. In the population of this hybrid, the molecular marker linked to *Gro1* gene was amplified in all resistant clones. However, it was also amplified in a few phenotypically susceptible plants. Milczarek *et al.* (2011) reported examination of two markers linked to the gene *GroVI*, which failed to discriminate susceptible and resistant clones. The authors explained this phenomenon as occurring due to the highly heterozygous genetic background of tetraploid *S. tuberosum*. In the hybrid gr × Bd.R5 derived from the hexaploids *S. guerreroense* and *S. demissum* and the tetraploid *S. tuberosum*, marker *Gro1* was amplified, but in a few cases was not correlated with phenotypic resistance. The markers of the *H1* gene conferring resistance to *G. rostochiensis* pathotype Ro1 and *Gro1-4* revealed differing predictive abilities (Antonova *et al.*, 2016). To obtain reliable molecular screening results for disease resistance of potato clones, several markers should be used.

Plants of hybrids derived from *S. guerreroense* and *S. neoantipoviczii* (= *S. stoloniferum*) were mainly found to be susceptible. In our study, few of the tested clones showed combined phenotypic resistance to *P. infestans* and *G. rostochiensis*. The reason for the difficulty of identification of sources of combined resistance to both pathogens is the different geographical area of distribution of species characterised by resistance to *Globodera rostochiensis* and those with resistance to late blight. Species resistant to PCN predominate in southern Bolivia and Argentina, where 78% of accessions were resistant, in contrast to 25% from Mexico, where highly late blight resistant wild potato germplasm is distributed (Castelli *et al.*, 2003). In field and laboratory assessment, accessions of several Bolivian species were identified as highly or partially foliar late blight resistant (Zoteyeva *et al.* 2004, Zoteyeva *et al.* 2012). South-American species *Solanum spegazinii*, *S. famatinae* and *S. kurtzianum* resistant to *G. rostochiensis* with a distribution area in Argentina were also identified as tuber resistant to *P. infestans* (Zoteyeva 2006; 2012). Potato plants combining foliar late

blight resistance with resistance to *G. rostochiensis* can be found among the Bolivian species. It can be assumed that the combination of tuber late blight and nematode resistance can be found among the species with a distribution area in Argentina.

Currently potato breeding aims at expanding diversity of resistance sources to economically important pathogens. This goal is being achieved by using interspecific hybridisation. Wild potato species are mainly characterised by small tuber size and long stolons. Plants with improved agronomic characteristics and high resistance rating occur after backcrosses to cultivated potatoes within the populations of interspecific hybrids. The process of hybridisation using plant material included in current evaluation resulted in a number of multispecies hybrids with resistance to late blight and good tuber characteristics (Zoteyeva *et al.*, 2017). In our pre-breeding research the germplasm of *S. guerreroense*, *S. neoantipoviczii*, *S. kurtzianum* and *S. microdontum* × *S. tarijense* were involved. The first hybrid of *S. guerreroense* was obtained in crossing with Swedish variety Superb. This hybrid produced middle size tubers, a part of which had irregular shape and long stolons. The next hybrid progenies obtained through crossing of abovementioned hybrid with *S. tuberosum* breeding clone had short stolons and produced large size tubers of good morphology (Zoteyeva and Carlson-Nilsson, 2013). The hybrid derived from cross between *S. guerreroense* × 2 *S. tuberosum* and *S. kurtzianum* kVIR-12488 had a short stolon and produced over 50 regular shape tubers per plant (Zoteyeva, 2018). Interspecific hybrids with detected markers of genes conferring resistance to late blight and Potato Virus Y were obtained using *S. neoantipoviczii* (Zoteyeva *et al.*, 2017).

The data obtained in our study provided additional information on the resistance to *G. rostochiensis* (pathotype Ro1) of interspecific potato hybrids derived from crosses with species of differing geographical distribution. Summarising the results from the literature and our own data obtained in current and previous studies, we can conclude that the accessions and interspecific potato hybrid clones with combined resistance to late blight and to *G. rostochiensis* belong mostly to South American species with a distribution area in Bolivia and in Argentina. These species are characterised by presence of haplotype *Gro1-4*. Hybrids obtained in crosses with the Mexican species *S. neoantipoviczii* (= *S. stoloniferum*) from the taxonomic series *Longipedicellata* Buk. exhibited resistance when derived from combinations with PCN resistant germplasm.

This study verified the good correlation of the markers 57R and *Gro1* with resistance to PCN, but revealed the inconsistency between phenotypic resistance and screening for resistance to *G. rostochiensis* using the marker CP113. The identified interspecific crosses and markers 57R and *Gro1* can be further used in potato breeding for backcrossing into elite germplasm.

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KARTUPEĻU STARPSUGU HIBRĪDU AR IZTURĪBU PRET LAKSTU PUVI (*PHYTOPHTHORA INFESTANS*) UN NEMATODI (*GLOBODERA ROSTOCHIENSIS*) IDENTIFICĒŠANA

Lakstu puve (*Phytophthora infestans*) un zeltītā kartupeļu cistu nematode (*Globodera rostochiensis*) ir kartupeļu patogēni, kuri var ievērojami samazināt kartupeļu ražu. Šajā pētījumā kartupeļu starpsugu hibrīdi izmantoti kā rezistences avots, lai iegūtu augus ar izturību pret abiem ekonomiski nozīmīgajiem kartupeļu patogēniem: lakstu puvi un zeltīto kartupeļu cistu nematodi. Pētījuma mērķis bija identificēt hibrīdus ar izturību pret abiem patogēniem un pārbaudīt vairāku molekulāro marķieru piemērotību zeltītās cistu nematodes patotipa Ro1 izturīgu augu izlasei. Devīdesmit divi kloni no vienpadsmit kartupeļu starpsugu hibrīdiem, kas iegūti, krustojot kultivēto *S. tuberosum* grupu *tuberosum*, *S. tuberosum* grupu *Andigena*, *S. tuberosum* grupu *Phureja* un savvaļas *S. guerreroense*, *S. microdontum*, *S. kurtzianum*, *S. neoantipoviczii* un *S. tarijense* kartupeļu sugas, tika pārbaudīti, inokulējot un ar molekulārajiem marķieriem. Ar inokulēšanas metodi zeltītās cistu nematodes izturība tika identificēta deviņu hibrīdu pēcnācējos. Ar inokulāciju iegūtie rezultāti lielā mērā sakrāja ar molekulāro marķieru noteikto rezistences statusu, izmantojot marķierus, kas saistīti ar *H1* gēnu (marķieris 57R) un *Gro1-4* gēnu (marķieris Gro1). AR *H1* gēnu saistītais marķieris CP113 nebija polimorfs un nespēja noteikt rezistences statusu. Lakstu puves un zeltītās kartupeļu cistu nematodes izturība tika identificēta hibrīdos, kas iegūti, krustojot *S. microdontum*, *S. tarijense* un *S. phureja* sugu augus, kā arī *S. guerreroense* un Bleka *P. infestans* rasu diferenciāļos, kas nēsā gēnu *R-5*.