Late blight resistance of Solanum species and potato hybrids: the evidence from coupled phytopathological and molecular study

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SUMMARY
The panel of wild *Solanum* species and potato hybrid clones was studied by coupled phytopathological and SCAR-marker analyses. Late blight (LB) resistance was assessed in individual plants under the field conditions and in the laboratory, with the detached-leaf test. The novel lines derived from previously unexplored sources of LB resistance among the Bolivian diploid species *S. alandiae* manifested a successful field resistance in the trials through two epidemic years. SCAR markers were developed using the R1, R3a and RB gene sequences. By screening wild *Solanum* species and clones of potato hybrids after natural and artificial infestation by *P. infestans*, we established that the presence of SCAR markers R1-1205 was significantly related to LB resistance. This evidence suggests the practicability of employing this marker as a breeding tool for early prediction of LB resistance in a wide range of *Solanum* germplasms.

KEYWORDS
Phytophthora infestans, wild tuber-bearing *Solanum* species, clones of interspecific potato hybrids, R-genes, SCAR-markers

INTRODUCTION
The most efficient and ecologically sustainable way to thwart the global threat of LB is to breed new potato varieties manifesting durable resistance to a wide range of *Phytophthora infestans* races. Initial programs of breeding for potato LB resistance were based on germplasm introgression from *Solanum demissum*. Nowadays, breeders try to incorporate germplasms of other *Solanum* species recognized as promising sources of LB resistance. Robust and reliable DNA markers greatly facilitate mapping and

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isolation of new LB resistance genes and help identify and track them in the germplasm collections.

MATERIALS AND METHODS

Seeds and microtubers of wild *Solanum* species were obtained from the Vavilov Institute of Plant Industry, Russia (VIR), the Centre for Genetic Resources, the Netherlands (CGN) and NRSP-6 Potato Genebank, USA (PI), and potato tubers, from VIR and the Institute of Potato Husbandry, Russia. The plant genotypes used in this study belong to 213 accessions representing 21 wild tuber-bearing *Solanum* species and 26 potato interspecific hybrid clones which incorporate germplasm of *S. bulbocastanum*, *S. verrucosum*, *S. stoloniferum*, *S. polytrichon*, *S. pinnatisectum*, *S. acaule*, *S. alandiae*, *S. spegazzinii*, *S. microdontum*, *S. berthaultii*, *S. andigenum*, *S. rybinii*, *S. phureja* and demissoid cultivars. To check the response to LB in individual plants, the field and detached-leaf trials were conducted (for the protocols see Rogozina *et al.*, 2010). We developed SCAR markers for the genes R1, R3 of *S. demissum* and RB of *S. bulbocastanum* and for the corresponding germplasms (for markers design, amplification and cloning see Khavkin *et al.*, 2010).

RESULTS AND DISCUSSION

Field trials

Conducive weather conditions enabled rapid establishment of LB in both years (2008-2009) of trial, so that susceptible standards (cv. Bintje, accessions of *S. kurtzianum*, *S. acaule*) were defoliated finally by the end of July. In 2009, disease progress was much slower than in 2008; nevertheless, some cultivars previously considered resistant (Nevsky, Najada) were noticeable affected. Field trials demonstrated that *Solanum* species and hybrid clones showed a range of variability for infestation. Among *Solanum* species, individuals of *S. stoloniferum* and *S. pinnatisectum* showed higher levels of foliar resistance, the individuals of *S. demissum*, *S. bulbocastanum* and *S. polyadenium* showed marked differences in the LB lesions, the response of *S. jamesii* and *S. cardiophyllum* individuals depended on particular year conditions, whereas the individuals of *S. fendleri*, *S. brachystotrichum* and *S. stenophyllidum* were susceptible. The factorial analysis indicated that both factors, plant species and the year of testing, significantly affected the final defoliation of tested plants, and these two factors did not interact.

Potato hybrid clones and varieties were divided into two groups according to average AUDPC values in three-year trial (2007-2009). The first group included 12 hybrid clones with high levels of resistance (not more than 20% foliar infection by the end of growth period). The second group included rest of hybrid clones and cvs. Peterburgsky and Najada wherein the extent of the damage was more severe and variable. These genotypes manifested intermediate resistance (20% to 50% foliar infestation depending on the year, Fig.1). Potato hybrid clones and varieties demonstrated the same rank order of LB resistance across three trial years indicating stability of this trait. The growth period of potato hybrid clones and varieties was significantly shorter than that of wild *Solanum* species. Potato crop was removed by the end of August to early September. Wild *Solanum* grew for another month and therefore were more affected with LB.

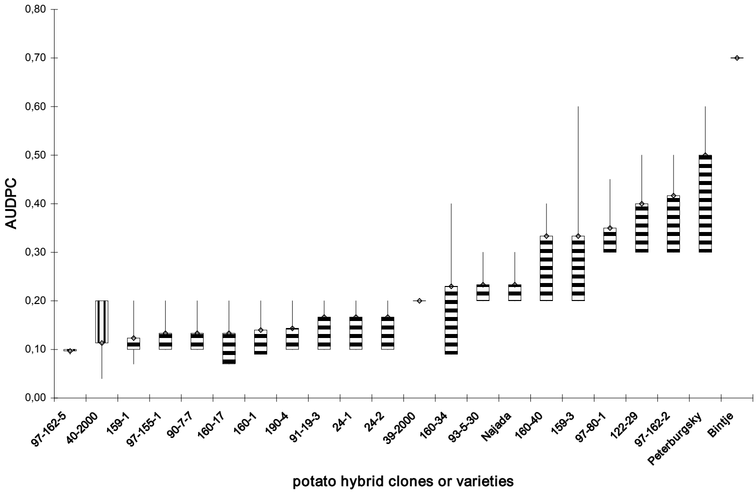

Figure 1. rAUDPC values for potato hybrid clones and varieties across the three-years trial

Detached-leaf trials

Reaction of some *Solanum* genotypes to artificial infection by *P. infestans* was quite remarkable. Leaves of *S. polyadenium* were not affected at all or manifested only point necroses, leaves of *S. verrucosum* showed large necrotic lesions, and leaves of individual plants of *S. demissum*, *S. stoloniferum* and *S. bulbocastanum* variable responses to infestation similar to those observed with whole plants in the field trial. Leaves of all individuals of *S. stenophyllidum* and *S. fendlerii* produced large necrotic spots with sporulation indicating high susceptibility to *P. infestans*. Leaves of most potato hybrid clones showed necrotic reactions without sporulation indicating the horizontal type of resistance. A single clone 97-162-5 was not damaged by artificial infestation; such evidence matches the durability of its LB resistance in the field (Fig.1).

Marker analysis

The specificity of markers based on the R-gene sequences was verified with the cultivars reportedly free of wild *Solanum* germplasm and potato cultivars comprising the germplasm of *S. demissum*. All markers reliably discerned cultivated potato from wild *Solanum* species (Sokolova *et al.* 2011). Markers specific for the R-genes introgressed from *S. demissum* were found in not only *S. demissum* genotypes and cultivars reportedly originating from *S. demissum*. The marker R1-1205 was present in the accessions of *Solanum iopetalum*, *S. polytrichon*, and *S. stoloniferum*. The marker R3-1380 was present in the accessions of *S. hougasii*, *S. stoloniferum*, *S. cardiophyllum*, *S. ehrenbergii* and *S. bulbocastanum* (Table 1). Our study demonstrates the presence of R-like sequences in genotypes of a wide range of *Solanum* species. RB-like sequences were exposed in an especially wide range of *Solanum* species section Petota (Pankin *et al.* 2010).

Comparison of the data obtained by phytopathological tests and marker analyses for wild *Solanum* shows that the presence of the marker for the genes R1 and R3a initially characterized in *S. demissum* in most cases matched the evidence for LB resistance. The agreement was higher for R1-1205 than for R3-1380 (Table 1). The association between the presence of R1-1205 marker and LB resistance was significant according to Pearson chi-square test: $\chi^2 = 6,63<3,84$. The association between the presence of R3-1380 and LB resistance was not significant: $\chi^2 = 1,63>3,84$.

Table 1. The presence of markers for R-genes in Solanum genotypes with diverse reactions to P. infestans

Solanum species	Number of tested accessions	Including resistant accessions	Presence of SCAR markers in resistant accessions *		Including susceptible accessions	Presence of SCAR markers in susceptible accessions *	
			R1-1205	R3-1380		R1-1205	R3-1380
S. verrucosum	7	0			7	0	0
S.demissum	20	17	10	1	3	0	0
S. iopetalum	1	1	1	0	0		
S. hougasii	2	2	0	1	0		
S. fendlerii	4	0			4	0	0
S. hjertingii	3	1	0	0	2	0	0
S. papita	3	2	0	0	1	0	0
S. polytrichon	8	2	1	0	6	0	1
S. stoloniferum	20	5	1	1	15	3	2
S. brachystotrichum	3	0			3	0	0
S. jamesii	8	3	0	0	5	0	0
S. pinnatisectum	4	2	0	0	2	0	0
S. tarnii	3	2	0	0	1	0	0
S. polyadenium	6	6	0	0	0		
S. cardiophyllum	4	3	0	2	1	0	0
S. ehrenbergii	11	2	0	0	9	0	2
S. bulbocastanum	18	16	0	5	2	0	0
Total	125	64	13	10	61	3	5

*Note: the number of genotypes comprising the marker

To link the presence of the R-genes to LB resistance scores in hybrid clones, we used the non-parametric Mann-Whitney test. The statistical analysis demonstrates highly significant association (z=-1, 97, p=0, 04) between the presence of R1-1205 marker and LB resistance. The frequency of R1-1205 was notably higher in the first group of hybrid clones with stable LB resistance (Fig.1, Table 2). The association of high LB resistance with the presence of two other gene markers was not that evident.

None of analyzed markers were detected in two hybrid clones under study: 24-2 and 97-155-1. Both these clones possess high LB resistance; therefore, we presume that R-genes other than R1, R3a and RB or some yet unidentified QTLs for LB resistance contribute to their sustainability in the field. Our study demonstrates the suitability of novel lines derived from the Bolivian diploid species *S. alandiae* for use as a source of LB resistance.

It is noteworthy that, opposite to the evidence discussed above, we failed to relate LB resistance of wild *Solanum* species to the presence of R-genes recognized with the simple races of *P. infestans*, apparently because of more complicated virulence patterns of these isolates. To illustrate, we found that these races comprised the IpiO gene alleles encoding the effectors recognized by Rpi-blb1/RB gene from *S. bulbocastanum* and *S. stoloniferum* (Pankin *et al.*, this volume).

The association of particular *Solanum* germplasms in hybrid clones with the presence of the markers for R1 and R3a was found in most cases. Markers specific for the R-genes introgressed from *S. demissum* were found most often due to pedigree of all hybrid clones tracing back to demissoid cultivars. SCAR markers for RB were found in hybrid clones comprising the germplasm of *S. bulbocastanum* and in hybrid clones free of such germplasm (Table 2). In the latter case, some of these hybrids were derived from *S. stoloniferum* germplasm, which is known to contain the R*pi-blb*1 ortholog.

Table 2. The presence of markers for R-genes in potato interspecific hybrid clones

Hybrid clones	Wild *Solanum* species in potato pedigrees[1]	Presence of SCAR-markers[2]		
		R1-1205	R3-1380	RB-638
24-1	*aln, dms*	1	0	0
24-2	*aln, dms*	0	0	0
190-4	*and, dms*	1	0	1
97-155-1	*and, dms*	0	0	0
160-1	*and, dms*	1	0	0
160-17	*and, dms*	1	0	0
160-34	*and, dms*	0	0	1
160-36	*and, dms*	0	0	0
160-40	*and, dms*	0	0	1
89-1-12	*and, sto, dms*	1	0	0
90-7-7	*and, sto, dms*	1	0	1
159-1	*and, sto, dms*	1	0	0
159-3	*and, sto, dms*	1	0	0
159-31	*and, sto, dms*	0	1	0
97-162-2	*and, sto, dms*	0	0	0
97-162-5	*and, sto, dms*	1	0	0
97-80-1	*and, vrn, dms*	1	0	0
122-29	*and, spg, mcd*	0	0	0
91-19-3	*and, blb, sto, acl, dms*	0	1	1
93-5-30	*and, phu, blb, sto, acl, dms*	0	0	0
11-1	*and, ber, dms*	0	0	1
11-2	*and, ryb, ber, dms*	1	0	0
12-2	*and, ryb, sto, ber, dms*	0	0	0
13-1	*and, ryb, sto, mcd, dms*	1	0	0
39-2000	*smp, dms*	n.d.	n.d.	n.d
40-2000	*smp, plt, dms*	1	0	1

[1] *acl – S. acaule, aln – S. alandiae, and – S. andigenum, ber – S. berthaultii, blb - S. bulbocastanum, dms – S. demissum, plt – S. polytrichon, phu – S. phureja, sml – S. simplicifolium, sto – S. stoloniferum, vrn – S. vernei*

[2] n.d. –no data

CONCLUSION

Our results indicate that R genes for LB resistance or their structural homologues are universally distributed across wild *Solanum* section Petota and in the progenies of crosses between wild species and cultivated potato. The presence of R1-1205 marker in LB resistant material with diverse background indicates the practicability of this marker as a breeding tool for early prediction of LB resistance in a wide range of *Solanum* germplasms.

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