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In quest for new sources of late blight resistance in the VIR collection of wild potato germplasm

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SUMMARY

Seedlings of 40 species wild tuber-bearing *Solanum* spp. maintained in VIR or introduced to VIR from other potato genebanks were assessed for late blight resistance under the field and laboratory conditions. Among 287 *Solanum* accessions, few were resistant (6-16%), half of accessions were susceptible, whereas about one third of accessions were heterogeneous seed populations segregating for resistant, moderately resistant and susceptible individuals. Newly isolated accessions of *S. doddsii* and *S. okadae* are prospective initial sources for breeding late blight resistant potato varieties. When the resistance indices obtained in these studies were compared to the previously obtained data, we observed medium correlation, with the Spearman's coefficient of 0.56 at $p<0.05$. The specific problems of maintaining and reproducing seed collections of wild potato species are under discussion.

KEYWORDS

Phytophthora infestans, wild tuber-bearing *Solanum* spp., *ex situ* population, potato late blight*,* durable resistance

INTRODUCTION

Rapid evolution of the pathogenic oomycete *Phytophthora infestans* Mont. De Bary presents the main obstacle on the road to breeding new potato varieties with high and durable resistance to late blight (LB). The best way to confront this threat is by developing the initial breeding material - potato parental lines that carry several resistance genes, providing in this way for the broad range of specificity towards diverse races of *P. infestans.* Wild tuber-bearing *Solanum* species, which have not yet been involved in commercial breeding, are of particular interest. The VIR collection comprises 2100 accessions of 140 tuber-bearing relatives of *Solanum tuberosum* L. (Kiru, Rogozina, 2017). This gene pool, which has been accumulated for almost a century, has been evaluated annually for numerous agronomic traits including many disease resistances. The objective of this study was to assess the samples of *Solanum* spp. for LB resistance and elucidate whether this resistance is maintained in accessions characterized as resistant in previous studies, a decade or over.

MATERIALS AND METHODS

Plant material and assessment of LB resistance

Nine of 19 tuber-bearing series of Subsection Potato G. Don. represented 40 *Solanum* species were assessed for LB resistance in the field and in the laboratory. Botanical seeds were either the samples (accessions) from VIR or were donated by others potato genebanks: NRSP-6 Potato Genebank (the USA), CGN (Centre for Genetic Resources, the Netherlands) and the Gross Lüesewitz Potato Collection of the IPK Genebank (GLKS, Germany). Species are listed in Table 1 according to J.G. Hawkes (Hawkes, 1990).

Table 1. *Plant material tested for LB resistance*

Solanum group	Series	*Solanum* spp. (number of accessions)
North American	Bulbocastana	*S. bulbocastanum* (4)
	Pinnatisecta	*S. cardiophillum* (10), subsp. *ehrenbergii* (2), *S. jamesii* (6), *S.× michoacanum* (6), *S. pinnatisectum* (8), *S. trifidum* (4)
	Polyadenia	*S. lesteri* (2), *S. polyadenium* (2)
	Tuberosa (wild)	*S. verrucosum* (6)
	Longipedicellata	*S. fendleri* (18), *S. hjertingii* (6), *S. papita* (4), *S. polytrichon* (15), *S. stoloniferum* (31)
	Demissa	*S. brachycarpum* (4), *S. demissum* (26)
South American	Acaulia	*S. acaule* (2)
	Yungasensa	*S. chacoense* (14)
	Tuberosa (wild)	*S. abancayense* (4), *S. leptophyes* (3), *S. medians* (4), *S. mochiquense* (3), *S. multidissectum* (5), *S. multiinterruptum* (4), *S. sparsipilum* (5), *S. alandiae* (15), *S. avilesii* (3), *S. berthaultii* (7), *S.×doddsii* (7), *S. gandarillasii* (5), *S. gourlayi* (5), *S. hondelmannii* (6), *S. incamayoense* (4), *S. kurtzianum* (2), *S. microdontum* (8), *S. okadae* (8), *S. oplocense* (4), *S. spegazzinii* (4), *S.×sucrense* (4), *S. vernei* (7)

The number of tested accessions of each *Solanum* species ranged between 2 and 31 (Table 1). For each accession, 10 to 20 seedlings were grown in the field, and 3 to 5 seedlings in the greenhouse. LB resistance of wild potato plants was assessed in the field trials at Pushkin (St. Petersburg, Russia) under conditions of natural infestation and scored by 1 to 9-point scale, where 9 corresponds to no visible lesions. The sightings of LB distribution were started when the first lesions appeared on wild potato plants. The final index of LB resistance of each tested accession was defined as the maximum damage score at the end of the growing season. LB resistance of greenhouse plants were assessed in the laboratory test conducted in VIR. Detached leaves test was performed according to the Eucablight protocol (www.euroblight.net) using highly virulent and aggressive isolate of *P. infestans* collected in the Leningrad region. Leaflets collected from 30-55-day-old plants were infected by pathogen isolates containing the race 1,2,3,4,5,6,7,8,9,10,11. Three leaflets per each tested genotype were inoculated with zoospore suspension at the concentration of 30-40 ×1000 per ml according to standard protocol. Scoring by 1 to 9-point scale was carried out 5-7 days after inoculation.

RESULTS AND DISCUSSION

Results of a current study of Solanum spp.

In 2016-2018 a total 287 accessions representing 40 *Solanum* spp. were assessed in the field. During the field trials, the weather conditions in the growing season were not the same; the onset of lesions and the pattern of LB development also varied. The earliest LB manifestation was registered in 2016, when the first symptoms appeared in July, and by the beginning of August all plants of *S. acaule*, *S. fendleri*, *S. hondelmannii*, *S. kurtzianum* and *S. oplocense* were completely affected. Under such conditions of early emergence and rapid LB development, high resistance was observed in accessions of *S. doddsii* k-18240, *S. lesterii* k-24475, and *S. polyadenium* k-24461: here the leaf area affected by LB did not exceed 50% by the end of the growing season. In 2017, the initial LB symptoms were noted in the first decade of August, and disease developed slowly; as a result, by early October, resistant accessions *S. berthaultii* k-19961, *S. famatinae* (*S. spegazzinii* syn.) k-7466, *S. lesterii* k-24475, *S. polyadenium* k-24461, *S. stoloniferum* k-5431, *S. vernei* k-23771, and *S. verrucosum* k-23015, k-24315, k-24427 were scored as 5-7 points. Early in the 2018 growing season, hot weather with heavy if short rainfalls favored the development of *Alernaria* ssp. A significant number of *S. demissum* and *S. fendleri* accessions were completely defeated by the first decade of August, which indicates their extreme susceptibility to this disease. Seedlings of *S. okadae* differed in their response to *Alernaria*. In September, with the onset of LB, we noted disease-resistant accessions of *S. demissum* k-24378, *S. lesterii* k-24232, *S. stoloniferum* k-24189, *S. okadae* k-25394 and *S. tarnii* k-23936. Every year of field testing, responses to LB widely varied among species and among accessions within species. The proportion of highly resistant (7-9 point) and susceptible (1-3 point) accessions notably differed in North and South American groups of *Solanum* species: the percentage of highly resistant accessions was 16 and 6, respectively, whereas for susceptible accessions, the respective indices were 47 and 54%. Both in the North American and South American groups of *Solanum* species under study, we registered the same proportion of accessions (34%) to segregate by resistance in the range of 1 to 7 points. Segregation in LB resistance was observed within accessions of *S. microdontum*, *S. okadae*, *S. vallis-mexici*, and *S. vernei* (Table 2).

Table 2. *Solanum accessions manifesting high LB resistance*

Solanum **spp.**	**Catalogue numbers of VIR accessions**	**LB resistance score (field\ laboratory test)**	**The corresponding accessions numbers in other collections***	**Previously reported data ****
S. berthaultii	k-19961	5\n.d	PI 473331	R
S. chacoense	k-7394	9\7	PI 320292	S
	k-2861	8\2.3-2.5	no	
S. demissum	k-2353	9\3.7	no	
"	k-20000	7\n.d.	PI 498230	R
"	k-24378	3-8\n.d.	PI 160221	R
S.× doddsii	k-18240	5-7\n.d.	PI 442690	n.d.
S. famatinae	k-7466	3-7\n.d.	no	
S. lesterii	k-24232	3-7\n.d.	GLKS 2782	n.d.
"	k-24475	5-7\n.d.	no	
S.× michoacanum	k-5763	6\5-7	no	
S. microdontum	k-25385	1-7\n.d.	CGN 20597	VR\M
S. stoloniferum	k-5135	7\n.d.	no	
"	k-5431	5-7\n.d.	no	
"	k-24189	7\n.d.	GLKS 66	n.d.
S. okadae	k-25394	5-8\5.5-7.7	CGN 17998	VR
"	k-25395	1-8\n.d.	CGN 17999	R
"	k-25397	3-9\2.7-8.0	CGN 18279	VR\M
S. tarnii	k-23936	7\n.d.	PI 498043	n.d.
S. trifidum	k-24984	7\n.d.	PI 283064	R
S. vallis-mexici	k-8473	1-7\4-5.7	no	
S. vernei	k-23771	5\n.d.	PI 458373	R
"	k-25413	1-7\n.d.	CGN 18112	R\R
S. verrucosum	k-23015	5-7\n.d.	PI 558482	R
"	k-24315	5\ n.d.	n.d.	
"	k-24427	7\n.d.	PI 275260	R

**PI, accession from NRSP-6 Potato Genebank, the USA; CGN, Centre for Genetic Resources, the Netherlands; GLKS, Gross Lüesewitz Potato Collection of the IPK Genebank, Germany*

*** R, resistant; VR, very resistant; M, moderately resistant; S, susceptible (see https://www.ars-grin.gov/nr6/ and Pel, 2010); n.d., no data*

Detached leaf tests were carried out in 2017. Variation among species and among accessions within species was also observed in this test. Resistance for LB artificial infestation (5-7 score) was found in accessions *S. chacoense*, *S.× michoacanum, S. mochiquense, S. okadae*. Detached leaf test confirmed the field data for LB resistance in some promising accessions of *S. chacoense* k-7394, *S.× michoacanum* k-5763, *S. okadae* k-25394, whereas *S. okadae* k-25397 and *S. vallis-mexici* k-8473 accessions segregated for resistance (Table 2).

Results in comparison with previous data

Our data do not completely match the evidence obtained with *Solanum* accessions from other collections. While we confirmed high LB resistance in accessions of *S. demissum* k-20000, *S. trifidum* k-24984, *S. verrucosum* k-23015 and k-24427, the accessions of *S. demissum* k-24378 and *S. vernei* k-25413, which were previously reported as resistant, were found to segregate for LB response in our field tests. Meanwhile the accession *S. chacoense* PI 320292 reported as susceptible in the USA tests behaved as resistant in our field and laboratory experiments (Table 2). We compared our current data with the evidence composed in the previous studies of the VIR collection (Zoteyeva *et al.* 2004). The data for LB resistance from two sources covering 82 accessions of 33 *Solanum* species matched moderately (the Spearman's correlation coefficient of 0.56 at $p<0.05$). This bulk of these data let us select 20 accessions of nine *Solanum* species with the stable level of LB resistance: *S. demissum* (4), *S. stoloniferum* (5), *S. pinnatisectum* (5), *S. jamesii* (1), *S. cardiophyllum* (1), *S.*× *michoacanum* (1), *S. avilesii* (1). *S. microdontum* (1) and *S. okadae* (1). Of special interest are the accessions of *S. chacoense* k-2861, *S. demissum* k-2353, *S. famatinae* k-7466, *S.*× *michoacanum* k-5763, *S. stoloniferum* k-5135, k-5431 and *S. vallis-mexici* k-8473, which were acquired by the VIR collection in 1953-1968 following the introduction of genetic materials from the collection of J. Hawkes and the Gross Lüesewitz Potato Collection. Through past half of a century, many accessions from this pool passed through several multiplication cycles. VIR Pushkin laboratories is the only place engaged in reproduction of wild potato species. Botanical seeds are gathered from plants grown in field and glasshouse. In field, the environmental conditions are most advantageous for LB development. *P. infestans* populations in the Pushkin potato stands changed similarly to those in commercial potato fields (Patrikeeva *et al.*, 2011; Vedenyapina *et al.*, 2002). Such accessions revealed in the VIR collection are extremely valuable for researching into the fundamentals of durable LB resistance of wild potato relatives, which is most important for their further deployment as the initial breeding sources.

Wild relatives of potato are *ex situ* conserved in germplasm banks as samples of seed populations (accessions). A key operation of a genebank that maintains botanical seeds is regeneration (also called "multiplication") in order to expand the amount of stored seeds and/or to increase their viability. This process includes the multiplication of seeds sampled *in situ* from an initial generation (original sample) or from the next generation sample donated by another germplasm holder. Multiplication is an activity that could easily affect the genetic composition of a resulting accession. Genebank Standards for Plant Genetic Resources prepared by FAO (2013) lay down the key principles that make up the core of genebank operations, i.e. the preservation of germplasm identity, maintenance of seed viability and genetic integrity, and the promotion of access. New consumer demands, climatic changes and shifts in the populations of pathogens cohabiting with potatoes as well as enhanced pathogenicity of previously innocuous microorganisms - all these factors call for a systematic study of the diversity in the collections of wild and cultivated potatoes. Today genebanks should take on a new role- not just being a repository providing germplasm resources, but also a research center to advance deeper understanding of genetic diversity (Bethke *et al.*, 2019; Mascher *et al.* 2019). A coupled study of genotypic and phenotypic traits of *Solanum* tuber-bearing species and *P. infestans* strains colonizing potato plants in the VIR collection is an important focus of multi-year research in this country (Chizhik *et al.*, this issue; Fadina *et al.*, 2017). This study produced the characteristics of individual genotypes (clones) representing the wide scope of wild and cultivated *Solanum* species. The next step will be to study the seed populations from the VIR collection in order to better comprehend the diversity of crop plants and their wild relatives and in this way develop new strategies to maintain these collections and successfully deploy them in breeding.

CONCLUSION

Among 287 accessions representing 40 wild tuber-bearing *Solanum* species from the VIR collection, only a small number (6-16%) are homogeneous populations resistant to late blight. About one third of the accessions under study are heterogeneous populations manifesting a wide range of diversity in plant response to late blight: resistant, moderately resistant and susceptible genotypes. The results of previous studies evaluating late blight resistance of potato accessions from the VIR collection and other potato genebanks are not fully consistent: newly obtained data and the evidence on accessions from the VIR collection assessed earlier did not strongly correlate. Further research will hopefully help to elucidate the causes of these discrepancies.

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