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Pyramiding *R* genes: genomic and genetic profiles of late-blight resistant interspecific potato hybrids

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SUMMARY

Clones of potato hybrids comprising genetic material from up to seven wild *Solanum* species were studied with phytopathological methods and DNA markers for *Solanum* genomes A, B and D and for late blight resistance genes *R1*, *R2/Rpi-blb3, R3a, R3b* and *RB/Rpi-blb1.* Late blight resistance of these clones was obviously associated with the presence of DNA markers for the *R* genes and significantly related to the number of these markers discerned in particular interspecific hybrids.

KEYWORDS

Phytophthora infestans, wild *Solanum* species, potato hybrids, late blight resistance, stacking genes, DNA markers.

INTRODUCTION

Stacking (pyramiding) several resistance genes of diverse race specificity by hybridization or genetic transformation is presently seen as an upcoming venue to broad-spectrum and durable late blight (LB) resistance (Tan *et al.*, 2010; Kim *et al.*, 2012; Zhu *et al.*, 2012). Here we describe two sets of interspecific potato hybrids, which comprise germplasms from several *Solanum* species per genotype, manifest high LB resistance and therefore are promising sources for breeding new potato cultivars with wide-range LB resistance (Rogozina *et al.*, 2013). SCAR markers for *Solanum* genomes A, B and D and for five LB resistance genes were used to screen these hybrids. We found that the profiles of genome markers were in good agreement with the reported pedigrees and breeding histories of the hybrids. In parallel, we demonstrated that the patterns of *R* genes in the hybrids were in most cases explained by the evidence on the *R* genes in wild diploid and polyploid *Solanum* species that were reportedly involved in breeding these hybrids. The data from collated molecular and phytopathological studies infer that stacking *R* genes in interspecific hybrids significantly fortifies their LB resistance.

MATERIALS AND METHODS

Two sets of interspecies hybrids (Table 1) comprising germplasms from two to ten *Solanum* species were developed in the Institute of Potato Husbandry, Korenevo, Moscow (IPH) and the Institute of Plant Protection, Pushkin. St. Petersburg (IPP). Most hybrids displayed high foliage LB resistance in the field trials under natural infection conditions and high-to-moderate resistance in the laboratory assays with detached leaves infected with highly virulent complex race isolates of *Phytophthora infestans*. As far as it was possible, our study also included wild *Solanum* species reportedly involved in breeding these hybrids (Table 2).

Standard protocols were employed to extract DNA from young leaves and PCR-amplify genome fragments. Hybrids were screened with SCAR markers for *Solanum* genomes A, B and D developed from low-copy *LEAFY* (Drobyazina and Khavkin, 2012) and COSII genes (Wu *et al.*, 2006). In the latter case, SCAR markers were designed using the sequences from several *Solanum* species cloned by the authors with the previously described primers (Wu *et al.*, 2006; Rodriguez *et al.*, 2009). The profiles of CC-NBS-LRR genes for LB resistance (*R* genes) were assessed with SCAR markers specific for the genes *R1, R2/Rpi-blb3, R3a, R3b*, and *RB/Rpi-blb1* (Sokolova *et al.,* in press).

The data were processed using the Statistica 6 package (StatSoft, http://www.statsoft.com/).

RESULTS AND DISCUSSION

All interspecific potato hybrids under study comprise markers for genomes A and D derived from *S. tuberosum* and *S*. *demissum*, and some hybrids contain the markers for genome B apparently transferred with the germplasm of *S. polytrichon* = *S. stoloniferum*. The hybrids comprising genome B markers contain the marker for *Rpi-blb1*, the gene found only in *S. bulbocastanum* and *S. stoloniferum* (Fadina *et al.*, in press). There were two exceptions: hybrids IPP10 and IPP12 reportedly comprising the AB germplasm from *S*. *stoloniferum* and *S. vallis-mexisi*; here we find the *Rpi-blb1* marker in the absence of the genome B marker (Table 1). The evidence on genomes and *R* genes in the hybrids agrees fairly well with the profiles previously established in wild *Solanum* species (Table 2), thus supporting the pedigrees of the hybrids.

***Table 1**. Genome and R-gene patterns in interspecies hybrids bred in the Institute of Potato Husbandry (IPH) and the Institute of Plant Protection (IPP)*

Hybrids	Hybrid pedigrees (*Solanum* species)*	*Solanum* genomes recognized with SCAR markers	*R* genes for LB resistance established with SCAR markers	LB resistance***
IPH1	*chc, cmm, dms, mga, tbr*	A1-A3, D	*R3a, R3b*	MS/4
IPH2	*adg, chc, dms, sem, tbr*	A1-A3, D	*R3b*	S/3
IPH3	*chc, cmm, dms, mga, tbr*	A1-A3, D	*R1, R3a, R3b*	MR/7
IPH4	*dms, tbr*	A, D	*R1, R2/Rpi-blb3, R3a, R3b*	MS/4
IPH5	*adg, chc, dms, sto, tbr*	A1, A3, D	*R1, R2/Rpi-blb3, R3a, R3b*	MR/6
IPH6	*adg, chc, dms, tbr*	A1, A3, D	*R2/Rpi-blb3, R3a, R3b*	MS/5
IPH7	*adg, chc, cmm, dms, edn, mga, ryb=phu, tbr*	A, A1, A3, D	*R2/Rpi-blb3, R3a, R3b*	MR/6
IPH8	*dms, tbr*	A, A1, A3, D	*R2/Rpi-blb3, R3b*	MS/5
IPH9	*dms, tbr*	A, A1, A3, D	*R1, R2/Rpi-blb3, R3a, R3b*	R/8
IPP10	*adg, dms, mcd, plt=sto, tbr, vlm*	A1-A3, D	*R2/Rpi-blb3, R3a, R3b, RB/Rpi-blb1*	MS/4
IPP11	*adg, dms, mcd, plt=sto, tbr, vlm*	A1-A3, B, D	*R2/Rpi-blb3, R3a, RB/Rpi-blb1*	S/3
IPP12	*adg, ber, dms, mcd, plt = sto, pnt, tbr, vlm*	A, A1, A3, D	*R2/Rpi-blb3, R3a, RB/Rpi-blb1*	MR/6
IPP13	*adg, dms, pnt, tbr*	A, A1, A3, D	*R2/Rpi-blb3, R3a*	MS/5
IPP14	*adg, ber, dms, mcd, plt=sto, pnt, tbr, vlm*	A1-A3, B, D	*R2/Rpi-blb3, R3a*	MS/4
IPP15	*adg, ber, dms, mcd, phu, plt=sto, pnt, tbr, vlm, vrn*	A1-A3, B, D	*R2/Rpi-blb3, R3a, RB/Rpi-blb1*	MR/6.5
IPP16	*adg, ber, dms, mcd, plt=sto, tbr*	A1-A3, D	*R1, R2/Rpi-blb3, R3a, RB/Rpi-blb1*	MR/7
IPP18	*dms, mcd, pnt, tbr*	A1, A2, D	*R1, R2/Rpi-blb3, R3a*	MS/4
The EUCABLIGHT standard cultivars				
Alpha	*tbr*	A	none	S/3
Bintje	*tbr*	A	none	S/3
Eersteling	*tbr*	A	none	S/3
Robijn	*tbr*	A	none	MS/4
Escort	*dms, tbr*	A, D	*R2/Rpi-blb3, R3a, R3b*	MR/7
Sarpo Mira	*dms?, tbr*	A, D	*R3a, R3b***	R/8

**Abbreviations of Solanum species. adg - S. andigenum, ber - S. berthaultii, chc - S. chacoense, cmm - S. commersonii, dms - S. demissum, edn - S. edinense, mcd – S. microdontum, mga - S. megistacrolobum, phu – S. phureja, plt - S. polytrichon (= S. stoloniferum), pnt - S. pinnatisectum, ryb - S. rybinii = S. phureja, sem – S. semidemissum, sto - S. stoloniferum; tbr - S. tuberosum, vlm - S. vallis-mexisi, vrn – S. vernei.*

*** According to Rietman et al. (2012), Sarpo Mira comprises at least four R genes.*

****Grades/points of LB resistance in detached leaf assays: R, resistant (points 8-9), MR, moderately resistant (points 6-7), MS, moderately susceptible (points 4-5), S, susceptible (points ≤3).*

We also compared the patterns of *R*-gene markers in the interspecific hybrids with their LB resistance evaluated in the detached leaf assays. The EUCABLIGHT standard cultivars free of wild *Solanum* germplasm (Alpha, Bintje, Eersteling, and Robijn) served as a negative control, and cultivars Escort and Sarpo Mira, as a positive control group with high LB resistance. The *R* genes obviously fortified LB resistance of potato hybrids, and the correlation between the number of *R*-gene markers and LB resistance was highly significant, with the Spearman's coefficient of 0.63 (Table 3; Fig. 1).

Table 2. *Some wild Solanum species reported in pedigrees of interspecies potato hybrids*

Series	Species	Genomes established		*R* genes established by molecular studies**
		by classical genome analysis	by molecular technologies*	
Tuberosa	*S. berthaultii*		A1A1, A3A3	*R1, R3b*
	S. microdontum	AA	A1A1, A3A3	*R1, R3a, Rpi-mcd1*
Longipedicellata	*S. stoloniferum, S. vallis-mexisi*	AABB	AABB, A1A1BB	*R1, R2/Rpi-blb3, R3a, R3b, Rpi-blb1*
Demissa	*S. demissum*	AADDD'D', A1A4[B,C,D]	AAPPPP, AABB, A1A1D	*R1, R2, R3a, R3b*
Bulbocastana	*S. bulbocastanum*	AbAb	BB	*R2/Rpi-blb3, R3a, R3b, Rpi-blb1, Rpi-blb2, RB-bt1*
Pinnatisecta	*S. pinnatisectum*	ApiApi	BpiBpi, BB	*R2, R3a, R3b*

**Compiled from Rodriguez et al. (2009): Drobyazina and Khavkin (2012).*

*** For more details see Sokolova et al. (in press).*

Table 3. *LB resistance of potato hybrids as affected by pyramiding the R genes*

R-gene markers per plant	Groups of genotypes	Average resistance, points*
0	Standard *R*-gene free cultivars Alpha, Bintje, Eeertstellung, Robijn	3.3
1	IPH2	3
2	IPH1, IPH8, IPP13, IPP14	4.5
3	IPH3, IPH6, IPH7, IPP11, IPP12, IPP15, IPP18	5.3
4	IPH4, IPH5, IPH9, IPP10, IPP16	5.8

**Detached-leaf assays.*

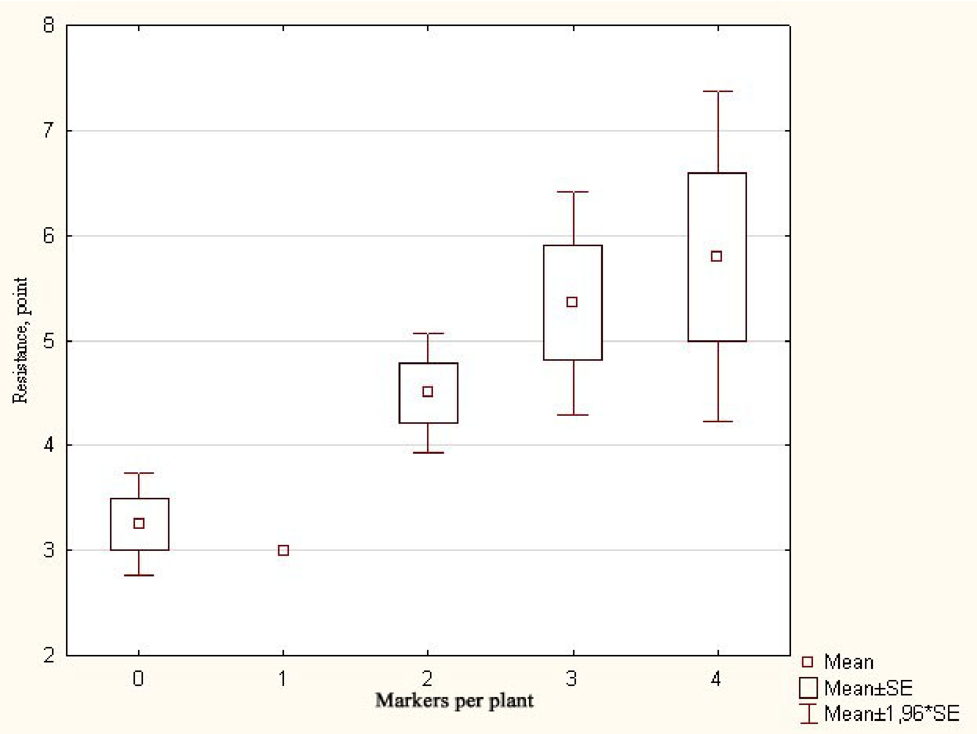

***Figure 1**. The effect of stacking R genes in interspecific potato hybrids on their LB resistance in detached-leaf assays*

CONCLUSION

While the race-specific *R* genes are commonly held to be overcome (defeated) by new virulent races of *P. infestans,* our data support the hypothesis that these genes provide a discernible input to LB resistance (Gebhardt, 2013). Stacking *R* genes in interspecific hybrids obviously promotes their LB resistance.

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