

## Structural homologues of CC-NBS-LRR genes for potato late blight resistance in wild *Solanum* species

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### SUMMARY

Breeding potato for durable resistance to late blight (LB) greatly benefits from expanding the resource of resistance genes provided by wild *Solanum* species. SCAR markers for race-specific CC-NBS-LRR resistance genes (*R* genes) have been employed to screen *Solanum* accessions representing six series of section *Petota*. Structural homologues of particular genes were found in many taxonomically distant species and widely differed in their distribution patterns. Such evidence suggests that the *R*-gene structures evolved before the divergence of genomes A and B and preceded *Solanum* speciation.

### KEYWORDS

*Phytophthora infestans*, *Solanum* spp., CC-NBS-LRR genes, late blight, race-specific resistance genes.

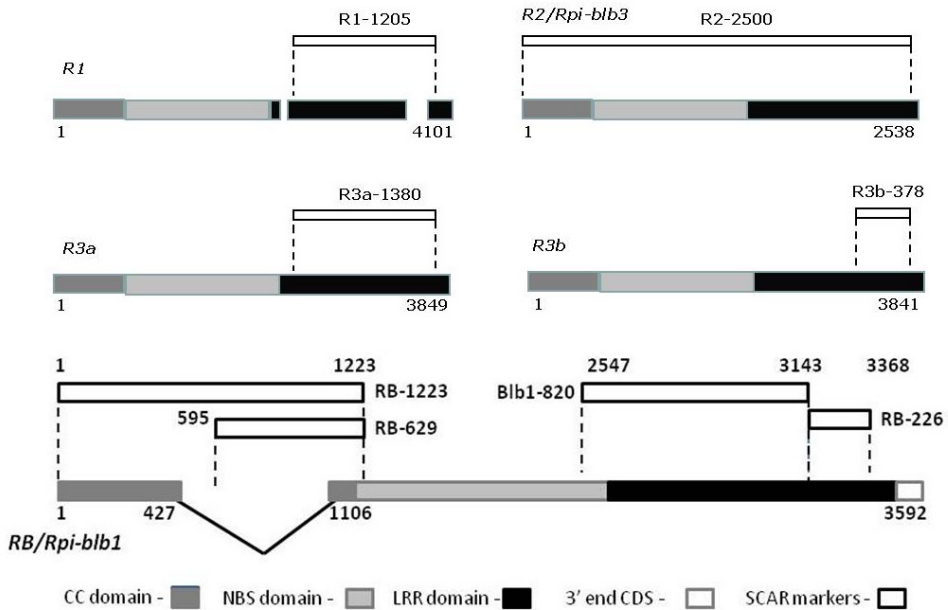
### INTRODUCTION

Late blight (LB) caused by *Phytophthora infestans* (Mont.) de Bary is among the most devastating potato diseases. New races of *P. infestans* rapidly defeat potato resistance based on germplasm transferred from *Solanum demissum*, and breeders search for new sources of durable LB resistance in genetic collections of wild *Solanum* species. *R* genes are highly conserved, and the candidate-gene approach, by using PCR amplification, opens the way to mine orphan *Solanum* species for new homologues of already known resistance genes (Hein *et al.*, 2009). We used five SCAR markers recognizing the race-specific resistance genes *R1*, *R2/Rpi-blb3*, *R3a*, *R3b* of *S. demissum* and *RB/Rpi-blb* of *S. bulbocastanum* to screen a collection of wild *Solanum* accessions representing six series from section *Petota* and clones developed from these accessions. The pattern thus produced will facilitate further search for new *R* genes for LB resistance and promote the comparative studies of rapidly evolving CC-NBS-LRR genes.

## MATERIALS AND METHODS

### Plant Material

*Solanum* accessions for this study arrived as microtubers from the collections of the Institute of Plant Industry (St. Petersburg, Russia) and the Institute of Phytopathology (Bol'shiye Vyazemy, Moscow region, Russia) and as seeds from the collections of the Institute of Plant Industry, Centre for Genetic Resources, the Netherlands, and the United States Potato Genebank, NRSP-6, Sturgeon Bay, WI. As a whole, we screened over 200 *Solanum* accessions.



**Figure 1.** SCAR markers for *R* genes for LB resistance. Primer positions (base pairs) are shown against the structures of CC-NBS-LRR kinases for prototype genes *R1*, *R2/Rpi-blb3*, *R3a*, *R3b* and *RB/Rpi-blb1*. For more details see Table 1

**Table 1.** SCAR markers of *R* genes

Genes	Markers and their sizes, bp	5'-3' primer sequences	Chromosome	Prototype clone	Position in the prototype clone	Annealing temp., °C	References
<i>R1</i>	R1-1205	5'-CACTCGTGACATATCCTCACTA-3' 5'-GTAGTACCTATCTTATTTCTGCAAGAAT-3'	5	AF447489	5126-6331	61	Sokolova <i>et al.</i> , 2011
<i>R3a</i>	R3a-1380	5'-TCCGACATGTATTGATCTCCCTG-3' 5'-AGCCACTTCAGCTTCTTACAGTAGG-3'	11	AY849382	1677-3056	64	<i>ibid.</i>
<i>R3b</i>	R3b-378	5'-GTCGATGAATGCTATGTTTCTCGAGA-3' 5'-ACCAGTTTCTTGCAATTCAGATTG-3'	11	JF900492	94818-95195	64	Rietman <i>et al.</i> , 2012
<i>R2</i>	R2-2500	5'-ATGGCTGATGCCTTTCTATCATTTCG-3' 5'-TCACAACATATAATTCCGCTTC-3'	4	FJ536325	1-2538	62	Kim <i>et al.</i> , 2012
<i>RB/Rpi-blb1</i>	RB-629	5'-GAATCAAATTATCCACCCCAACTTTTAAAT-3' 5'-CAAGTATTGGGAGGACTGAAAGGT-3'	8	AY336128	595-1223	65	Pankin <i>et al.</i> , 2011
<i>RB/Rpi-blb1</i>	RB-226	5'-CACGAGTGCCCTTTTCTGAC-3' 5'-TTCAATTGTGTTGCGCACTAG-3'	8	<i>ibid.</i>	3143-3368	50	Colton <i>et al.</i> , 2006
<i>RB/Rpi-blb1</i>	Bib1-820	5'-AACCTGTATGGCAGTGGCATG-3' 5'-GTCAGAAAAGGGCACTCGTG-3'	8	<i>ibid.</i>	2547-3143	62	Wang <i>et al.</i> , 2008

#### Development of DNA markers



Standard protocols were employed for genomic DNA isolation from young plant leaves, PCR analysis, and cloning and identifying genome fragments. Some specific primers for SCAR markers were already reported elsewhere, and some were designed following multiple alignment of the prototype gene sequences, their structural homologues and anonymous genome fragments lifted from the NCBI Genbank using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Vector NTI Suite 8 package (Invitrogen). The markers, primers and their positions as related to the prototype genes are shown in Fig. 1 and Table 1.

## RESULTS AND DISCUSSION

SCAR markers for CC-NBS-LRR resistance genes have been employed to screen 14 wild *Solanum* species (Table 2). Structural homologues of particular *R* genes widely differ in their distribution patterns in the section *Petota*. The genes *R1*, *R2*, *R3a* and *R3b* initially identified in *S. demissum* were found in many taxonomically distant species. Structural homologues of the *RB/Rpi-blb1* gene, which was initially identified in genome B of *S. bulbocastanum* and *S. stoloniferum* (for review see Vleeshouwers *et al.*, 2011), were found in many genome A species; however, only *RBver* in *S. verrucosum* was shown to participate in LB resistance (Liu and Halterman, 2006). Such evidence suggests that many *R*-gene structures evolved before the divergence of genomes A and B and subsequent *Solanum* speciation. The presence of markers RB-226 and RB-820 consistently corresponds to the functional gene *Rpi-blb1*; whereas marker RB-629 may tag RB structural homologues poorly related to LB resistance (see also Fadina *et al.*, in press).

**Table 2.** Distribution of markers R genes in wild *Solanum* species

Series and species	The total number of screened accessions	The numbers of accessions comprising the particular markers						
		R1-1205	R2-2500	R3-1380	R3b-378	RB-629	RB-226	Blb1-820
<i>Bulbocastana</i> ( <i>S. bulbocastanum</i> )	28	0						
<i>Demissa</i> <i>S. demissum</i>	38		n.d.					0
<i>S. hougasii</i>	7							0
<i>Longipedicelata</i> <i>S. hjertingii</i>	6	0		0			0	n.d.
<i>S. polytrichon</i>	12		0				0	
<i>S. stoloniferum</i>	51		0					
<i>Pinnatisecta/Cardiophylla</i> <i>S. cardiophyllum</i>	7	0	n.d.				0	0
<i>S. ehrenbergii</i>	17	0					0	0
<i>S. jamesii</i>	13	0	0	0			0	0
<i>S. pinnatisectum</i>	10	0		0			0	0
<i>Polyadenia</i> <i>S. polyadenium</i>	8	0	n.d.		0		0	n.d.
<i>Tuberosa</i> <i>S. verrucosum</i>	12	0	0	0		0	0	0
<i>S. berthaultii</i>	7		n.d.	0	0		0	0
<i>S. microdontum</i>	6		0		0		0	

 - frequent occurrence of the marker; 
  - rare occurrence of the marker; 
 n.d. - no data.

When using this evidence for selecting the genotypes of paramount interest for further introgression programs, the crucial issue is the functional identity of newly found CC-NBS-LRR homologues. In some cases, the evidence for new alleles of the *R* genes in orphan *Solanum* species is supported by our data obtained by cloning and sequencing: thus cloned markers R1-1205 and R3a-1380 in wild *Solanum* species were 98-99% similar to the corresponding regions in the prototype genes. Therefore, we presumed that they represented the active genes (Sokolova *et al.*, 2011).

Other laboratories provide even more convincing data from co-expression studies in *Nicotiana benthamiana* and potato transformation (Table 3). To illustrate, the presence of the marker R3a-1380 in *S. stoloniferum*, *S. cardiophyllum*, *S. ehrenbergii*, and *S. microdontum* confirms the evidence by Champouret (2010), who found orthologues of the *R3a* gene in this species. The presence of the marker R2-2500 in *S. bulbocastanum* and *S. hjertingii* (Table 2) is in line with the report that the functional R2/Rpi-blb3 gene is present in these species (Champouret, 2010; Lokossou *et al.*, 2010). The presence of the markers RB-629 and Blb1-820 in the species *S. bulbocastanum*, *S. stoloniferum* and *S. polytrichon* is supported by the evidence from several studies using *Nicotiana* and *Solanum* transformation.

**Table 3.** Late blight resistance genes in wild *Solanum* species

Genes- prototype	Species where SCAR markers were found	Mapped and cloned genes and their homologues	Genes were cloned from the following species	Co-expression or transient complementation in <i>N. benthamiana</i>	Potato transformation
R1	<i>dms, hou, plt, sto, ber, mcd</i>	R1 (Ballvora <i>et al.</i> , 2002; Kuang <i>et al.</i> , 2005)	<i>dms</i> (Ballvora <i>et al.</i> , 2002; Kuang <i>et al.</i> , 2005)	-	R1 <i>dms</i> (Ballvora <i>et al.</i> , 2002)
R2/Rpi- <i>blb3</i>	<i>blb, hou, pnt, hjt, ehr</i>	R2/Rpi- <i>blb3</i> <i>Rpi-abpt</i> <i>R2-like</i> (Lokossou <i>et al.</i> , 2010) <i>Rpi-edn1.1</i> <i>Rpi-hjt1.1, Rpi-hjt1.2, Rpi-hjt1.3</i> <i>Rpi-snk1.1, Rpi-snk1.2</i> (Champouret, 2010)	<i>tub, dms, edn, snk</i> <i>hjt, blb, vrn</i> (Champouret, 2010; Lokossou <i>et al.</i> , 2010)	R2, Rpi- <i>blb3, Rpi-abpt, R2-like</i> (Lokossou <i>et al.</i> , 2010)	R2 Rpi- <i>abpt R2-like</i> (Lokossou <i>et al.</i> , 2010)
R3a	<i>blb, hou, dms, cph, ehr, pld, mcd, plt, sto</i>	R3a (Huang <i>et al.</i> , 2005) <i>Rpi-sto2</i> (Champouret, 2010)	<i>tub, dms</i> (Liu, Halterman, 2006) <i>sto</i> (Champouret, 2010; Sokolova <i>et al.</i> , 2011) <i>blb, cph, hou, plt</i> (Sokolova <i>et al.</i> , 2011)	<i>Rpi-sto2, dms, sem, mcd, sto, cph, ehr</i> (Champouret, 2010)	
R3b	<i>blb, hou, dms, cph, hjt, jam, pnt, ver, sto, plt,</i>	R3b (Li <i>et al.</i> , 2011 )	<i>dms</i> (Lokossou <i>et al.</i> , 2010)	-	
RB/Rpi- <i>blb1</i>	<i>blb, hou, dms, cph, hjt, jam, pnt, pld, ber, sto, plt, mcd,</i>	RB/Rpi- <i>blb1</i> (van der Vossen <i>et al.</i> , 2003; Song <i>et al.</i> , 2003) <i>Rpi-bt1</i> (Oosumi <i>et al.</i> , 2009) RB <i>ver</i> (Liu, Halterman, 2006) <i>Rpi-sto1, Rpi-pta1, Rpi-plt1</i> (Wang <i>et al.</i> , 2008)	<i>blb, sto, ver, plt</i> (Wang <i>et al.</i> , 2008; Liu, Halterman, 2006; van der Vossen <i>et al.</i> , 2003; Song <i>et al.</i> , 2003; Oosumi <i>et al.</i> , 2009; Vleeshouwers <i>et al.</i> , 2008)		RG3 (van der Vossen <i>et al.</i> , 2003), RB (Song <i>et al.</i> , 2003; Vleeshouwers <i>et al.</i> , 2008) <i>Rpi-bt1</i> (Oosumi <i>et al.</i> , 2009) <i>RBver</i> (Liu, Halterman, 2006)

*ber* - *S. berthaultii*, *cph (ehr)*- *S. cardiophyllum*=*S. ehrenbergii*, *dms* - *S. demissum*, *edn* - *S. edinense*, *hou*- *S. hougasii*, *hjt* - *S. hjertingii*, *jam* - *S. jamesii*, *tbr* -*S. tuberosum*, *mcd* - *S. microdontum*, *pnt* - *S. pinnatisectum*, *plt* - *S. polytrichon* = *S. stoloniferum*, *pld* - *S. polyadenium*, *sem* - *S. semidemissum*, *snk* - *S. schenckii*, *sto* - *S. stoloniferum*, *tbr* - *S. tuberosum*, *vrn* - *S. vernei*, *ver* - *S. verrucosum*.

## CONCLUSIONS

The present results confirm the previously known phytopathological data on the presence of *R1*, *R2*, *R3a* and *R3b* genes, initially identified in *S. demissum*, beyond the series *Demissa*. Structural homologues of the *RB/Rpi-blb1* gene, which was initially identified in genome B of *S. bulbocastanum* and *S. stoloniferum*, were found in many species comprising genomes A and B. The presence of markers RB-226 and RB-820 consistently indicates the functional gene *Rpi-blb1*. These results imply that the markers described in this communication can be used to search for new *R* genes homologues in genetic collections and to estimate their breeding potential.

## ACKNOWLEDGEMENTS

The study was supported by the ISTC - ARS-USDA (project 3714p), the Ministry of Education and Science, Russian Federation (contract No.16. 04.12.0007), and the Russian Foundation for Basic Research (project 13-04-00163a).

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