SCAR markers for the *RB/Rpi-blb1* gene of potato late blight resistance

OKSANA A. FADINA[1], TATIANA V. BELYANTSEVA[1], EMIL E. KHAVKIN[1], ARTEM A. PANKIN[1] , ELENA V. ROGOZINA[2], MARIYA A. KUZNETSOVA[3], RICHARD W. JONES[4], KENNETH L. DEAHL[4]

[1]Institute of Agricultural Biotechnology, Moscow, Russia; e-mail: fadinaokcaha@gmail.com

[2]N.I.Vavilov Institute of Plant Industry, St. Petersburg, Russia

[3]Institute of Phytopathology, Bol'shiye Vyazemy, Moscow region, Russia

[4] USDA-ARS BARC, Beltsville, MD, USA

The *RB/Rpi-blb1* gene of broad-spectrum late blight (LB) resistance initially isolated from *Solanum bulbocastanum* has been successfully employed in breeding for durable LB resistance. Breeder-friendly DNA markers of this gene would considerably promote breeding programs. We employed four SCAR markers: RB-226 (Colton et al., 2006) and Blb1-820 (Wang et al.., 2008) representing the LRR region of *Rpi-blb1* and RB-629 (Beketova et al., 2007) and RB-1223 (Pankin et al., 2010) representing the CC region of the gene (Fig. 1). Using the clonal collection of wild *Solanum* species, we collated the frequencies of RB-629, RB-226 and Blb1-820 with the indices of LB resistance for particular clones. The markers RB-226 and Blb1-820 were found only in genome B of *S. bulbocastanum* and *S. stoloniferum.* By several nonparametric statistics, these two markers were significantly coupled and fairly well predicted LB resistance (Table). In contrast, RB-1223 and its fragment RB-629 were widely distributed in *Solanum* species section *Petota* and were poor predictors of LB resistance (Fig. 2, Table).

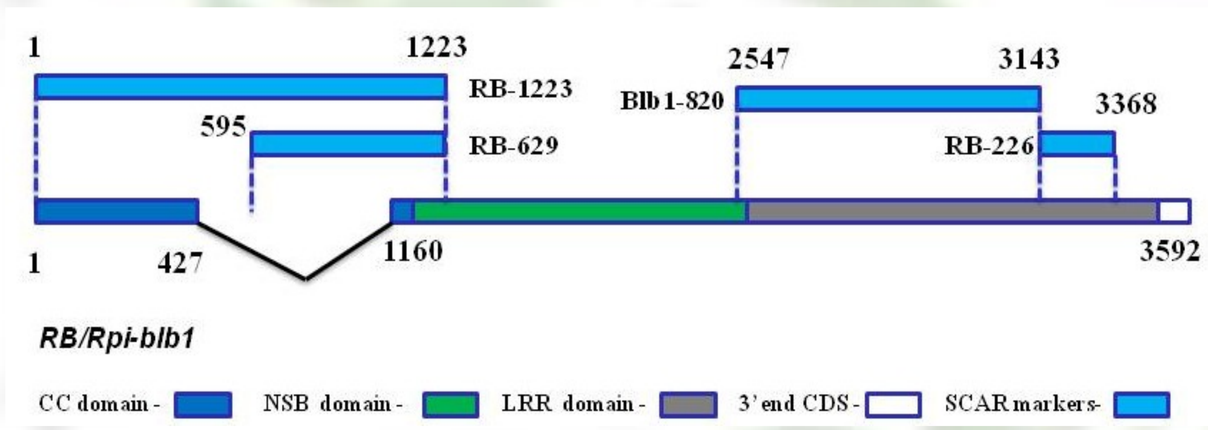

Fig. 1. Markers recognizing functional gene *RB/Rpi-blb1* and its structural homologs.

Three markers of the gene *RB/Rpi-blb1* in *Solanum* species

Genome*	Functional *RB* gene**	Genotypes, accessions, clones	RB-629	RB-226	Blb1-820	LB resistance, points***
B	*Rpi-blb1, Rpi-bt1*	*S. bulbocastanum* VIR24866, CD-76-1	1	1	1	9
B	same	*S. bulbocastanum* PI255516, CD-75-1	1	1	1	9
B	same	*S. bulbocastanum* VIR24866, CD-76-3	0	0	0	9
B	same	*S. bulbocastanum* VIR21266, S-137	1	1	1	9
B	same	*S. bulbocastanum* VIR23181, 511-1	0	0	0	1
B	same	*S. bulbocastanum* VIR23181, 511-2	0	0	0	1
B	same	*S. bulbocastanum* VIR19981, 431	0	0	0	9
B	same	*S. bulbocastanum* PI24866, CD-76-5	1	1	1	9
B	same	*S. bulbocastanum* VIR24866, CD-76-3	0	0	0	9
B	same	*S. bulbocastanum* VIR23181, 511-1	0	0	0	1
B	same	*S. bulbocastanum* VIR21274, 509-1	0	0	0	9
B	same	*S. bulbocastanum* VIR21274, 509-3	0	0	0	7
B	same	*S. bulbocastanum* VIR21266, 432-1	1	1	0	7
A1B	*Rpi-blb1*	*S. stoloniferum* PI275248, CD-360-5	1	1	1	9
A1B	same	*S. stoloniferum* PI255525, CD-356-2	1	0	0	2
A1B	same	*S. stoloniferum* VIR23652, PI195169, D-481	1	0	0	9
A1B	same	*S. stoloniferum* PI365401, CD-362-1	1	1	1	8
A1B	same	*S. stoloniferum* VIR24263, D-99	1	0	0	3
A1B	same	*S. stoloniferum* PI255534, D-482	1	1	1	9
B	-	*S. cardiophyllum* VIR21301,PI279272, 403	0	0	0	8
B	-	*S. cardiophyllum ehr.* VIR23277, PI251725, S-123	0	0	0	4
B	-	*S. cardiophyllum* VIR18225, PI274213, S-122	0	0	0	5
B	-	*S. cardiophyllum* PI347759, D-609	0	0	0	5
B	-	*S. cardiophyllum* VIR24373, PI275213, 425	0	0	0	8
B	-	*S. ehrenbergii* VIR24373, PI275213i, D-629	0	0	0	2
B	-	*S. ehrenbergii* PI275216i, D-616	1	0	0	4
B	-	*S. ehrenbergii* PI255520, D-625	0	0	0	4
B	-	*S. ehrenbergii* PI278216, D-610	0	0	0	4
A1	*RBver*	*S. verrucosum* PI275260, CD-410	0	0	0	7
A1	same	*S. verrucosum* PI161173, CD-407-1	0	0	0	8
A1	same	*S. verrucosum* VIR24313, PI365404, CD-401-1	0	0	0	5
A3	-	*S. microdontum* VIR5399, D-262-09	1	0	1	9
A3	-	*S. microdontum* VIR12658, D-264-09	0	0	0	9
A3	-	*S. berthaltii* PI473331, CD-38-2	0	0	0	9
A1?D?	-	*S. demissum* VIR18487, Och14156, CD-130-2	1	0	0	9
A1?D?	-	*S. demissum* PI161167, CD-142-2	1	0	0	6
A1?D?	-	*S. demissum* VIR15174, S-98	0	0	0	4
A1?D?	-	*S. hougasii* VIR24389, CD-188-2	1	0	0	7
B?	-	*S. jamesii* PI275265, CD-210-1	0	0	0	7
B?	-	*S. jamesii* PI275265, CD-210-2	1	0	0	6
B?	-	*S. stenophyllidium* PI24255, D-574	1	0	0	3
B?	-	*S. stenophyllidium* PI255530, D-564	1	0	0	2
Api/B?	-	*S. pinnatisectum* VIR24239, D-564	0	0	0	9
Api/B?	-	*S. pinnatisectum* VIR21955, D-560	1	0	0	5

*Based on cytogenetic and molecular evidence.
**(+) Established by independent methods
*** Artificial infection of detached leaves with the complex race of *P. infestans* (Institute of Phytopathology).

Two highly resistant clones of *S. bulbocastanum* and *S. stoloniferum* are devoid of the markers RB-226 and Blb1-820. Their resistance may depend on the presence of other *R* genes identified in these two species, such as *Rpi-blb2*, *Rpi-blb3* and *R3a* (Sokolova et al., 2011; Vleeshouwers et al., 2011). High resistance of several other clones apparently devoid of *Rpi-blb1* indicates that other R genes are presumably present in these species (see the poster by Sokolova et al. at this Workshop).

By several nonparametric statistics, RB-226 and Blb1-820s were significantly coupled and fairly well predicted LB resistance (Table). High LB resistance was usually linked to the simultaneous presence of both these markers. Rare cases of high resistance in genotypes comprising only one of two markers probably resulted from allelic polymorphisms affecting the region recognized by a primer.

Two other markers, RB-629 and RB-1223, are present in genomes A and B screened in over 100 *Solanum* genotypes (Table, Fig. 2). These markers are not specific for *Rpi-blb1* and fail to predict LB resistance.

A clonal collection of wild *Solanum* species (section *Petota*) was used to directly compare the late blight (LB) resistance of detached leaves with the patterns of SCAR markers for the *RB/Rpi-blb1* gene initially identified in *S. bulbocastanum* (Table).

The markers RB-226 and Blb1-820 are specific for *Rpi-blb1*. These markers are found only in *S. bulbocastanum* and *S. stoloniferum* (which presumably received its genome B from *S. bulbocastanum*) and were absent from other *Solanum* species comprising genome B, such as *S. cardiophyllum, S. ehrenbergii* and probably *S. pinnatisectum*. These data support the evidence by Wang et al. (2008) that the marker Blb1-820 was found only in *S. bulbocastanum* and *S. stoloniferum.* The presence of the functional gene *RB/Rpi-blb1* in *S. stoloniferum* was demonstrated by independent methods (Lokossou et al., 2010; Sokolova et al., 2011; Vleeshouwers et al., 2011). The markers RB-226 and Blb1-820 do not recognize *Rpi-blb1* orthologs *Rpi-bt1* (Oozumi et al.,) and *RBver* (Liu and Halterman, 2006), and we therefore do not find them in *S. verrucosum*.

Two other markers, RB-629 and RB-1223, are present in genomes A and B screened in over 100 *Solanum* genotypes (Table, Fig. 2). These markers are not specific for *Rpi-blb1* and fail to predict LB resistance.

S. bulbocastanum
(photo by A. Pankin)

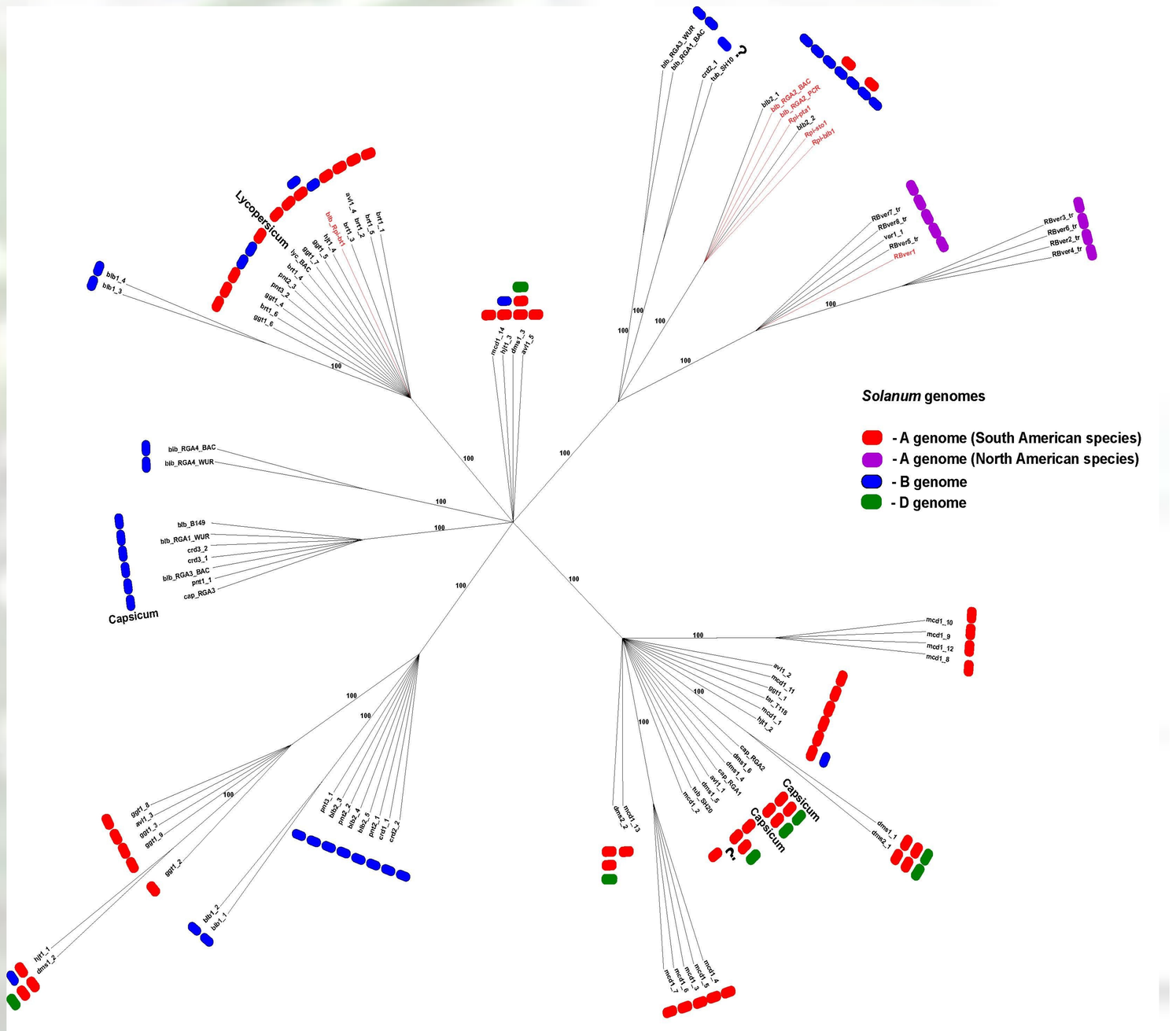

Fig. 2. Maximum likelihood phylogram (strict consensus tree) of the coding regions of *RB* family genes covered by SCAR marker RB-1223 in *Solanum* and *Capsicum* species:
avl - S. avilesii, blb - S. bulbocastanum , ber - S. berthaultii, can - C. annuum, cba - C. baccatum , cch - C. chinense, cph - S. cardiophyllum, dms - S. demissum, hjt - S. hjertingii, lyc - S. lycopersicum, mcd - S. microdontum, phu - S. phureja , pld - S. polyadenium, pnt - S. pinnatisectum, sto - S. stoloniferum, tar - S. tarijense , tbr - S. tuberosum, ver - S. verrucosum

Red branches and sequence labels correspond to genes known to confer resistance to *P. infestans* isolates. Bootstrapping was performed using rapid bootstrap algorhithm (Stamatakis et al., 2008), and values are shown at the nodes.

REFERENCES

Colton L.M. et al., 2006. Marker-assisted selection for the broad-spectrum potato late blight resistance conferred by gene *RB* derived from a wild potato species. Crop Sci., 46:589-594.

Liu Z., Halterman D., 2006. Identification and characterization of *RB* orthologous genes from the late blight resistant wild potato species *Solanum verrucosum*. Physiol. Mol. Plant Pathol., 69: 230-239.

Lokossou A.A. et al, 2010. Diversity, distribution, and evolution of *Solanum bulbocastanum* late blight resistance genes. MPMI, 23: 1206–1216.

Oosumi T. et al., 2009. Gene *Rpi-bt1* from *Solanum bulbocastanum* confers resistance to late blight in transgenic potatoes. Am. J. Potato Res., 86: 1–10.Pankin A.A. et al., 2010. Searching among wild *Solanum* species for homologues of *RB/Rpi-blb1/Rpi-bt1* gene conferring durable late blight resistance. In: Schepers HTAM (ed.) PPO-Special Report no. 14, 277 - 284.

Sokolova, E. et al., 2011. SCAR markers of the *R*-genes and germplasm of wild *Solanum* species for breeding late blight-resistant potato cultivars. Plant Gen. Res., 9: 309–312.

Stamatakis A. et al., 2008. A rapid bootstrap algorithm for the RAxML web servers. Syst. Biol., 57: 758-771.

Vleeshouwers V.G.A.A. et al., 2011. Understanding and exploiting late blight resistance in the age of effectors. Annu. Rev. Phytopathol. 49: 507–531.

Wang M. et al., 2008. Allele mining in *Solanum*: Conserved homologues of *Rpi-blb1* are identified in *Solanum stoloniferum*. Theor. Appl. Genet. 116: 933-943.

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