Searching among wild *Solanum* species for homologues of *RB/Rpi-blb1/Rpi-bt1* gene conferring durable late blight resistance

A Pankin[1], E Sokolova[1], E Rogozina[2], M Kuznetsova[3], K Deahl[4], R Jones[4], E Khavkin[1]

[1]Institute of Agricultural Biotechnology, Moscow, Russia
[2]Institute of Plant Industry, St. Petersburg, Russia
[3]Institute of Phytopathology, Bol'shiye Vyazemy, Russia
[4]USDA-ARS, Agriculture Research Center, Beltsville, MD, USA

Summary

A CC-NBS-LRR gene *RB/Rpi-blb1* initially isolated from *S. bulbocastanum* confers broad-spectrum resistance to *Phytopthora infestans* and is currently employed in potato breeding for durable late blight (LB) resistance. *RB/Rpi-blb1* homologues were reported from *S. bulbocastanum*, *S. papita*, *S. stoloniferum* and *S. verrucosum*; some of them retained defence function when introgressed into potato cultivars by trans- and cisgenesis. A cisgenic potato variety with improved resistance to *Ph. Infestans* is currently on the field trials in the European Union (BASF Plant Science GmbH, 2005). *RB*-mediated resistance has not been defeated in the field tests as yet; however, Champouret et al. (2009) reported on two Mexican *Phytopthora* isolates virulent on *RB* potato. Pyramiding several *RB*-like genes from various *Solanum* species in potato cultivars would promote durable LB resistance. Here we report an early evidence on *RB*-like sequences in the wide range of orphan *Solanum* species section *Petota*. The panel of wild *Solanum* species was screened with *RB/Rpi-blb1* locus-specific RB-629, RB-226 (modified from Colton et al., 2006), RB-1223 and RB-1096 markers. Markers RB-1223 and RB-1096 tagging several *RB*-like loci were found in every tested *Solanum* accession suggesting universal distribution of *RB* structural homologues among *Solanum* genomes. Effectiveness of these markers in allele mining for new sources of durable resistance in *Solanum* specie was proved by sequencing. Phylogenetic analysis of RB-629 sequences produced four distinct clusters. It is noteworthy that the RB-226 marker was found only in the cluster I genotypes. The pattern of polymorphisms was neither species- nor series-specific, and we conclude that duplication and evolution of *RB*-like loci preceded *Solanum* speciation. Marker presence and particular haplotypes were not immediately associated with high LB resistance. It is therefore apparent that RB-226 cannot universally diagnose the active *Rpi-blb1/RB* allele.

1

R gene-mediated resistance to *Phytophthora infestans*

R genes encode proteins specifically recognising pathogen effectors (Avr) and activating hypersensitive response to the pathogen attack. Eleven *R* genes of *S. demissum* were identified, and some of them (*R1, R2, R3, R4* and *R10*) were introgressed into potato cultivars. However, *S. demissum*-derived resistance is apparently defeated in the field by rapidly evolving *Ph. infestans* races. In contrast, *Rpi-blb1/RB* gene initially cloned from *S. bulbocastanum* encodes a ligand recognising a diversity of Avr effectors (ipO family) and confers broad-spectrum late blight (LB) resistance.
Several genetic loci carrying broad-spectrum LB resistance genes were mapped on linkage groups of various *Solanum* species, but only nine functionally active genes have been cloned from *S. bulbocastanum*, *S. papita*, *S. stoloniferum* and *S. verrucosum*; six of them are homologous *RB*-like loci.

R genes from wild *Solanum* species conferring resistance to broad spectrum of *Ph. infestans* races

Gene	Species	LG	Reference	Genbank acc.#
Rpi-blb1	*S. bulbocastanum*	8	van der Vossen et al., 2003	AY426259
RB/RGA2	*S. bulbocastanum*	8	Song et al., 2003	AY336128
Rpi-bt1	*S. bulbocastanum*	8	Oosumi et al., 2009	FJ188415
Rpi-sto1	*S. stoloniferum*	8	Vleeshouwers et al., 2008	EU884421
Rpi-pta1	*S. papita*	8	Vleeshouwers et al., 2008	EU884422
Rb**ver	*S. verrucosum*	nd	Liu and Halterman, 2006	EF202333
Rpi-blb2	*S. bulbocastanum*	6	van der Vossen et al., 2005	DQ122125
Rpi-vnt1.1	*S. venturii*	9	Foster et al., 2009, Pel et al., 2009	FJ423044
Rpi-vnt1.3	*S. venturii*	9	Foster et al., 2009, Pel et al., 2009	FJ423046

Homologous *RB*-like loci are in bold. LG – linkage group, nd – not determined

2

Why do we need more broad-spectrum resistance genes from wild *Solanum* species?

Rationale:

S. bulbocastanum RB-derived late blight resistance has not been overcome in the field as yet, however the genome of *Ph. infestans* is prone to rapid evolution under increased adaptive selection (Haas et al., 2009).

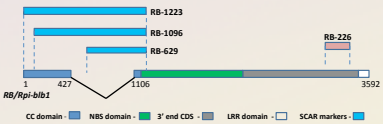
Champouret et al. (2009) reported on two Mexican *Ph. infestans* isolates lacking Avr effectors compatible with RB/Rpi-blb1 ligand and thus virulent on *RB* plants.

Introgression of single *Rpi-bt1* gene into a potato cultivar did not confer field resistance at the level observed in the wild *Solanum* parent (Oosumi et al., 2009).

Pyramiding broad-spectrum resistance genes from various sources in potato genome by means of trans- and cisgenesis and marker-assisted selection is an effective approach to durable late blight resistance of potato cultivars (Tan et al., 2010). Allele mining using DNA markers tagging *RB*-like loci can be successfully applied to the identification of new sources of broad spectrum resistance genes among wild *Solanum* species (Wang et al., 2008).

3

Structure of *RB/Rpi-blb1* gene and locations of the RB-related SCAR markers



Numbers are nucleotide positions in base pairs (bp) in relation to the full-length *RB/Rpi-blb1* gene sequence (AY426259, 3529 bp). Line angled downwards indicates the position of intron.

A *RB/Rpi-blb1* gene consists of two exons encoding coiled-coil (CC) domain split by a 680-bp length intron, nucleotide-binding site (NBS) and leucine-rich repeat (LRR) domain. In order to explore the diversity of the *RB*-like genes in orphan *Solanum* species, three SCAR markers were designed: RB-629 spanning intron and second exon fragments, RB-1096 spanning first exon, intron and second exon fragments and RB-1223 covering the stretch from the start codon until the second exon. The RB-226 marker (modified from Colton et al., 2006) is located in the LRR domain and reportedly tags the active allele of *bulbocastanum RB*.

4

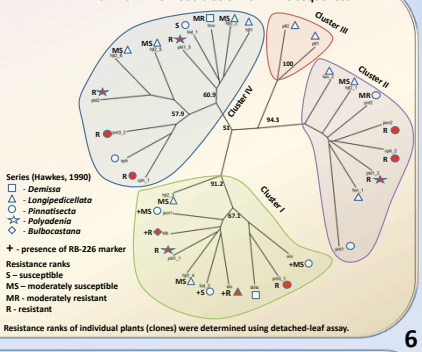
The panel of North and Central American *Solanum* species screened with RB-629 and RB-226 markers

Series (Hawkes, 1990)	Species	Abbreviation	Number of accessions		
			Total	RB-629	RB-226
Bulbocastana	*S. bulbocastanum*	blb	18	11	6
Demissa	*S. brachycarpum*	bcp	1	0	0
Demissa	*S. demissum*	dms	16	5	3
Demissa	*S. hougasii*	hou	3	2	0
Demissa	*S. lopetalum*	iop	1	0	0
Longipedicellata	*S. fendleri*	fen	4	3	0
Longipedicellata	*S. hjertingii*	hjt	7	4	0
Longipedicellata	*S. papita*	pta	4	2	0
Longipedicellata	*S. polytrichon*	plt	10	7	1
Longipedicellata	*S. stoloniferum*	sto	21	17	3
Pinnatisecta	*S. brachistotrichum*	bst	4	3	3
Pinnatisecta	*S. cardiophyllum*	cph	4	3	0
Pinnatisecta	*S. cardiophyllum subsp. ehrenbergii*	ehr	13	4	2
Pinnatisecta	*S. jamesii*	jam	9	4	1
Pinnatisecta	*S. pinnatisectum*	pnt	13	11	2
Pinnatisecta	*S. stenophyllidium*	sph	3	2	1
Pinnatisecta	*S. tarnii*	trn	2	0	0
Polyadenia	*S. polyadenium*	pld	5	2	0
Tuberosa	*S. verrucosum*	ver	3	0	0
6 Series	19 species		141	80 (57%)	22 (16%)

The panel of 141 accessions of 19 tuber-bearing *Solanum* species was screened with the *RGA2* locus-specific marker RB-629 and allele-specific marker RB-226. RB-629 was present in 57% of accessions representing 15 *Solanum* species whereas allele-specific RB-226 was present only in 16% of accessions from eight *Solanum* species. Curiously, *RB*-like sequences are widely distributed in section Petota among both wild *Solanum* species of Mexican/Central American origin and South American species such as *S. tarijense* (Genbank acc. AY426266) and cultivated *S. tuberosum* (AY426265). However, the presence of *RB*-related markers does not immediately associate with high LB resistance suggesting structural redundancy of *RB*-like structures in *Solanum* genomes.

5

Maximum-likelihood tree of *RGA2*-like sequences

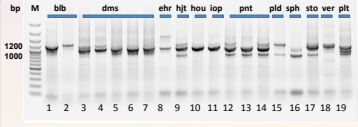


RB-629 was cloned from 16 accessions representing 12 wild *Solanum* species. Comparative analysis of the 29 RGA2-like sequences revealed 14 polymorphic haplotypes comprising 69 SNP markers and one *S. polytrichon*-specific insertion. Phylogenetic analysis of RB-629 sequences produced four distinct clusters: cluster I of *S. bulbocastanum*-like haplotypes, cluster II of pseudogenes, cluster III specific for *S. polytrichon* and cluster IV combining other *RB* homologues with open reading frame. The marker RB-226 was found only in the cluster I genotypes. The pattern of polymorphisms was neither species- nor series-specific, and we conclude that observed diversity of *RGA2*-like loci emerged before *Solanum* speciation. Marker presence and particular haplotypes were not immediately associated with high LB resistance.

Resistance ranks of individual plants (clones) were determined using detached-leaf assay.

6

Polymorphism of the RB-1223 marker in wild *Solanum* species



1, 2 - *S. bulbocastanum* (PI243510, PI243512); 3-7 - *S. demissum* (PI160221, PI161674, PI161176, PI175404, PI218047); 8 – *S. c ardiophyllum subsp. ehrenbergii* (PI255520); 9 - *S. hjertingii* (PI283130); 10 – *S. hougasii* (PI161727); 11 – *S. lopetalum* (PI230459); 12-14 - *S. pinnatisectum* (PI186553, PI275233, PI275234); 15 – *S. polyadenium* (PI275237); 16 – *S. stenophyllidium* (PI320265); 17 – *S. stoloniferum* (PI205510); 18 – *S. verrucosum* (PI365404); 19 – *S. polytrichon* (VIR8815); M – 100bp DNA Ladder; bp – base pairs.

Markers RB-1223 and RB-1096 tagging several *RB*-like loci were found in every tested *Solanum* accession corroborating widespread distribution of the *RB*-like structures in *Solanum* genomes. Sequencing experiments revealed that polymorphic bands presumably correspond to several RB-like loci and observed variation in size was mainly due to polymorphisms in the introns. These markers proved to be instrumental in identification of new sources of durable RB-related resistance to LB in various *Solanum* species using efficient candidate gene approach.

Conclusions

RGA2- and *Rpi-bt1*-like loci are widely distributed in various tuber-bearing *Solanum* species and co-exist in *S. cardiophyllum* and *S. pinnatisectum* individual genomes. Both conservative pattern of polymorphism and abundant distribution of *RB*-like loci presumably indicate that *RB*-like loci diverged in ancient *Solanum* species before speciation and dissemination over American continent. Despite the defence function against *Ph. infestans* unequivocally demonstrated in complementation experiments with *RB-like* genes isolated from several accessions of *S. bulbocastanum*, *S. papita*, *S. stoloniferum* and *S. verrucosum*, the presence and polymorphisms of *RB*-like sequences in various *Solanum* species do not immediately associate with higher LB resistance. *RB*-specific DNA markers are useful tools for identifying new sources of *RB*-related broad-spectrum LB resistance by allele mining and candidate gene approach.

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R genes

See the Supplemental materials envelope for the list of the cited literature.