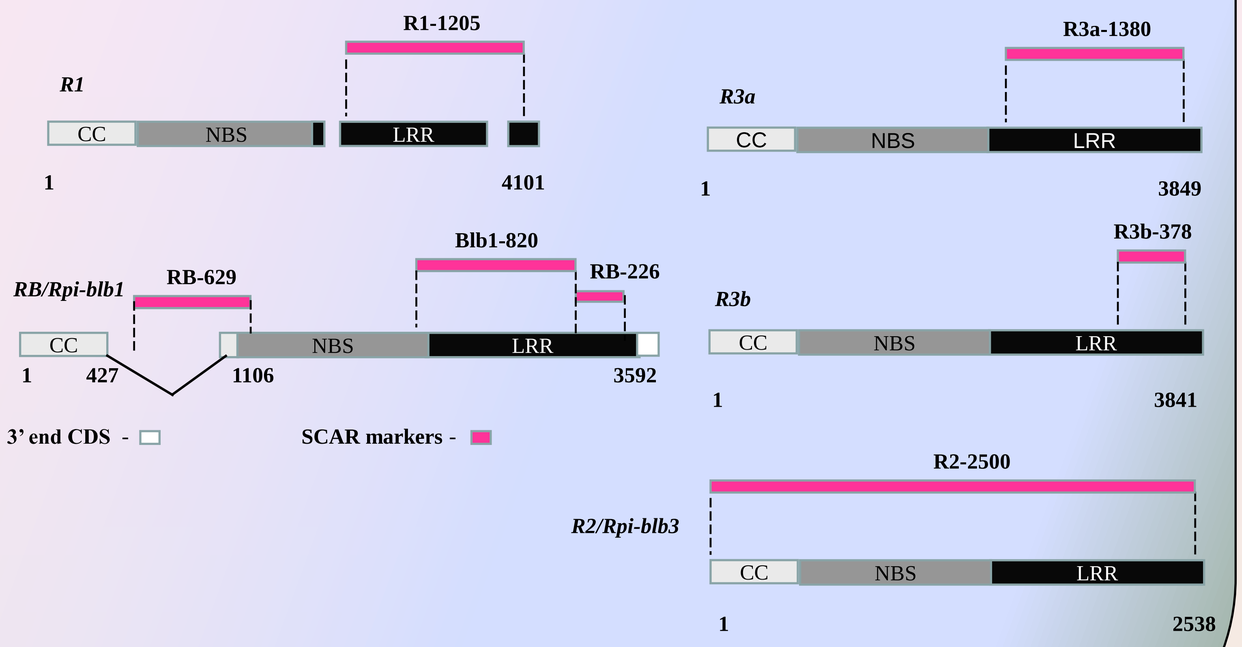
Structural homologues of CC-NBS-LRR genes for potato late blight resistance in wild *Solanum* species

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

[1]Institute of Agricultural Biotechnology, Moscow, Russia; [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

New races of *Phytophthora infestans* rapidly defeat potato late blight (LB) resistance based on germplasm transferred from *Solanum demissum*, so breeders search for new sources of durable LB resistance in genetic collections of wild *Solanum* species. SCAR markers for five race-specific genes initially characterized in *S. demissum* and *S. bulbocastanum*: *R1, R2/Rpi-blb3, R3a, R3b* and *RB*/*Rpi-blb1* – were employed 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 mining for new *R* genes for LB resistance and promote the evolutionary studies of CC-NBS-LRR resistance genes.

Markers and their sizes, bp	Prototype clone	Chromosome	Position in the prototype clone	References
RB-629	AY336128	8	595-1223	[1]
RB-226	AY336128	8	3143-3368	[2]
Blb1-820	AY336128	8	2547-3143	[3]
R1-1205	AF447489	5	5126-6331	[1]
R2-2500	FJ536325	4	1-2538	[4]
R3a-1380	AY849382	11	1677-3056	[1]
R3b-378	JF900492	11	94818-95195	[4]



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*.

When using this evidence for selecting the genotypes of paramount interest for further breeding program, the crucial problem is the functional identity of newly found CC-NBS-LRR homologues. In some cases, we have supported the evidence for new alleles of the *R* genes in orphan *Solanum* species by cloning and sequencing [1], while other laboratories provide even more convincing data from co-expression studies in *Nicotiana benthamiana* and potato transformation.

Genes-prototype	SCAR markers found in species	Mapped and cloned genes and their homologues	Genes cloned from the following species	Co-expression or transient complementation in *N. benthamiana*	Transformation of potato
R1	*dms, hou, plt, sto, ber, mcd*	*R1* [11,12]	*dms* [11,12]	-	*R1 dms* [11]
R2/Rpi-blb3	*blb, hou, pnt*	*R2/Rpi-blb3* *Rpi-abpt* *R2-like* [8] *Rpi-mcd1* [9] *Rpi-edn1.1* *Rpi-hjt1.1, Rpi-hjt1.2, Rpi-hjt1.3* *Rpi-snk1.1, Rpi-snk1.2* [10]	*tub, dms, edn, snk* *hjt, blb, vrn, mcd* [8-10]	*R2 Rpi-blb3 Rpi-abpt R2-like* [8] *Rpi-mcd1* [9]	*R2 Rpi-abpt R2-like* [8] *Rpi-mcd1* [9]
R3a	*blb, hou, dms, cph, pld, mcd, plt, sto*	*R3a* [13] *Rpi-sto2* [10] *R8 [*19] *R3b* [14] *R5, R6, R7, R9, R10, R11* [13]	*tub, dms* [6] , *sto* [10,1], *blb, cph, hou, plt* [1]	*Rpi-sto2, dms, sem, mcd, sto, cph, ehr* [10]	
R3b	*blb, hou, dms, cph, hjt, jam, pnt, ver, sto, plt*	*R3b* [14] *R3a* [13] *R8* [19] *R5, R6, R7, R9, R10, R11* [13]	*dms* [8]	-	
RB/Rpi-blb1	*blb, hou, dms, cph, hjt, jam, pnt, ver, pld, ber, sto, plt, mcd*	*RB/Rpi-blb1* [15,16] *Rpi-bt1* [17] *RB ver* [6] *Rpi-sto1, Rpi-pta1, Rpi-plt1* [3]	*blb, sto, ver, plt* [3,6,15-18]		*RGA3* [15], *RB* [16, 18], *Rpi-bt1* [17], *RBver* [6]

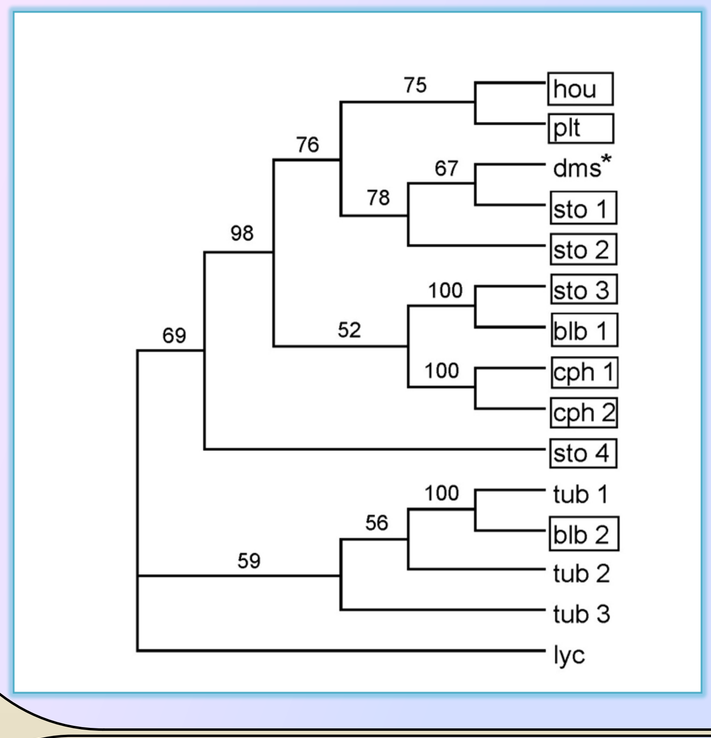
ber - S. berthaultii, cph (ehr)- S. cardiophyllum=S. ehrenbergii, dms - S. demissum, edn - S. edinense, hou- S. hougasii, hjt - S. hjertingii, jam - S. jamesii, tbr -S. tuberosum, mcd – S. microdontum, pnt - S. pinnatisectum, plt - S. polytrichon = S. stoloniferum, pld - S. polyadenium, sem – S. semidemissum,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

SCAR markers for CC-NBS-LRR resistance genes have been employed to screen 218 *Solanum* accessions of 14 wild *Solanum* species. 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 [5], were found in many genome A species, and only *RBver* in *S. verrucosum* was found to participate in LB resistance [6]. 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 presence of the functional gene *Rpi-blb1*, whereas marker RB-629 presents its structural homologue poorly related to LB resistance (for further details see the poster by Fadina et al. at this meeting).

Series and species [20]		The total number of screened accessions	Occurrence of *R*-gene markers						
			R1-1205	R2-2500	R3a-1380	R3b-378	RB-629	RB-226	Blb1-820
Bulbocastana (*S. bulbocastanum*)		28	0						
Demissa	*S. demissum*	38		nd					0
	S. hougasii	7							0
Longipedicellata	*S. hjertingii*	6	0		0			0	nd
	S. polytrichon	12		0				nd	nd
	S. stoloniferum	51		nd					
Pinnatisecta/ Cardiophylla	*S. cardiophyllum*	7	0	nd				0	nd
	S. ehrenbergii	17	0						0
	S. jamesii	13	0	0	0			0	nd
	S. pinnatisectum	10	0		0			0	0
Polyadenia	*S. polyadenium*	8	0	nd		0		0	nd
Tuberosa	*S.verrucosum*	12	0	0	0		0		0
	S. berthaulti	7		nd	0	0		0	nd
	S. microdontum	6		0		0		0	nd

- frequent occurrence of the marker **- less frequent occurrence of the marker**

Cloned markers R1-1205 in *S. polytrichon* and *S. stoloniferum* and R3a-1380 in *S. bulbocastanum, S. cardiophyllum, S. hougasii, S. polytrichon* and *S. stoloniferum* were 98-99% identical to the corresponding regions in the prototype genes. The phylogenetic analysis of the R3a-1380 fragments from wild *Solanum* species sequenced in our laboratory when compared to those of the *demissum R3a* gene, tomato *I2* gene and *R3a*-like inactive homologues from the NCBI Genbank demonstrated that the R3a-1380 sequences belonged to the same cluster as *R3a* presuming that they represented the active genes [1].

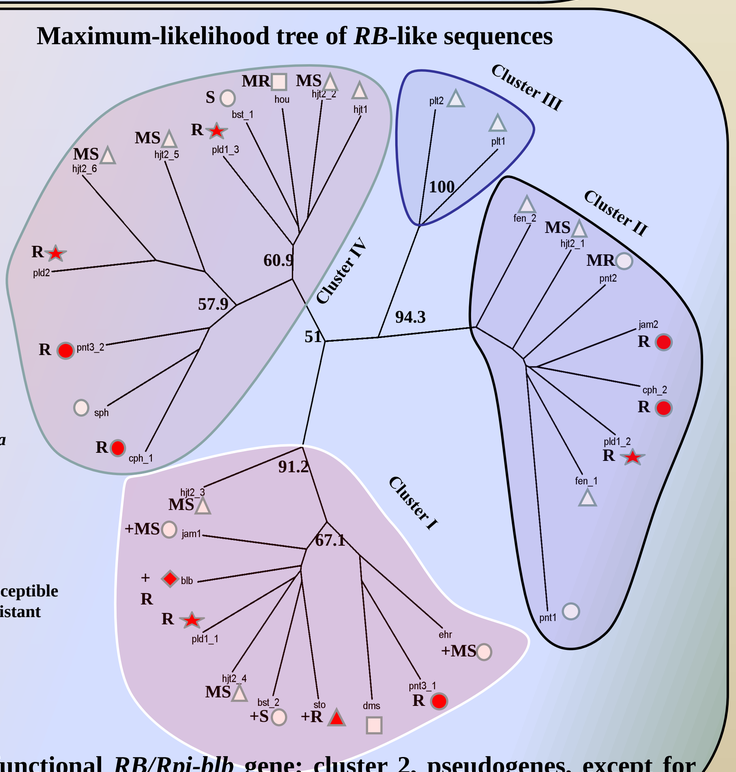


blb - *S. bulbocastanum;*
cph - *S. cardiophyllum;*
dms*- *S. demissum* gene *R3a;*
hou - *S. hougasii;*
lyc - *S. lycopersicum* gene *I2;*
plt - *S. polytrichon;*
sto - *S. stoloniferum;*
tub - *S. tuberosum.*

Maximum Likelihood tree comparing the R3a-1380 sequences from wild *Solanum* species (in boxes) to the functional gene *R3a*, gene *I2* and *R3a* pseudogenes.

When RB-629 was cloned from 16 accessions representing 12 wild *Solanum* species, the pattern of polymorphisms was neither species- nor series-specific, and we conclude that observed diversity of *RGA2*-like loci emerged before *Solanum* speciation. The marker RB-226 was found only in the cluster I genotypes. Marker presence and particular haplotypes were not immediately associated with high LB resistance [7].

Maximum-likelihood tree of *RB*-like sequences



Series [20]
- *Demissa*
- *Longipedicellata*
- *Pinnatisecta*
- *Polyadenia*
- *Bulbocastana*

Resistance ranks
S – susceptible
MS – moderately susceptible
MR - moderately resistant
R - resistant
+ - presence of RB-226 marker

Cluster 1, haplotypes that joined with functional *RB/Rpi-blb* gene; cluster 2, pseudogenes, except for pnt2 haplotype; cluster 3, *S. polytrichon*-specific haplotypes; cluster 4, other haplotypes.

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