

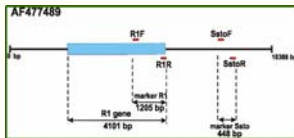
Potato resistance to late blight as related to the *R1* and *R3* genes introgressed from *S. demissum* and *S. stoloniferum*.

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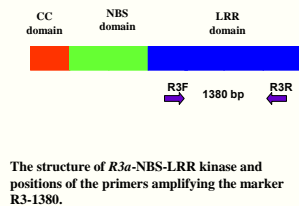
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For several decades, breeding potato for late blight (LB) resistance heavily relied on germplasm introgressed from wild *Solanum* species, primarily *S. demissum*, the source of race-specific resistance genes (*R*-genes). New pathogen races are generally assumed to rapidly defeat such monogenic resistance; however, the presence of the SCAR marker for the major *demissum* gene *R1* in potato cultivars was shown to correlate with higher field indices of LB resistance (Gebhardt et al., 2004; Beketova et al., 2006). Such evidence adds to persistent discussion on the role of race-specific *R*-genes in plant defense response (Stewart et al., 2003; Tan et al., 2008). We developed three SCAR markers recognizing the race-specific genes *R1* and *R3* of *S. demissum* and *S. stoloniferum* and the germplasm of these species.

Marker and its size, bp	Prototype clone	Chromosome	Position in the prototype clone
R1-1205	AF447489	5	5126-6331
R3-1380	AY849382	11	1677-3056
Ssto-448	EU041625	5	100-548



Relative positions of the markers R1-1205 and Ssto-448 on the chromosome 5 fragment AF477489



The structure of *R3a*-NBS-LRR kinase and positions of the primers amplifying the marker R3-1380.

Marker verification. By screening wild *Solanum* species and potato accessions reportedly free from wild *Solanum* germplasm (Chilotanum forms and heirloom cultivars), we demonstrated that these markers reliably discerned between germplasms of *Solanum tuberosum* ssp. *tuberosum* and wild sources of LB resistance.

Frequencies of markers for *R*-genes and *dms/sto* germplasm in potato and its wild relatives

Genotypes (the number of accessions)	SCAR markers		
	R1-1206	R3-1380	Ssto-448
<i>S. demissum</i> (26)	0.42	0.08	0.8
<i>S. stoloniferum</i> (41)	0.20	0.22	0.8
<i>S. tuberosum</i> ssp. <i>tuberosum</i> : Chilotanum (5)	0.0	0	0
<i>S. tuberosum</i> ssp. <i>tuberosum</i> : heirloom cultivars reportedly free of <i>dms/sto</i> germplasm (6)	0.0	0	0.0
Demissoid potato cultivars (161)	0.35	0.34	0.77

The markers R1-1205 and R3-1380 were also verified by cloning and sequencing the genome fragments equivalent to the markers from potato cultivars, including differentials, and *S. stoloniferum*; their sequences were 99% homologous to the corresponding regions of the genes *R1* and *R3a* in *S. demissum*.

The marker R1-1205 represents the active *R1* allele, and the marker Ssto-448 resides in both *R1* and *r1* alleles. It follows that the frequency of the *R1* allele in *S. demissum* and derived potato cultivars is about 0.5. Following introgression, only some markers of wild *Solanum* germplasm crossed several meiotic generations and were maintained at high frequencies in modern potato cultivars. The frequencies of other markers characteristic of *S. demissum* and *S. stoloniferum* developed from the ITS sequence of rDNA (AY875783), *waxy* (*GBSSI*) gene (AY875455, AY875536) and *LEAFY* intron 2 sequence (DQ256075) dropped from 0.4-1.0 in the wild species to 0.06-0.34 in potato cultivars.

In addition to *S. demissum* and *S. stoloniferum*, homologues of *R1* and *R3* were found in few accessions of *S. bulbocastanum*, *S. cardiophyllum*, *S. hougasii*, *S. iopetalum*, *S. pinnatisectum*, and *S. polytrichum*.

Markers for the *R* genes and germplasm of the species conferring LB resistance in the series of the section *Petota*

Series and species	The total number of screened accessions	The total numbers of accessions comprising the particular markers		
		R1-1205	R3-1380	Ssto
tetraploid <i>Tuberosa</i> :	17	0	0	0
<i>S. tuberosum</i> ssp. <i>tuberosa</i> (Chilotanum and heirloom cultivars reportedly free of <i>dms/sto</i> germplasm)				
diploid <i>Tuberosa</i> : (<i>verrucosa</i>)	2	0	0	1
Demissoid potato cultivars	161	57	55	51 (of 61)
<i>Demissa</i> (four species)	31	12	4	21
<i>Longipedicellata</i> (five species)	66	9	11	44
<i>Pinnatisecta/Cardiophylla</i> (five species)	47	0	3	1
<i>Polyadenia</i> (one species)	6	0	0	1
<i>Bulbocastana</i> (one species)	2	0	5	0

Screening about 200 potato cultivars demonstrated that the R1-1205 and R3-1380 markers were significantly associated with higher LB resistance (Mann-Whitney U-test) presuming that both *R1* and *R3* genes contribute to LB resistance of potato cultivars. The control subpopulations 1 and 3 lacking the markers R1-1205 and R3-1380 manifest higher LB resistance than the control 2 free of the germplasm of *S. demissum* и *S. stoloniferum*. Such difference presumes the presence of *demissum* *R*-genes other than *R1* and *R3*.

Association of potato late blight resistance with the presence of the *R1* and *R3* genes. Marker analysis. Average score by 9-score scale. The number of potato accessions in each subpopulation is given in parentheses. (A) Passport indices of late blight resistance; (B) Field and laboratory evaluations at the Institute of Phytopathology.

Subpopulations of potato cultivars	Average score		
	A		B
	Foliage	Tubers	Foliage/leaves
comprising R1-1205	6.0 (26) ^{bc}	6.42 (26) ^{bc}	5.3 (22) ^c
comprising R3-1380	5.6 (20) ^d	6.06 (17)	5.4 (23) ^c
comprising both R1-1205 and R3-1380	6.07 (13) ^e	6.46 (13)	5.9 (20) ^{bc}
all accessions comprising the <i>R</i> -gene markers	5.86 (59) ^{bc}	6.32 (56) ^{bd}	5.51 (65) ^{bc}
control 1: accessions devoid of R1-1205 and R3-1380 markers	5.0 (59) ^f	5.62 (53)	4.76 (44) ^f
control 2: <i>tuberosum</i> accessions free of the germplasm of <i>S. demissum</i> and <i>S. stoloniferum</i>	3.8 (17) ^{ac}	5.1 (11)	3.2 (8) ^{ac}
control 3: cultivars comprising the germplasm of <i>S. demissum</i> and <i>S. stoloniferum</i> but devoid of R1-1205 and R3-1380 markers	5.4(42) ^e	5.8 (42)	5.1 (36) ^e

- a Significantly different from the control 1 at 1% confidence level
 b Significantly different from the control 1 at 5% confidence level
 c Significantly different from the control 2 at 1% confidence level
 d Significantly different from the control 2 at 5% confidence level
 e Significantly different from the control 3 at 5% confidence level

Association of potato late blight resistance with the presence of the *R1* and *R3* genes. Analysis with *Ph. infestans* races.

The assessment of late blight resistance of potato cultivars using simple races of *Ph. infestans* (race 1 to 4) also demonstrated the significant input of the *R*-genes into potato resistance. In most cases, the data obtained by such phytopathological *R*-genotyping confirmed the evidence for the presence of one or both *R* genes recognized with the molecular markers. The agreement was higher for *R1* than for *R3* because the marker R3-1380 can probably recognize the related *R5-R11*. The discrepancies between two independent methodologies could also result from the changes in the structure and expression of *R* genes.

Association of potato late blight resistance (average of field and laboratory indices assessed in the Institute of Phytopathology in 2008-2009) with the presence of *R*-genes discerned with *Ph. infestans* races 1 to 4. Average score by 9-score scale. The number of potato accessions in each subpopulation is given in parentheses.

Subpopulations of potato cultivars	Average resistance score
comprising the <i>R1-R4</i> genes	6.1 (57) ^a
free of the <i>R1-R4</i> genes	4 (29)

Significant at 1% confidence level by Mann-Whitney U-test

Agreement between the phytopathological genotyping and molecular marker analysis (71 potato cultivar)

The number of matches between two assessment methods	Genes		
	<i>R1</i>	<i>R3</i>	<i>R1&R3</i>
	53	44	33
%	75	62	47

The described markers would promote searching for new *R*-genes and breeding for LB resistance, especially when screening cross and backcross segregants

The authors cordially thank all colleagues who kindly provided *Solanum* accessions and the relevant information.

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