

A complex resistance locus in *Solanum americanum* recognizes a conserved *Phytophthora* effector

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Abstract

Late blight caused by *Phytophthora infestans* greatly constrains potato production. Many *Resistance (R)* genes were cloned from wild *Solanum* species and/or introduced into potato cultivars by breeding. However, individual *R* genes have been overcome by *P. infestans* evolution; durable resistance remains elusive. We positionally cloned a new *R* gene, *Rpi-amr1*, from *Solanum americanum*, that encodes an NRC helper-dependent CC-NLR protein. *Rpi-amr1* confers resistance in potato to all 19 *P. infestans* isolates tested. Using association genomics and long-read RenSeq, we defined eight additional *Rpi-amr1* alleles from different *S. americanum* and related species. Despite only ~90% identity between *Rpi-amr1* proteins, all confer late blight resistance but differentially recognize *Avramr1* orthologs and paralogs. We propose that *Rpi-amr1* gene family diversity assists detection of diverse paralogs and alleles of the recognized effector, facilitating durable resistance against *P. infestans*.

Introduction

Potato is the third most important directly-consumed food crop world-wide¹. *Phytophthora infestans*, an oomycete pathogen, causes late blight disease in potato, and can result in complete crop failure. Disease management is primarily based on repeated fungicide applications (10-25 times per season in Europe). However, fungicide-resistant races have emerged².

To elevate late blight resistance, *Resistance to Phytophthora infestans (Rpi)* genes were identified in wild relatives of potato and used for resistance breeding³. More than 20 *Rpi* genes have been mapped and cloned from different *Solanum* species, e.g. *R2 (Rpi-blb3)*, *R3a*, *R8*, *Rpi-blb1*, *Rpi-blb2* and *Rpi-vnt1*⁴⁻¹⁰. All encode coiled-coil (CC), nucleotide binding (NB), leucine-rich repeat (LRR) (NLR) proteins¹¹ and some require helper NLR proteins of the NRC family¹². However, most cloned *Rpi* genes can be overcome by at least one strain of *P. infestans*¹³. Provision of durable late blight resistance for potato remains a major challenge.

NLR-mediated immunity upon effector recognition activates “effector-triggered immunity” (ETI)¹⁴. In oomycetes, all identified recognized effectors, or avirulence (AVR) proteins, carry a signal peptide and an RXLR motif¹⁵. 563 RxLR effectors were predicted from the *P. infestans* genome, enabling identification of the recognized effectors^{16,17}. Many *P. infestans* effectors show signatures of selection to evade recognition by corresponding NLR proteins¹⁸. *NLR* genes also show extensive allelic and presence/absence variation in wild plant populations^{19,20} and known *Resistance (R)* gene loci like *Mla*, *L*, *Pi9*, *RPP1* and *RPP13* from barley, flax, rice and Arabidopsis show substantial allelic polymorphism²¹⁻²⁴. Remarkably, different barley *Mla* and flax *L* gene alleles can recognize sequence-unrelated effectors^{25,26}.

Technical advances like RenSeq (Resistance gene enrichment and Sequencing) and PenSeq (Pathogen enrichment Sequencing) enable rapid definition of allelic variation and mapping of plant *NLRs*, or discovery of variation in pathogen effectors²⁷⁻²⁹. Combined with single-molecule real-time (SMRT) sequencing, SMRT RenSeq enabled cloning of *Rpi-amr3* from *Solanum americanum*³⁰. Similarly, long read and cDNA

PenSeq enabled us to identify *Avramr1* from *P. infestans*³¹.

In this study, we further explored the genetic diversity of *S. americanum*, and by applying sequence capture technologies, we fine-mapped and cloned *Rpi-amr1* from *S. americanum*, (usually) located on the short arm of chromosome 11. Multiple *Rpi-amr1* homologs were found in different *S. americanum* accessions and in relatives, including *Solanum nigrescens* and *Solanum nigrum*. Functional alleles show extensive allelic variation and confer strong resistance to all 19 tested diverse *P. infestans* isolates. Although differential recognition was found between different *Rpi-amr1* and *Avramr1* homologs, all *Rpi-amr1* alleles recognize the *Avramr1* homologs from *Phytophthora parasitica* and *Phytophthora cactorum*. Our study reveals unique properties of genetic variation of *R* genes from “non-host” species.

Results

***Rpi-amr1* maps to the short arm of chromosome 11**

We previously investigated *S. americanum* and isolated *Rpi-amr3* from an accession 944750095 (SP1102)³⁰. To discover new *Rpi-amr* genes, we characterized additional 14 lines of *P. infestans*-resistant *S. americanum* and close relatives *S. nigrescens* and *Solanum nodiflorum* by crossing them to a susceptible (S) *S. americanum* line 954750186 (hereafter SP2271) (Table 1, Fig. S1). To avoid self-pollination, a resistant parent was always used as a pollen donor. All the corresponding F1 plants (6-10 per cross) were resistant in a detached leaf assay (DLA) (Table 1). Around 60-100 F2 progeny derived from each self-pollinated F1 plant were phenotyped by DLA using *P. infestans* isolate 88069³². The F2 progenies that derived from the resistant parents with working numbers SP1032, SP1034, SP1123, SP2272, SP2273, SP2360, SP3399, SP3400, SP3406, SP3408 and SP3409 segregated in a ratio suggesting the presence of a single (semi-) dominant resistance gene (fitting 3:1 or 2:1 [likely due to segregation distortion], R:S - resistant to susceptible - ratio). Two crosses showed a 15:1 segregation (resistant parent SP2300 and SP2307), suggesting the presence of two unlinked resistance genes. Investigating resistance in SP1101 required two backcrosses to SP2271 prior to selfing of resistant progeny to reveal a 3:1 R:S segregation.

To identify *Rpi* genes from these resistant *S. americanum* accessions, we prioritized an F2 population derived from resistant parent SP2273 and named the corresponding gene *Rpi-amr1*. Using markers from RenSeq, genotyping by sequencing (RAD markers) and Whole Genome Shotgun sequencing (WGS), the *Rpi-amr1* gene was mapped in a small population (n=188 gametes) to the short arm of chromosome 11, between markers RAD_3 and WGS_1 (Fig. 1a, Table S1, S2). We expanded the mapping population and developed a PCR marker WGS_2 that co-segregated with resistance in 3,586 gametes (Fig. 1b, Table S2). To generate the physical map of the target interval from SP2273, a BAC library was generated. Two BAC clones (12H and 5G) covering the target interval were identified by a PCR screen with the above linked marker, sequenced on the PacBio RSII platform, and assembled into a single contig of 204,128 bp (Fig. 1c). We predicted 11 potential coding sequences on the assembled contig, nine of which encode *NLR* genes (Fig. 1c). These *NLR* genes belong to the CNL class and have 80-96% between-paralog identity.

To define which of these *NLR* genes are expressed, cDNA RenSeq data of the resistant parent SP2273 were generated and mapped to the BAC_5G sequence. Seven out of nine *NLR* genes were expressed. These genes - *Rpi-amr1a*, *b*, *c*, *d*, *e*, *g* and *h* - were tested as candidate genes for *Rpi-amr1* (Fig. 1c).

***Rpi-amr1e* confers resistance in *Nicotiana benthamiana* and cultivated potato**

To test the function of the seven candidate genes, we cloned their open reading frames from genomic DNA inclusive of introns into a binary expression vector under control of the 35S promoter. *Rpi-amr3* was used as a positive control and the non-functional *Rpi-amr3-S* was used as a negative control. The constructs carrying each of the seven candidate genes were transiently expressed after *Agrobacterium* infiltration into *N. benthamiana* leaves, which were subsequently inoculated with the *P. infestans* isolate 88069 as described previously³⁰. *P. infestans* growth was observed six days post inoculation (dpi). Only 35S::*Rpi-amr1e*-infiltrated leaves showed reduced pathogen growth at 6 dpi compared to other candidate genes like *Rpi-amr1c*, or negative control *Rpi-amr3-S*. (Fig. 1e). Hence, we conclude that *Rpi-amr1e* is the functional *Rpi-amr1* (hereafter) gene from *S. americanum* SP2273.

To test if *Rpi-amr1* confers late blight resistance in potato, we cloned it with its native promoter and terminator, and generated transgenic potato cultivar Maris Piper plants carrying *Rpi-amr1*. A non-functional paralog *Rpi-amr1a* was also transformed into Maris Piper as a negative control. As in the transient assay, stably transformed *Rpi-amr1* lines resisted *P. infestans* 88069 in potato (Fig. 1f), but *Rpi-amr1a*-transformed plants did not (Fig. 1g).

***Rpi-amr1* is a four exon CC-NLR**

To characterize the structure of *Rpi-amr1*, we mapped the cDNA RenSeq data to the full length *Rpi-amr1* gene and found four alternatively spliced forms of *Rpi-amr1*. The most abundant form, supported by >80% of reads, comprises four exons encoding a protein of 1,013 amino acids. The remaining three forms had shifts in reading frames, leading to premature stop codons or absence of some exons. This was confirmed with 3' RACE PCR (Fig. 1d). The *Rpi-amr1* is a typical CC-NB-LRR resistance protein, with a coiled-coil domain (CC; amino acids 2-146), nucleotide binding domain (NB-ARC; amino acids 179-457) and leucine-rich repeats (LRR; located between amino acids 504-900) which are all positioned in the first exon (1-918 aa, Fig. 2a). The remaining three short exons (amino acids 919-943, 944-1002 and 1,003-1,013) lack homology to any known domains. No integrated domains³³ were found in the *Rpi-amr1* protein.

Functional *Rpi-amr1* homologs are present in were identified from multiple resistant *S. americanum* and relatives

Previously, we found at least 14 *S. americanum* accessions and related species that resist late blight (Table 1). To test if *Rpi-amr1* contributes to late blight resistance in other resistant *S. americanum* accessions, we genotyped 10-50 susceptible F2 plants of the populations derived from resistant accessions, with a marker positioned in *Rpi-amr1* gene (56766, Fig. 1 and Table S2). We found that the marker is absent in all tested susceptible descendants of accessions SP1032, SP1034, SP1123, SP2272, SP2307, SP2360, SP3399, SP3400, SP3406 and SP3408, suggesting that the resistance is linked to the *Rpi-amr1* locus. To test if in these accessions the resistance is conferred by functional *Rpi-amr1* homologs, we performed SMRT RenSeq-based *de novo* assembly

of each resistant accession, and looked for homologs with the greatest identity to *Rpi-amr1*. For accessions SP2307, SP3399 and SP3406, we also used cDNA RenSeq to monitor their expression. We mapped *de novo* contigs to the coding sequence of *Rpi-amr1* allowing for 15% mismatches and gaps, and selected the closest homolog as a candidate *Rpi-amr1* ortholog (Table S3). In three resistant parents, namely SP1034, SP2360 and SP3400, the functional alleles showed 100% identity at the amino acid level to *Rpi-amr1*, while amino acid sequences from the remaining accessions had as little as 89% identity to the functional *Rpi-amr1* (Table S3). As described previously, we transiently expressed the closest related candidate *Rpi-amr1* homologs in *N. benthamiana* leaves followed by DLA with *P. infestans* isolate 88069, and verified their functionality. The unique homologs of *Rpi-amr1-2273* were named as *Rpi-amr1-1032*, *Rpi-amr1-1123*, *Rpi-amr1-2272*, *Rpi-amr1-2307* and *Rpi-amr1-3408*.

For some accessions, like SP1101 and SP2300, the *Rpi-amr1*-linked markers gave ambiguous results, so we directly performed bulked segregant analysis (BSA) and RenSeq. Additional *Rpi-amr1* co-segregating paralogs, *Rpi-amr1-1101* and *Rpi-amr1-2300*, were identified and verified in transient assays as above (Fig. 2b).

Similarly, we inspected an F2 population derived from *S. nigrescens* accession SP3409 (Table 1). We applied BSA RenSeq and SMRT RenSeq to the resistant parents and F2 segregating population, and we found five candidate *NLRs* belonging to the same *Rpi-amr1* clade, all of which are expressed. The five candidates were cloned, and transient assays verified one of them as a functional *Rpi-amr1* homolog, *Rpi-amr1-3409*. However, *Rpi-amr1-3409* does not co-segregate with *Rpi-amr1*-linked markers. We used GenSeq sequence capture-based genotyping (Chen *et al.* 2018), and found that *Rpi-amr1-3409* locates on chromosome 1, based on the potato DM reference genome³⁴. This result suggests that a fragment of DNA that locates on distal end of the short arm of chromosome 11 in other resistant accessions was translocated to the distal end of the long arm of chromosome 1 in SP3409.

When the full-length amino acid sequences of nine *Rpi-amr1* homologs were aligned, the polymorphisms between different functional alleles were found to be distributed through all domains including the LRR region (Fig. 2a and Fig. S2).

Taken together, by using BSA RenSeq, SMRT RenSeq, cDNA RenSeq, association genomics and GenSeq, we cloned eight additional functional *Rpi-amr1* homologs from different resistant accessions, of which all confer resistance to *P. infestans* 88069 in transient assays. The closest *Rpi-amr1* homolog from susceptible parent SP2271 does not confer resistance (Fig. 2b).

***Rpi-amr1* confers broad-spectrum late blight resistance in cultivated potato**

To test the scope of late blight resistance conferred by *Rpi-amr1* and its homologs, we generated stably transformed transgenic potato cv Maris Piper plants carrying *Rpi-amr1-2272* and *Rpi-amr1-2273*, the most diverged of the homologs (Table S3), and inoculated them by DLA with 19 *P. infestans* isolates from UK, the Netherlands, Belgium, USA, Ecuador, Mexico and Korea (Table 2). Many of the tested *P. infestans* isolates can defeat multiple *Rpi* genes (Table 2). Our DLAs show that Maris Piper carrying *Rpi-amr1-2272* or *Rpi-amr1-2273* resist all 19 tested *P. infestans* isolates, while the wild-type Maris Piper control is susceptible to all of them. This indicates that *Rpi-amr1* confers broad-spectrum resistance against diverse *P. infestans* races.

Differential recognition by *Rpi-amr1* alleles of *Avramr1* homologs

Avramr1 (PITG_07569) was identified in *P. infestans* race T30-4 by long-read and cDNA PenSeq, and multiple *Avramr1* homologs were identified in four *P. infestans* isolates and classified into four subclades³¹. To investigate if all nine cloned *Rpi-amr1* homologs could recognize diverse *Avramr1* homologs from different *P. infestans* isolates, in addition to *Avramr1* from race T30-4 that corresponds to clade A, we synthesized three *Avramr1* homologs *Avramr1-13B1*, *Avramr1-13C2* and *Avramr1-13D1* from isolate 3928A (EU_13_A2, commonly known as “Blue 13”), corresponding to clades B, C and D, respectively (Fig. 3). We also synthesized the *Avramr1* homologs from *P. parasitica* and *P. cactorum*³¹. These six *Avramr1* homologs were co-expressed in *N. benthamiana* by agro-infiltration in all possible combinations with nine functional *Rpi-amr1* homologs and the non-functional *Rpi-amr1-2271* as a negative control (Fig. 3).

We found that different combinations of *Rpi-amr1* alleles and *Avramr1* homologs led either to strong, weak or no HR phenotype in transient assay, but the non-functional *Rpi-amr1-2271* allele failed to recognize any *Avramr1* homologs (Fig. 3) The representative HR phenotype and the scoring of HR indices are shown in Fig. S3. *Rpi-amr1-2300* and *Rpi-amr1-2307* recognized one *Avramr1* homolog each, but others detected *Avramr1* homologs from more than one clade. Clade C, represented here by *Avramr1-13C2*, is usually not expressed³¹, and when expressed from 35S promoter, this effector was not recognized by most *Rpi-amr1* homologs, though a weak HR was observed upon co-expression with *Rpi-amr1-2272*. *Avramr1-13D1* belongs to Clade D, which is absent in T30-4 but present in four other sequenced isolates³¹, and was recognized by all but one (*Rpi-amr1-2300*) homologs in the transient assay. Surprisingly, two *Avramr1* homologs from *P. parasitica* and *P. cactorum* are strongly recognized by all functional *Rpi-amr1* homologs, apart from *Rpi-amr1-2272* which showed a weaker HR (Fig. 3).

Collectively, our data shows that *Rpi-amr1/Avramr1* homolog pairs provoke quantitatively and qualitatively different HRs, but all functional *Rpi-amr1* homologs detect at least one *Avramr1* homolog from *P. infestans* isolate 3928A.

Both *Rpi-amr1*-mediated resistance and effector recognition are NRC2 or NRC3 dependent

We generated a phylogenetic tree for representative *Solanaceae* NLR proteins. *Rpi-amr1* is grouped with clade CNL-3, from which no functional resistance genes were previously cloned (Fig. 4a). The closest related cloned functional gene is *Rpi-amr3* (31.2% identity on aa level) belonging to clade CNL-13 and located on chromosome 4. The phylogenetic affiliation suggested that *Rpi-amr1* is likely to depend on the helper NRC clade because CNL-3 is among the large super-clade of NRC-dependent sensors (Fig. 4a)¹².

To test this hypothesis, we transiently expressed *Rpi-amr1-2273* together with *PpAvramr1* in NRC4, NRC2/3 or NRC2/3/4 knock out *N. benthamiana* leaves^{35,36} (Fig. S4). The HR phenotype was abolished in NRC2/3 and NRC2/3/4 knockout plants (Fig. S5c and b), but not in NRC4 knock-out or wild-type plants (Fig. S5d and a). The HR

was recovered when NRC2 or NRC3 was co-expressed in the NRC2/3/4 or NRC2/3 knock out plants, but co-expression of NRC4 did not complement the loss of HR phenotype in NRC2/3/4 knockout plants. (Fig. S5b and c). We further showed that also *Rpi-amr1* mediated resistance is dependent on NRC2 or NRC3 but not NRC4, as transient expression of *Rpi-amr1-2273* followed by *P. infestans* infection restricted pathogen growth only in NRC4 knockout *N. benthamiana* plants (Fig. 4b-d). These data indicate that both the effector recognition and resistance conferred by *Rpi-amr1* are NRC2 or NRC3 dependent.

Functional *Rpi-amr1* homologs are present in hexaploid *S. nigrum* accessions

Most *S. nigrum* accessions are highly resistant to *P. infestans* and *S. nigrum* has been reported to be a “non-host” to *P. infestans*³⁷, even though rare accessions are susceptible³⁸. *S. americanum* may be the diploid ancestor of hexaploid *S. nigrum*³⁹. To test if *Rpi-amr1* also contributes to late blight resistance in *S. nigrum*, we designed nested PCR primers based on the *Rpi-amr1-2273* sequence, and amplified and sequenced the coding sequence of *Rpi-amr1* homologs from two resistant and one reported susceptible *S. nigrum* accessions³⁸. From two resistant accessions (SP1088 and SP1097; Table S4), we amplified sequences with >99% nucleotide identity to *S. americanum* *Rpi-amr1-2273*, namely *Rpi-nig1-1088* and *Rpi-nig1-1097*. The protein sequences of *Rpi-nig1-1088* and *Rpi-nig1-1097* are identical, with only one amino-acid (225 R->Q) change compared to *Rpi-amr1-2273* (Fig. S6a). The primers used for allele mining did not amplify a product of the expected size for *Rpi-amr1* from the susceptible line SP999. To test their function, we performed transient assay for HR and disease resistance on *N. benthamiana*. We found both *Rpi-nig1-1088* and *Rpi-nig1-1097* show strong HR when co-expressed with PpAvramr1 and PcAvramr1. However, they activate a weaker HR to Avramr1 and Avramr1-13B1 compared to *Rpi-amr1-2273* (Fig. S6b). Like *Rpi-amr1-2273*, but not the negative control *Rpi-amr1-2271*, transiently expressed *Rpi-nig1-1088* and *Rpi-nig1-1097* confer resistance to *P. infestans* 88069 (Fig. S6c). It is the first report of functional *Rpi* genes from *S. nigrum*, and our finding suggests the strong late blight resistance of *S. nigrum* is determined or partially determined by the *Rpi-amr1* homologs, that were most likely inherited from *S. americanum*.

High allelic diversity at *Rpi-amr1* was generated through inter-paralog and ortholog sequence exchange

Rpi-amr1 alleles show relatively high nucleotide diversity ($\pi=0.04$), which could be an indication of balancing or diversifying selection (Table S5). In addition, *Rpi-amr1* alleles differ in their recognition of the *Avramr1* homologs (Fig. 3) which is also consistent with selection in a host-parasite co-evolutionary arms race. To test the hypothesis that allelic polymorphism at *Rpi-amr1* results from diversifying selection, we calculated diversity statistics and performed a McDonald-Kreitman test on both *Rpi-amr1* alleles and *Avramr1* homologs. As expected, *Avramr1* homologs show a signature consistent with balancing selection (Tajima's $D = 2.27$) (Table S5). Remarkably, despite the high nucleotide diversity, no clear signals of balancing or diversifying selection were detected for *Rpi-amr1* (Tajima's $D = 0.09083$) (Table S5). Aligning the *Rpi-amr1* alleles against the reference and scrutinizing the sequences in more detail provided further insights. The nucleotide similarity of alleles varies markedly across the *Rpi-amr1* homologs (Fig. 2a and Table S3); this pattern is consistent with occasional recombination between highly diverged alleles or paralogs.

To test whether recombination could explain the observed polymorphisms in *Rpi-amr1* alleles, we predicted the possible recombination events using 3SEQ. Several recombination events were detected between *Rpi-amr1* orthologs from different *S. americanum* accessions, and *Rpi-amr1* paralogs from SP2273 (Table S6). Some sequence exchanges were visualized using HybridCheck (Fig. S7)⁴⁰, and these data suggest that sequence exchange occurred between functional *Rpi-amr1* alleles and paralogs. To confirm these findings, we mapped all cloned *Rpi-amr1* CDS back to the BAC_5G sequence from accession SP2273 (Fig. S8). As expected, some *Rpi-amr1* homologs (e.g. SP2300 and SP2272) show a perfect match with the fourth NLR, and show a distribution of high identity that reflects the intron-exon structure. For some homologs (e.g. 2271), 5' end sequences match different NLR sequences on the BAC_5G and for others (e.g. 2275) part of the sequence is highly diverged from BAC_5G. Taken together, our results indicate that the polymorphism of *Rpi-amr1* alleles appears to have arisen partly due to sequence exchange between highly diverged alleles and paralogs, and not just through mutation accumulation.

Discussion

Achieving complete and durable resistance is the ultimate goal of resistance breeding. Here, we report significant progress towards durable resistance against potato late blight. Most cloned late blight resistance genes derive from wild tuber-bearing species of genus *Solanum*, and many have been overcome by one or more *P. infestans* strains⁴¹. Conceivably, resistance to *P. infestans* in nearly all *S. americanum* and *S. nigrum* accessions is due to multiple *NLR* genes, as zoospores from *P. infestans* can germinate on *S. nigrum* leaves but penetration is stopped by strong HR^{37,42}. *Rpi* genes from plant species that only rarely support pathogen growth have likely not participated, or are no longer participating, in an evolutionary arms race with *P. infestans*, and hence, the pathogen's effectors have not (yet) evolved to evade detection by these *Rpi* genes. Under this scenario, a pre-existing standing variation in the pathogen for overcoming such *Rpi* genes is either absent or extremely rare. This makes such genes promising candidates for provision of broad-spectrum and durable late blight resistance, provided they are not deployed alone which facilitates one-step genetic changes in the pathogen to evade them, but rather in combination with other genes, as in the source plant⁴³.

We report here a novel, broad-spectrum *S. americanum* resistance gene, *Rpi-amr1*. We also identified eight additional *Rpi-amr1* alleles from different *S. americanum* accessions and relatives, including one *Rpi-amr1* allele that translocated to the long arm of chromosome 1. Homology-based cloning also revealed the presence of functional *Rpi-amr1* homologs in *S. nigrum*. All nine cloned *Rpi-amr1* alleles confer late blight resistance in transient assays in *N. benthamiana*, and both *Rpi-amr1-2272* and *Rpi-amr1-2273* in potato cv Maris Piper background confer resistance to all 19 tested *P. infestans* isolates from different countries, many of which overcome other *Rpi* genes. Thus, *Rpi-amr1* is widely distributed in germplasm of *S. americanum*, its relatives and *S. nigrum*, and may contribute to the resistance of nearly all accessions to *P. infestans*.

Many plant *R* genes and their corresponding *Avr* genes evolved differential recognition specificities with extensive allelic series for both *R* gene and *Avr* genes. Examples include *ATR1* and *RPP1* or *ATR13* and *RPP13* from *Hyaloperonospora arabidopsidis* and *Arabidopsis*¹⁵, *Avr567* and *L* genes from the rust *Melampsora lini* and flax⁴⁴, and

multiple and diverse recognized effectors from barley powdery mildew and *Mla* from barley. Similarly, *Avramr1* and its homologs from several *P. infestans* races³¹ were found to be differentially recognized by alleles of the *Rpi-amr1* gene. Remarkably though, *Rpi-amr1* nucleotide diversity of the *R* gene did not show any of the hallmarks of diversifying or balancing selection.

Rather than through mutation accumulation, the high allelic variation observed at *Rpi-amr1* appears to have been generated partly by recombination between significantly diverged alleles and paralogs. The recombination events are likely to be rare relative to the mutation rate, given that the alleles carry many polymorphisms. This evolutionary scenario can explain the observed mosaic-like structure of high and low sequence similarities when the *Rpi-amr1* alleles were mapped against the contig based on two overlapping BAC clones. The deep coalescence of alleles that is implicit in this scenario can be generated by balancing selection, but we did not find evidence of such selection when analysing the nucleotide substitution patterns. Recombination between *Rpi-amr1* alleles could have eroded this signature of selection, as has been observed also in *Rpi1* resistance genes in grasses⁴⁵ and in the vertebrate immune genes of the major histocompatibility complex (MHC)^{46,47}. Nucleotide sequence diversity across the *Rpi-amr1* alleles is correlated with only slight differences in *Avramr1* recognition specificity. *Rpi-amr1* alleles can even recognize multiple *Avramr1* paralogs from a single *P. infestans* strain, a scenario that might elevate durability of resistance. Since the *S. americanum* population recognizes multiple *Avramr1* alleles and paralogs, small mutational changes in *Avramr1* gene are unlikely to suffice to escape detection, which makes resistance-breaking less likely, thus promoting evolutionary durability of *Rpi-amr1*. Remarkably, *Avramr1* (*PITG_07569*) was recently reported to regulate plant alternative splicing and promote the colonization of *P. infestans*⁴⁸, indicating *Avramr1* contributes an important function for the virulence of *P. infestans*. We hypothesise that this enhanced recognition capacity could be key to the evolution of “non-host” resistance, offering an escape for the plant from the coevolutionary arms race. Conceivably, stacking *Rpi-amr1* alleles *in cis* could extend the recognition specificities, which could potentially lead to even more durable late blight resistance.

Intriguingly, two *Avramr1* homologs from *P. parasitica* and *P. cactorum* are recognized by all *Rpi-amr1* homologs. Presumably, these genes have been under even

less selection pressure to evade *Rpi-amr1* recognition. This result indicates that *Rpi-amr1* has the potential to provide “non-host” type resistance in *S. americanum* against multiple oomycete pathogens like *P. parasitica* and *P. cactorum*, which can infect a wide range of hosts. As both the resistance and effector recognition of *Rpi-amr1* are *NRC2* or *NRC3* dependent, co-expression of *NRC2* or *NRC3* with *Rpi-amr1* might enable it to confer resistance to other *Phytophthora* species outside the *Solanaceae*.

In summary, we cloned *Rpi-amr1*, a broad-spectrum *Rpi* gene that contributes to the strong late blight resistance of nearly all *S. americanum* accessions to late blight. The apparent redundancy across the *Rpi-amr1* gene family may serve an evolutionary function by broadening the scope for recognizing multiple *Avramr1* alleles and paralogs, and potentially reducing the probability of evolution of resistance-breaking strains. Stacking this type of *Rpi* gene with additional *Rpi* genes might help to turn host plants such as potato into non-hosts for late blight, enabling broad-spectrum and durable resistance.

Materials and Methods:

Development of mapping populations

Fourteen *P. infestans* resistant diploid *Solanum americanum* and relatives were used in this study (Table 1). The F1 populations were generated by crossing with a susceptible *Solanum americanum* accession 954750186 (working name SP2271) as a female parent. Heterozygous F1 progeny was allowed to self-pollinate to generate F2 segregating populations, or further back-crossed to the susceptible parent and allowed to self-pollinate until resistance to *P. infestans* co-segregated as a monogenic trait.

***P. infestans* infection assay**

P. infestans isolates were cultured on rye and sucrose agar (RSA) medium at 18 °C for 10 days. Sporangia were washed off with cold water and incubated at 4°C for 1-2 h to induce zoospore release. Detached leaves were inoculated on the abaxial side with 10 µl droplets of zoospore suspension (50-100,000 per ml). The inoculated leaves were incubated at 18°C in high humidity under 16 h day/8 h night photoperiod conditions. Disease scoring was done at 5-7 days after infection.

DNA and RNA extraction

RenSeq experiments (both short- and long-reads protocols) were conducted on gDNA freshly extracted from young leaves using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. For the cDNA RenSeq experiment, RNA was extracted using TRI-Reagent (Sigma-Aldrich, MO, USA) and Direct-zol RNA MiniPrep Kit (Zymo Research, CA, USA), following manufacturer's recommendations.

Mapping of *Rpi-amr1*

To map the underlying resistance gene from the resistant parent 954750184 (working name SP2273), we generated an F2 segregating population which was phenotyped with *P. infestans* isolates EC1_3626 and 06_3928A. Selected resistant plants were self-pollinated and up to 100

plants from F3 populations were screened for resistance and susceptibility with *P. infestans* isolates EC1_3626 and 06_3928A. gDNA from susceptible F2 and F3 plants (BS pool), as well as gDNA from the resistant (R) and susceptible parent (S) were subjected to RenSeq using Solanaceae bait library²⁷ and sequenced with Illumina MiSeq 250 bp paired-end reads. Pre-processing, assembly, mapping and SNP calling was performed as described earlier^{27,30}.

The same gDNA samples were used in a RAD-seq experiment using PstI digestion and Illumina HiSeq sequencing, which was outsourced to Floragenex Inc. (OR, USA). Bioinformatic analysis was also performed by Floragenex using *Solanum lycopersicum* genome as a reference⁴⁹. SNP calling resulted in sixteen polymorphic sites with eleven of them locating at the top of chromosome 11 (Supplementary table 1). The remaining ones were randomly distributed on chromosomes 4 and 1.

We additionally outsourced Whole Genome Shotgun sequencing (WGS) of R and S samples to BGI (BGI, Shenzhen, China) for ~30 deep Illumina HiSeq sequencing with 100PE. Reads from the resistant parent were assembled as described in³⁰ and we used our previously published *in silico* trait mapping pipelines to perform SNP calling and detection of polymorphisms linked to disease resistance^{27,30}. Contigs polymorphic between R and S parents were further aligned to the DM reference genome to identify their position.

Screening a set of markers derived from these three approaches on gDNA of 94 susceptible F2 and F3 plants identified 12 markers linked to resistance response that flank the *R* locus between 7.5 cM to one side and 4.3 cM to the other side (WGS, Table S1). Four of these markers were found to co-segregate with the resistance, and two others located around 1 cM on either side, CAPS marker RAD_3 to the distal side and the PCR marker WGS_1 to the proximal side (Figure 1). Both 1 cM markers were subsequently used to genotype 1,793 F2 plants, and we identified 228 recombinants (118 homozygous susceptible to one side and heterozygous to the other, 110 homozygous resistant to one side and heterozygous to the other).

The 118 informative recombinants (homozygous susceptible / heterozygous) were further genotyped using eight linked markers (Figure 1b), and tested in detached leaf assays for their response to *P. infestans* isolates EC1_3626 and 06_3928A. This revealed that markers CLC_3 (WGS_3) and RAD1 are flanking with a single recombination event for each marker, and CLC_2 (WGS_2), 56766 and 46418 are co-segregating with the resistance locus (Figure 1b).

Comparison of the linkage map (Figure 1) with the potato reference genome³⁴ identified the homogeneous CNL-3 NLR gene sub-family to be within the co-segregating locus. This cluster comprises 18 members on potato reference chromosome 11.

BAC clones identification and analysis

Construction and screening of 5x BAC library from resistant parent SP2273 was outsourced to BioS&T company (Quebec, Canada). Two candidate BAC clones (5G and 12H) were identified in PCR screen with WGS_2 marker-specific primers. BAC sequencing with RSII PacBio platform and bioinformatic analysis was outsourced to Earlham Institute (Norwich, UK); both BACs were assembled into single contigs with length of 117,865 bp (5G) and 117,666 bp (12H). While the co-segregating marker WGS_2 was present on both BAC clones, a further co-segregating marker WGS_3 was only present on 12H. The BACs were further assembled into one 204,128 bp contig (available in GenBank under study number MW348763). NLRs on the contig sequence were annotated using NLR-annotator⁵⁰ and Geneious 8.1.2 build-in ORF prediction tool. Gene models were annotated manually using cDNA RenSeq data generated from *S. americanum* accession SP2273 as described below.

3' RACE

Total RNA was extracted using RNeasy Plant Mini Kit (Qiagen) and treated with RNase-Free DNase (Qiagen) following manufacturer's instructions. First strand cDNA was synthesized from total RNA using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen, CA, USA) with P7-oligoDT primer. The resulting product was amplified with P7- and gene specific primers by using KAPA HiFi HotStart ReadyMix PCR Kit (Kapa Biosystems, Cape Town, SA) and cloned into pCR™-Blunt II-TOPO vector by using Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen) and transformation was performed using One Shot™ TOP10 Chemically Competent *E. coli* (Invitrogen). Isolation of plasmid DNA was performed with NucleoSpin® Plasmid kit (MACHEREY-NAGEL, Duren, Germany).

Resistance gene enrichment sequencing (RenSeq) and Gene enrichment sequencing (GenSeq)

SMRT RenSeq, short-read RenSeq and cDNA RenSeq were performed as described previously² and enriched libraries were sequenced at Earlham Institute, Norwich, UK.

Illumina GenSeq was performed as described above (Illumina RenSeq), except GenSeq baits⁵¹ were used instead of RenSeq baits.

PacBio reads were processed and assembled using Geneious R8.1.8⁵² as described³⁰. NLR coding sequences were predicted with Geneious and AUGUSTUS⁵³ and annotated with NLR-parser⁵⁰.

To infer linked polymorphisms, the quality control for Illumina paired-end reads was performed using Trimmomatic⁵⁴ with standard settings. For the RenSeq, the paired reads were mapped to PacBio-assembled contigs from the resistant parent, while GenSeq reads were mapped to the reference DM genome (PGSC_DM_v4.03_pseudomolecules.fasta), using BWA mapper⁵⁵ with default settings. PCR duplicates and unmapped reads were removed and Mpileup files to find out potential linked SNPs were created using SAMtools⁵⁶. Mpileup files were processed with VarScan⁵⁷ set to minimum read depth 20, minimum variant allele frequency threshold 0.1, and minimum frequency to call homozygote 0.98. The candidate SNPs were manually inspected using Savant genome browser⁵⁸. TopHat⁵⁹ with default settings was used to map cDNA Illumina reads to assembled PacBio data. All the tools used in this study were embedded in The Sainsbury Laboratory (TSL) customized Galaxy instance, if not stated otherwise.

Transient complementation of a candidate genes in *N. benthamiana*

The candidate genes were PCR amplified from gDNA with their own promoters (1-2 kb upstream of start codon) and up to 1 kb terminator elements, and cloned into USER vector as described². Transient complementation assays followed by *P. infestans* inoculation were performed as described².

Stable transformation of susceptible potato cultivar Maris Piper

Stable transgenic plants with constructs carrying *Rpi-amr1-2272*, *Rpi-amr1-2273* or *Rpi-amr1a* under the control of their native regulatory elements were created in the background of

potato cultivar Maris Piper as described previously⁶⁰. At least 10 independent transgenic lines were generated for each construct and tested for the presence of the transgene using gene specific primers. All positive *Rpi-amr1-2272* and *Rpi-amr1-2273* lines showed resistance in DLA with *P. infestans* isolate 88069, while *Rpi-amr1a* transgenic plants were fully susceptible. Selected lines of *Rpi-amr1-2272* and *Rpi-amr1-2273* were tested in DLA with 19 additional *P. infestans* isolates (Table 2). WT Maris Piper plants were used as a negative control.

Generation of *NRC2/3* knockout *N. benthamiana*

NRC4 and *NRC2/NRC3/NRC4* knockout *N. benthamiana* lines were described previously^{35,36}. Knocking out of *NRC2/NRC3* in *N. benthamiana* were performed according to the methods described previously³⁵. Forward primers CHW_sgNbNRCs and reverse primer JC_sgrna_R³⁵ were used to clone sgRNA2.1-4, sgRNA3.1-4 into Golden Gate level 1 vectors for different positions. Constructs of sgRNAs targeting *N. benthamiana NRC2* and *NRC3* were assembled into level 2 vector pICSL4723 together with pICSL11017 (pICH47732::NOSp::BAR, Addgene no. 51145) and pICH47742::35S::Cas9⁶¹. Leaf discs of *N. benthamiana* were transformed with the binary vector pICSL4723 containing the BAR selection marker gene, Cas9 expression cassette, and sgRNAs targeting *NRC2* and *NRC3*. T0 transgenic plants were selected in the medium with phosphinothricin (2 mg/L) and then transferred into the soil. The progeny of the transformants were genotyped using amplicon sequencing as described previously³⁵ (Fig. S6a). T3 populations from the selected T2 plants were used for further experiments. *NRC2/3* knockout line (*nrc23_1.3.1*) did not exhibit any growth defects when compared to the wild type plants (Fig. S6b).

Cloning *Rpi-amr1* homologs from resistant *Solanum nigrum* accessions

To test if *Rpi-amr1* also contributes to late blight resistance in *S. nigrum*, we firstly amplified and sequenced the first exon of *Rpi-amr1* from two resistant and one reported susceptible *S. nigrum* accessions. From two resistant accessions (SP1088 and SP1097; Table S4), we amplified sequences with >99% nucleotide identity to *S. americanum Rpi-amr1-2273*.

To clone the full CDS of these *Rpi-amr1* homologs, we designed nested PCR primers based on *Rpi-amr1-2273* sequence (First PCR: *Rpi-amr1-77bp_F*: 5' ATGTGGACATAAATCATTTAGACAA 3' and *Rpi-amr1-USER_R*: 5' GGTTTAAUTTACACAGTGCCGTCCTGCTT 3' ; Second PCR: *Rpi-amr1-UF*: 5'

GGCTTAAUATGGCATATGCTGCTCTTTCTT 3' and Rpi-amr1-USER_R: 5' GGTTTAAUTTACACAGTGCCGTCCTGCTT 3'). The amplicons were cloned into USER vector with 35S promoter as described², the clones were sequenced and transformed into *Agrobacterium* strain AGL1 (pVirG), the transient HR and disease assays are as described previously.

Phylogenetic tree construction

Phylogenetic tree was generated from protein sequences of the cloned *Solanaceae* *R* genes obtained from NCBI. Full-length sequences were aligned using ClustalW 1.74⁶² and the alignments were imported to the MEGA7⁶³ to build a maximum-likelihood phylogenetic tree with Jones-Taylor-Thornton (JTT) substitution model and 100 bootstraps.

Evolutionary analyses of *Rpi-amr1* and *Avramr1* homologs

CDS were aligned using MUSCLE⁶⁴ as implemented in seaview⁶⁵ with and without outgroup (the closest homologs from *S. lycopersicum* and *P. cactorum* for *Rpi-amr1* and *Avramr1*, respectively). Calculations of diversity statistics and the MacDonald-Kreitmann Test were executed through DNAsp5.0⁶⁶; DAMBE⁶⁷ was used to rule out saturation. For *Rpi-amr1* homologs, the calculations were performed separately on annotated full-length sequences as well as the individual domains.

We used 3SEQ⁶⁸ to identify break points in the aligned CDS. To confirm gene conversion events in *Rpi-amr1*, we mapped the CDS back to the BAC_5G sequence using BLAT (minScore 1500, minMatch 93)⁶⁹. The resulting .psl files were converted into .bed files using a custom R script, prior to visualization using the R package Sushi⁷⁰.

HybridCheck

For each accession, FASTA files of all *Rpi-amr1e* orthologs or *Rpi-amr1* paralogs in combinations of three (triplets) were generated and aligned using MUSCLE v3.8.31⁶⁴. The sequence triplets were analysed using HybridCheck⁴⁰ to detect and date recombination blocks between *Rpi-amr1* orthologs (sliding windows = 200bp) or paralogs (sliding windows = 100

bp); non-informative sites were removed from the sequence triplets. Figures showing sequence similarity were plotted (MosaicScale = 50) with HybridCheck and formatted using R v3.2.0 (<https://www.r-project.org>). The colour of each sequence window was calculated based on the proportion of SNPs shared between pairwise sequences at informative sites.

Data availability

Supporting raw reads were deposited in European Nucleotide Archive (ENA) under project number PRJEB38240. BAC and Rpi-amr1 allele sequences were deposited in GenBank under accession numbers MW345286-95 and MW348763. Detailed accession information are shown in Table S7. All the materials in this study are available upon request.

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Author contributions:

K.W., X.L., F.J., R.S., C.O. and J.D.G.J. designed the study. K.W., X.L., H.S.K., F.J., A.I.W., S.B., R.H., W.B., L.T. and T.S., performed the experiments. K.W., X.L., H.S.K., F.J., A.I.W., B.S., R.S., C.O., S.F., and J.M.C. analysed the data. K.W., X.L., H.S.K., F.J. and J.D.G.J. wrote the manuscript with input from all authors. V.G.A.A.V., B.B.H.W., C.-H.W., H.A. and S.K. contributed resources. K.W., X.L. and H.S.K. made equivalent contributions and should be considered joint first authors. All authors approved the manuscript.

Conflict of interest:

K.W., H.S.K., F.G.J. and J.D.G.J. are named inventors on a patent application (PCT/US2017/066691) pertaining to *Rpi-amr1* that was filed by the 2Blades Foundation on behalf of the Sainsbury Laboratory. The other authors declare no competing interests.

Figure Legends:

Fig. 1. Map-based cloning of *Rpi-amr1* and its resistance to *P. infestans*.

- (a) Mapping of *Rpi-amr1* in a small F2 population (n=188 gametes); the names of the markers and genetic distances are shown above or below the bar.
- (b) Fine mapping of *Rpi-amr1* in the F2 population of 3,586 gametes. The names of the markers and the number of recombinants are shown above or below the bar.
- (c) Physical map of the target *Rpi-amr1* interval based on the assembled BAC contig. The markers present on the BAC are shown. The predicted NLR genes are depicted as black arrows (expressed NLRs) or empty arrows (pseudogenized NLRs). *Rpi-amr1* (formerly *Rpi-amr1e*) is indicated by a red arrow.
- (d) Four *Rpi-amr1* transcripts detected by 3' RACE PCR.
- (e) Leaves of *N. benthamiana* plants were infiltrated with the binary vector pICSLUS0003::35S overexpressing either the late blight resistance gene *Rpi-amr3* (positive control), one of seven *Rpi-amr1* candidates, or the non-functional *Rpi-amr3-S* (negative control). Leaves were inoculated with *P. infestans* strain 88069 24 h after infiltration. Only leaves infiltrated with *Rpi-amr3* and *Rpi-amr1e* (pictured) showed reduced pathogen growth, whereas *P. infestans* grew well in the presence of the remaining *Rpi-amr1* candidates. Only *Rpi-amr1c* is shown as the phenotype of all other non-functional candidate genes was indistinguishable. Photographs were taken 9 dpi.
- (f) Transgenic potato cv. Maris Piper which expresses *Rpi-amr1* under the native regulatory elements is resistant to *P. infestans* isolate 88069 (top), displaying no symptoms at the spot of inoculation. Each leaflet was inoculated with a droplet containing approximately 1,000 zoospores; photographs were taken 9 dpi.
- (g) The control plants carrying the non-functional candidate *Rpi-amr1a* show large necrotic lesions and sporulation. Each leaflet was inoculated with a droplet containing approximately 1,000 zoospores; photographs were taken 9 dpi.

Fig. 2. Schematic representation of amino acid sequence alignment of *Rpi-amr1* homologs (a) and *P. infestans* resistance in transient assay (b).

- (a) The exons and the conserved NLR domains are highlighted at the top of the alignment (exons, grey; CC, orange; NB-ARC, blue; LRR, green). Black bars in the alleles indicate the polymorphic amino acids and indels as compared with *Rpi-amr1-2273*. The

numbers next to the alleles refer to the accession working numbers (Table 1). Figure drawn to the scale.

- (b) Nine *Rpi-amr1* homologs provide resistance to *P. infestans* in transient complementation assay. *Rpi-amr1* genes with native regulatory elements were infiltrated into *N. benthamiana* leaves. At 1 dpi, leaves were cut off and drop inoculated with 10 µl of zoospore suspension (50,000 zoospores/mL) from *P. infestans* isolate 88069. The non-functional *Rpi-amr1-2271* homolog from susceptible accession SP2271 was used as negative control. Photographs were taken 8 dpi.

Fig. 3. Differential recognition of *Rpi-amr1* and *Avramr1* homologs.

Four *Avramr1* homologs representing clades A-D, and *P. parasitica* and *P. cactorum* homologs were co-infiltrated with ten *Rpi-amr1* homologs, including a non-functional homolog *Rpi-amr1-2271*, into *N. benthamiana* leaves. Colours from green to brown represent the strength of HR scored from 0 to 2 (see bottom panel). N=3. The representative HR phenotype and scoring are shown in Fig. S3.

Left: phylogenetic tree of nine functional *Rpi-amr1* homologs and non-functional homolog *Rpi-amr1-2271*. Top: phylogenetic tree of *Avramr1* homologs from four isolates of *P. infestans*. * Stable *Rpi-amr1-2307* *N. benthamiana* transformants show HR upon transient expression of *Avramr1* and *Avramr1-13B1*.

Fig. 4. *Rpi-amr1* is NRC2 or NRC3 dependent.

- (a) Phylogenetic analysis of *Rpi-amr1* protein and other functional Solanaceae NLR proteins. The NLR clades shown here are as described previously³⁰, the NRC-dependent sensor clades are marked by blue box.
- (b) Transient expression of *Rpi-amr1-2273* in *NRC2/NRC3* double knockout *N. benthamiana*, followed by zoospore inoculation of *P. infestans* isolate 88069, results in large necrotic lesions indicating lack of resistance.
- (c) Transient expression of *Rpi-amr1-2273* in *NRC2/NRC3/NRC4* triple knockout *N. benthamiana*, followed by zoospore inoculation of *P. infestans* isolate 88069, results in large necrotic lesions indicating lack of the resistance.
- (d) Transient expression of *Rpi-amr1-2273* in *NRC4* knockout *N. benthamiana*, followed by zoospore inoculation of *P. infestans* isolate 88069 results in small necrotic lesions indicating resistance.

Tables:

Table 1. *S. americanum*, *S. nodiflorum* and *S. nigrescens* accessions used in this study and the corresponding *Rpi-amr1* homologs

Table 1

Accession	Working name	Species	Reported origin	Source	Late blight resistance	<i>Rpi-amr1</i> homolog	Similarity	Cloning method
954750186	SP2271	<i>S. americanum</i>	Brazil	RU	Susceptible			
954750184	SP2273	<i>S. americanum</i> var. <i>patulum</i>	unknown	RU	Resistant	<i>Rpi-amr1-2273</i>	100%	Map-based cloning
sn27	SP1032	<i>S. americanum sensu lato</i>	China	BGS	Resistant	<i>Rpi-amr1-1032</i>	92.8%	Association genomics
Veg422	SP1034	<i>S. americanum sensu lato</i>	unknown	NN	Resistant	<i>Rpi-arm1-2273</i>	100%	Association genomics
A54750014	SP1101	<i>S. americanum sensu lato</i>	unknown	RU	Resistant	<i>Rpi-amr1-1101</i>	89.4%	SMRT RenSeq
A14750006	SP1123	<i>S. americanum sensu lato</i>	unknown	RU	Resistant	<i>Rpi-amr1-1123</i>	91.8%	Association genomics
954750174	SP2272	<i>S. americanum</i>	unknown	RU	Resistant	<i>Rpi-amr1-2272</i>	89.4%	Association genomics
SOLA 226	SP2300	<i>S. americanum</i>	Cuba	IPK	Resistant	<i>Rpi-amr1-2300</i>	90.4%	SMRT RenSeq
SOLA 425	SP2307	<i>S. americanum</i>	America	IPK	Resistant	<i>Rpi-amr1-2307</i>	91.7%	Association genomics
Wang 2059	SP2360	<i>S. americanum</i>	China	NHM	Resistant	<i>Rpi-arm1-2273</i>	100%	Association genomics
A14750138	SP3399	<i>S. americanum</i>	unknown	RU	Resistant	<i>Rpi-amr1-2272</i>	89.4%	Association genomics
A14750130	SP3400	<i>S. nodiflorum</i>	unknown	RU	Resistant	<i>Rpi-amr1-2273</i>	100%	Association genomics
944750261	SP3406	<i>S. nigrescens</i>	Bolivia	RU	Resistant	<i>Rpi-amr1-3406</i>	92.5%	Association genomics

954750172	SP3408	<i>S. nigrescens</i>	Bolivia	RU	Resistant	<i>Rpi-amr1-3408</i>	92.6%	Association genomics
A14750423	SP3409	<i>S. nigrescens</i>	Mauritius	RU	Resistant	<i>Rpi-amr1-3409</i>	89.5%	SMRT RenSeq

RU - Radboud University, Nijmegen, The Netherlands

IPK - IPK Gatersleben, Germany

NHM - Natural History Museum, London, United Kingdom

BGS - Shanghai Botanical Garden, Shanghai, China

NN - Nicky's Nursery Ltd, Kent, United Kingdom

Table 2. Phenotypes of potato plants stably transformed with *Rpi-amr1-2272* and *Rpi-amr1-2273* after inoculation with multiple isolates of *P. infestans*.

Isolate	<i>Rpi-amr1-2272</i>	<i>Rpi-amr1-2273</i>	Maris Piper	Origin	Race ^e
NL00228	R	R	S	The Netherlands	1.2.4.7
US23	R	R	S	USA	n.a.
3928A ^a	R	R	S	UK	1.2.3.4.5.6.7.10.11 ^f
EC3626 ^b	R	R	S	Ecuador	n.a.
NL14538 ^c	R	R	S	The Netherlands	n.a.
NR47UH ^d	R	R	S	UK	1.3.4.7.10.11 ^f
T30-4	R	R	S	The Netherlands	n.a.
USA618	R	R	S	USA	1.2.3.6.7.10.11
KPI15-10	R	R	S	Korea	n.a.
IPO-C	R	R	S	Belgium	1.2.3.4.5.6.7.10.11
PIC99189	R	R	S	Mexico	1.2.5.7.10.11
UK7824	R	R	S	UK	n.a.
PIC99177	R	R	S	Mexico	1.2.3.4.7.9.11
VK98014	R	R	S	The Netherlands	1.2.4.11
NL08645	R	R	S	The Netherlands	n.a.
PIC99183	R	R	S	Mexico	1.2.3.4.5.7.8.10.11
NL11179	R	R	S	The Netherlands	n.a.
EC1 ^b	R	R	S	Ecuador	1.3.4.7.10.11
NL01096	R	R	S	The Netherlands	1.3.4.7.8.10.11

^a Clonal lineage EU_13_A2, or “Blue13”

^b Overcomes *Rpi-vnt1*

^c Overcomes *Rpi-vnt1* and partially *Rpi-blb1*, *Rpi-blb2*

^d Clonal lineage EU_6_A1, commonly known as “Pink6”

^e Summarized in⁷¹

f See⁷²