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

Kamil Witek[1,13], Xiao Lin[1,13], Hari S. Karki[1,8,13], Florian Jupe[1,9], Agnieszka I. Witek[1], Burkhard Steuernagel[2], Remco Stam[3], Cock van Oosterhout[4], Sebastian Fairhead[1], Robert Heal[1], Jonathan M. Cocker[5,6], Shivani Bhanvadia[7], William Barrett[1,10], Chih-Hang Wu[1,11], Hiroaki Adachi[1], Tianqiao Song[1,12], Sophien Kamoun[1], Vivianne G. A. A. Vleeshouwers[7], Laurence Tomlinson[1], Brande B. H. Wulff[2] and Jonathan D. G. Jones[1]✉

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* orthologues and paralogues. We propose that *Rpi-amr1* gene family diversity assists detection of diverse paralogues and alleles of the recognized effector, facilitating durable resistance against *P. infestans*.

Potato is the third most important directly consumed food crop world-wide[1]. *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 emerged[2].

To elevate late blight resistance, *Resistance* to *P. infestans* (*Rpi*) genes were identified in wild relatives of potato and used for resistance breeding[3]. More than 20 *Rpi* genes have been mapped and cloned from different *Solanum* species, for example *R2* (*Rpi-blb3), R3a*, *R8*, *Rpi-blb1*, *Rpi-blb2* and *Rpi-vnt1* (refs. [4–10]). All encode coiled-coil (CC), nucleotide binding (NB), leucine-rich repeat (LRR) (NLR) proteins[11] and some require helper NLR proteins of the NRC family[12]. 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 motif[15]. A total of 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 proteins[18]. *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 polymorphism[21–24]. 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 effectors[27–29]. Combined with single-molecule real-time (SMRT) sequencing, SMRT RenSeq enabled cloning of *Rpi-amr3* from *Solanum americanum*[30]. Similarly, long-read and complementary DNA 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. Many *Rpi-amr1* homologues were found in different *S. americanum* accessions and in relatives, including *S. nigrescens* and *S. 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* homologues, all *Rpi-amr1* alleles recognize the *Avramr1* homologues from *P. parasitica* and *P. cactorum*. Our study reveals unique properties of genetic variation of *R* genes from non-host species.

[1]The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, Norwich, UK. [2]John Innes Centre, Norwich Research Park, Norwich, UK. [3]Phytopathology, Technical University Munich, Freising, Germany. [4]School of Environmental Sciences, University of East Anglia, Norwich Research Park, Norwich, UK. [5]Faculty of Biological Sciences, University of Leeds, Leeds, UK. [6]University of Hull, Hull, UK. [7]Plant Breeding, Wageningen University and Research, Wageningen, the Netherlands. [8]Present address: US Department of Agriculture–Agricultural Research Service, Madison, WI, USA. [9]Present address: Bayer Crop Science, Chesterfield, MO, USA. [10]Present address: The New Zealand Institute for Plant & Food Research Ltd, Nelson, New Zealand. [11]Present address: Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan. [12]Present address: Institute of Plant Protection, Jiangsu Academy of Agricultural Sciences, Nanjing, P. R. China. [13]These authors contributed equally: K. Witek, X. Lin, H. S. Karki. ✉e-mail: jonathan.jones@tsl.ac.uk

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

Accession	Working name	Species	Reported origin	Source	Late blight resistance	*Rpi-amr1* homologue	Similarity	Cloning method
954750186	SP2271	*S. americanum*	Brazil	RU	Susceptible			
954750184	SP2273	*S. americanum* var. *patulum*	Unknown	RU	Resistant	*Rpi-amr1-2273*	100%	Map-based cloning
sn27	SP1032	*S. americanum* sensu *lato*	China	BGS	Resistant	*Rpi-amr1-1032*	92.8%	Association genomics
Veg422	SP1034	*S. americanum* sensu *lato*	Unknown	NN	Resistant	*Rpi-arm1-2273*	100%	Association genomics
A54750014	SP1101	*S. americanum* sensu *lato*	Unknown	RU	Resistant	*Rpi-amr1-1101*	89.4%	SMRT RenSeq
A14750006	SP1123	*S. americanum* sensu *lato*	Unknown	RU	Resistant	*Rpi-amr1-1123*	91.8%	Association genomics
954750174	SP2272	*S. americanum*	Unknown	RU	Resistant	*Rpi-amr1-2272*	89.4%	Association genomics
SOLA 226	SP2300	*S. americanum*	Cuba	IPK	Resistant	*Rpi-amr1-2300*	90.4%	SMRT RenSeq
SOLA 425	SP2307	*S. americanum*	America	IPK	Resistant	*Rpi-amr1-2307*	91.7%	Association genomics
Wang 2059	SP2360	*S. americanum*	China	NHM	Resistant	*Rpi-arm1-2273*	100%	Association genomics
A14750138	SP3399	*S. americanum*	Unknown	RU	Resistant	*Rpi-amr1-2272*	89.4%	Association genomics
A14750130	SP3400	*S. nodiflorum*	Unknown	RU	Resistant	*Rpi-amr1-2273*	100%	Association genomics
944750261	SP3406	*S. nigrescens*	Bolivia	RU	Resistant	*Rpi-amr1-3406*	92.5%	Association genomics
954750172	SP3408	*S. nigrescens*	Bolivia	RU	Resistant	*Rpi-amr1-3408*	92.6%	Association genomics
A14750423	SP3409	*S. nigrescens*	Mauritius	RU	Resistant	*Rpi-amr1-3409*	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.

Results

***Rpi-amr1* maps to the short arm of chromosome 11.** We previously investigated *S. americanum* and isolated *Rpi-amr3* from accession 944750095 (SP1102)[30]. To discover new *Rpi-amr* genes, we characterized an additional 14 lines of *P. infestans*-resistant *S. americanum* and close relatives *S. nigrescens* and *S. nodiflorum* by crossing them to a susceptible (S) *S. americanum* line 954750186 (hereafter SP2271) (Table 1 and Supplementary Fig. 1). To avoid self-pollination, a resistant parent was always used as a pollen donor. All the corresponding F_1 plants (6–10 per cross) were resistant in a detached leaf assay (DLA) (Table 1). Around 60–100 F_2 progeny derived from each self-pollinated F_1 plant were phenotyped by DLA using *P. infestans* isolate 88069 (ref. [32]). The F_2 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 (probably 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 before 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 F_2 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 and Supplementary Tables 1 and 2). We expanded the mapping population and developed a PCR marker WGS_2 that cosegregated with resistance in 3,586 gametes (Fig. 1b and Supplementary Table 2). 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 base pairs (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-paralogue 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 previously[30]. *P. infestans* growth was observed 6 d post-inoculation (dpi). Only *35S::Rpi-amr1e*-infiltrated leaves showed reduced pathogen growth at 9 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 paralogue *Rpi-amr1a* was also transformed into

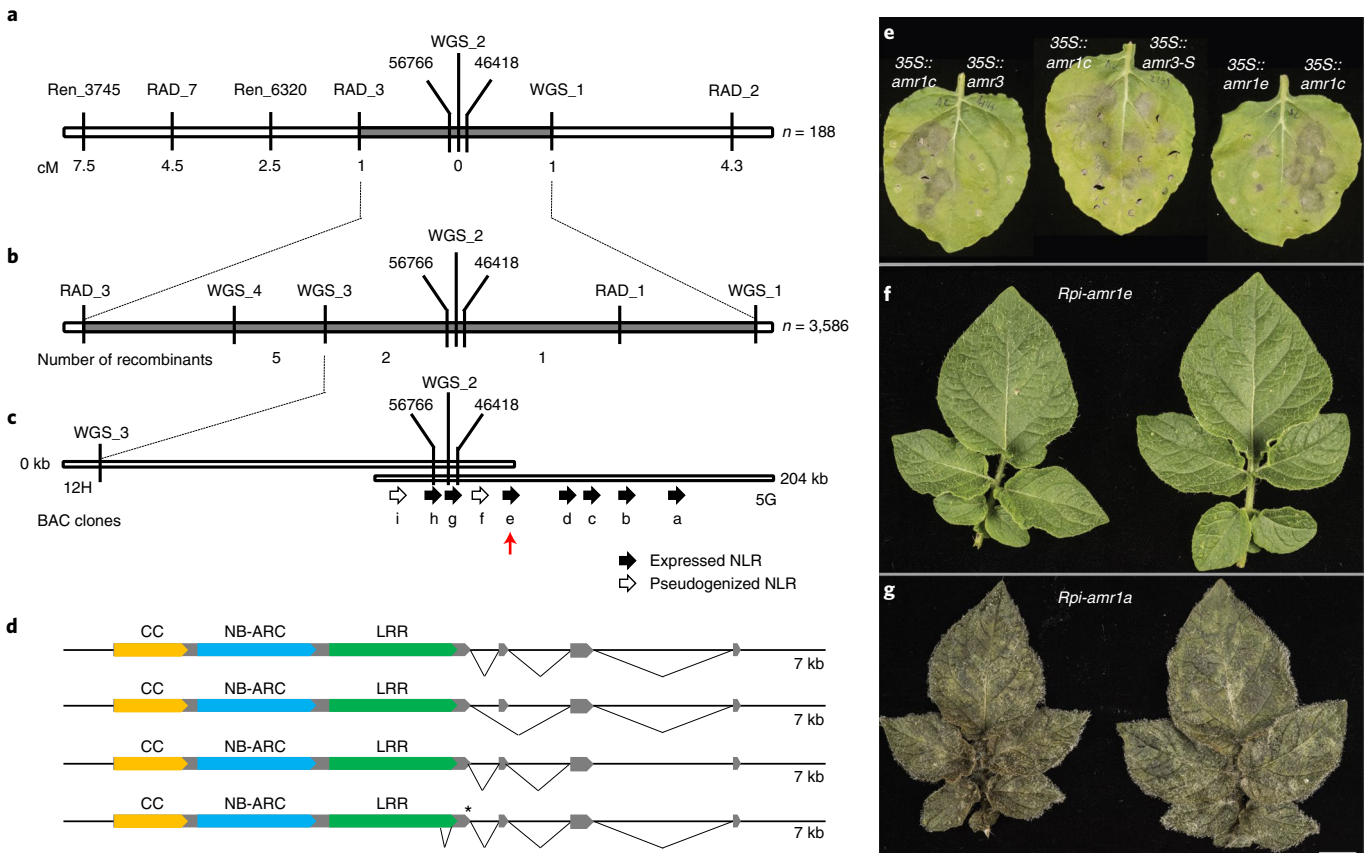

Fig. 1 | Map-based cloning of *Rpi-amr1* and its resistance to *P. infestans*. **a**, Mapping of *Rpi-amr1* in a small F_2 population ($n = 188$ gametes); the names of the markers and genetic distances are shown above or below the bar. **b**, Fine mapping of *Rpi-amr1* in the F_2 population of 3,586 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. The asterisk represents a premature stop codon. **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 cultivar Maris Piper which expresses *Rpi-amr1* under the native regulatory elements is resistant to *P. infestans* isolate 88069, displaying no symptoms at the spot of inoculation. Each leaflet was inoculated with a droplet containing ~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 ~1,000 zoospores; photographs were taken 9 dpi.

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 CC domain (amino acids 2–146), NB domain (NB-ARC; amino acids 179–457) and LRRs (located between amino acids 504 and 900) which are all positioned in the first exon (1–918 amino acids; Fig. 2a). The remaining three short exons (amino acids 919–943, 944–1,002 and 1,003–1,013) lack homology to any known domains. No integrated domains[33] were found in the Rpi-amr1 protein.

Functional *Rpi-amr1* homologues were identified from multiple lines of 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 F_2 plants of the populations derived from resistant accessions, with a marker positioned in *Rpi-amr1h* gene (56766, Fig. 1 and Supplementary Table 2). 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* homologues, we performed SMRT RenSeq-based de novo assembly of each resistant accession and looked for homologues 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 homologue as a candidate *Rpi-amr1*

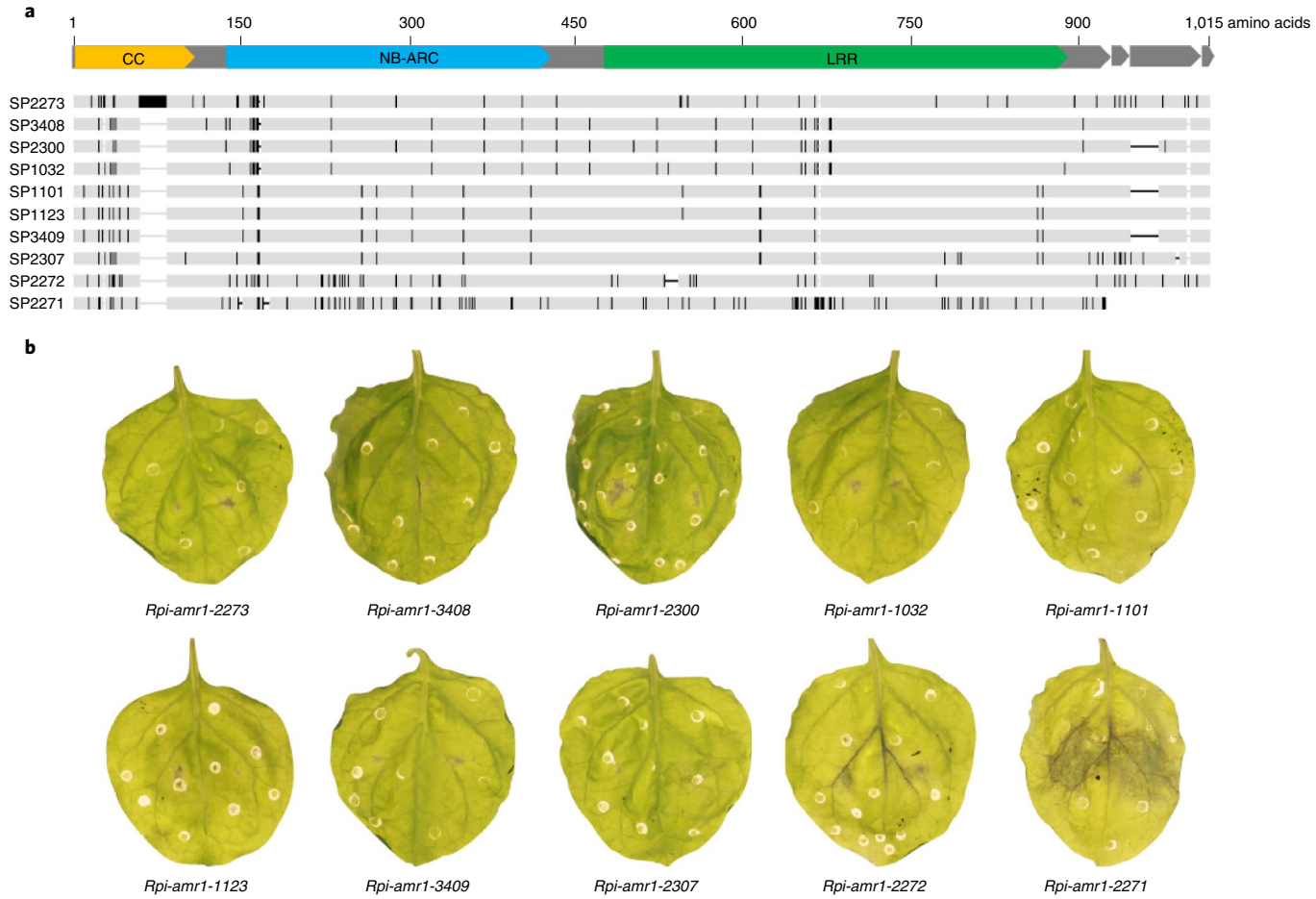

Fig. 2 | *Rpi-amr1* homologues and their phenotypes in transient assays. a, Schematic representation of amino acid sequence alignment of *Rpi-amr1* homologues. The exons and the conserved NLR domains are highlighted at the top of the alignment (exons, grey; CC, orange; NB-ARC, blue; LRR, green). Black 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). **b**, *P. infestans* resistance in transient assay. Nine *Rpi-amr1* homologues provide resistance to *P. infestans* in transient complementation assay. *Rpi-amr1* genes with native regulatory elements were infiltrated into *N. benthamiana* leaves. At 1dpi, leaves were cut off and drop inoculated with 10µl of zoospore suspension (50,000 zoosporesml^{-1}) from *P. infestans* isolate 88069. The non-functional *Rpi-amr1-2271* homologue from susceptible accession SP2271 was used as negative control. Photographs were taken 8dpi.

orthologue (Supplementary Table 3). 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 (Supplementary Table 3). As described previously, we transiently expressed the closest related candidate *Rpi-amr1* homologues in *N. benthamiana* leaves followed by DLA with *P. infestans* isolate 88069 and verified their functionality. The unique homologues 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* cosegregating paralogues, *Rpi-amr1-1101* and *Rpi-amr1-2300*, were identified and verified in transient assays as above (Fig. 2b).

Similarly, we inspected an F_2 population derived from *S. nigrescens* accession SP3409 (Table 1). We applied BSA RenSeq and SMRT RenSeq to the resistant parents and F_2 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* homologue, *Rpi-amr1-3409*. However, *Rpi-amr1-3409* does not cosegregate with *Rpi-amr1*-linked markers. We used gene enrichment sequencing (GenSeq) sequence capture-based genotyping[34] and found that *Rpi-amr1-3409* locates on chromosome 1, based on the potato DM reference genome[35]. This result suggests that a fragment of DNA that locates on the 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* homologues were aligned, the polymorphisms between different functional alleles were found to be distributed through all domains including the LRR region (Fig. 2a and Supplementary Fig. 2).

Taken together, by using BSA RenSeq, SMRT RenSeq, cDNA RenSeq, association genomics and GenSeq, we cloned eight additional functional *Rpi-amr1* homologues from different resistant accessions, of which all confer resistance to *P. infestans* 88069 in transient assays. The closest *Rpi-amr1* homologue 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 homologues, we generated stably transformed transgenic potato cultivar Maris Piper plants carrying *Rpi-amr1-2272* and *Rpi-amr1-2273*, the most diverged of the

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	*Rpi-amr1-2272*	*Rpi-amr1-2273*	Maris Piper	Origin	Race[e]
NL00228	R	R	S	the Netherlands	1.2.4.7
US23	R	R	S	United States	NA
3928A[a]	R	R	S	United Kingdom	1.2.3.4.5.6.7.10.11[f]
EC3626[b]	R	R	S	Ecuador	NA
NL14538[c]	R	R	S	the Netherlands	NA
NR47UH[d]	R	R	S	United Kingdom	1.3.4.7.10.11[f]
T30-4	R	R	S	the Netherlands	NA
USA618	R	R	S	United States	1.2.3.6.7.10.11
KPI15-10	R	R	S	Korea	NA
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	United Kingdom	NA
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	NA
PIC99183	R	R	S	Mexico	1.2.3.4.5.7.8.10.11
NL11179	R	R	S	the Netherlands	NA
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 commonly known as 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 in ref. [71]. [f]See ref. [72]. NA, information not available.

homologues (Supplementary Table 3) and inoculated them by DLA with 19 *P. infestans* isolates from United Kingdom, the Netherlands, Belgium, the United States, 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* homologues. *Avramr1* (*PITG_07569*) was identified in *P. infestans* race T30-4 by long-read and cDNA PenSeq, and multiple *Avramr1* homologues were identified in four *P. infestans* isolates and classified into four subclades[31]. To investigate if all nine cloned *Rpi-amr1* homologues could recognize diverse *Avramr1* homologues from different *P. infestans* isolates, in addition to *Avramr1* from race T30-4 that corresponds to clade A, we synthesized three *Avramr1* homologues, *Avramr1-13B1*, *Avramr1-13C2* and *Avramr1-13D1,* from isolate 3928A (EU_13_A2, commonly known as Blue13), corresponding to clades B, C and D, respectively (Fig. 3). We also synthesized the *Avramr1* homologues from *P. parasitica* and *P. cactorum*[31]. These six *Avramr1* homologues were co-expressed in *N. benthamiana* by agro-infiltration in all possible combinations with nine functional *Rpi-amr1* homologues and the non-functional *Rpi-amr1-2271* as a negative control (Fig. 3).

We found that different combinations of *Rpi-amr1* alleles and *Avramr1* homologues led either to strong, weak or no hypersensitive response (HR) phenotype in transient assay but the non-functional *Rpi-amr1-2271* allele failed to recognize any *Avramr1* homologues (Fig. 3). The representative HR phenotype and the scoring of HR indices are shown in Supplementary Fig. 3. *Rpi-amr1-2300* and *Rpi-amr1-2307* recognized one *Avramr1* homologue each but others detected *Avramr1* homologues from more than one clade. Clade C, represented here by *Avramr1-13C2*, is usually not expressed[31] and when expressed from 35S promoter, this effector was not recognized by most *Rpi-amr1* homologues, although 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 isolates[31] and was recognized by all but one (*Rpi-amr1-2300*) homologues in the transient assay. Surprisingly, two *Avramr1* homologues from *P. parasitica* and *P. cactorum* are strongly recognized by all functional *Rpi-amr1* homologues, apart from *Rpi-amr1-2272* which showed a weaker HR (Fig. 3).

Collectively, our data show that *Rpi-amr1*/*Avramr1* homologue pairs provoke quantitatively and qualitatively different HRs but all functional *Rpi-amr1* homologues detect at least one *Avramr1* homologue 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 amino acid 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 knockout *N. benthamiana* leaves[36,37] (Supplementary Fig. 4). The HR phenotype was abolished in NRC2/3 and NRC2/3/4 knockout plants (Supplementary Fig. 5b,c) but not in NRC4 knockout or wild-type plants (Supplementary Fig. 5a,d). The HR was recovered when NRC2 or NRC3 was co-expressed in the NRC2/3/4 or NRC2/3 knockout

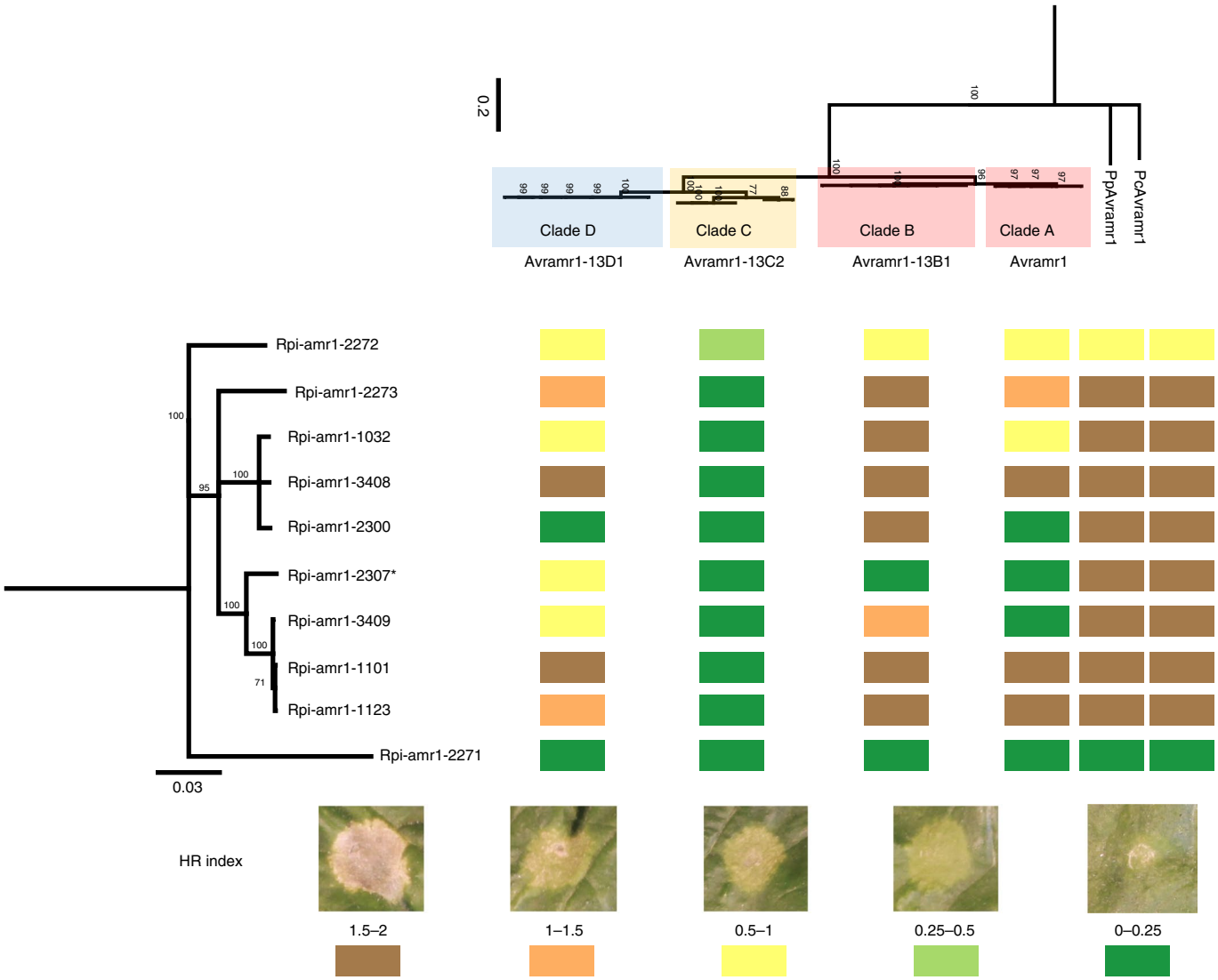

Fig. 3 | Differential recognition of *Rpi-amr1* and *Avramr1* homologues. Four *Avramr1* homologues representing clades A–D and *P. parasitica* and *P. cactorum* homologues were co-infiltrated with ten Rpi-amr1 homologues, including a non-functional homologue *Rpi-amr1-2271*, into *N. benthamiana* leaves. 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 Supplementary Fig. 3. Left: phylogenetic tree of nine functional *Rpi-amr1* homologues and non-functional homologue *Rpi-amr1-2271*. Top: phylogenetic tree of *Avramr1* homologues from four isolates of *P. infestans*. The scale bars represent the number of amino acid substitutions per site. *Stable *Rpi-amr1-2307 N. benthamiana* transformants show HR upon transient expression of *Avramr1* and *Avramr1-13B1*.

plants but co-expression of NRC4 did not complement the loss of HR phenotype in NRC2/3/4 knockout plants. (Supplementary Fig. 5b,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* homologues 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*[38], even though rare accessions are susceptible[39]. *S. americanum* may be the diploid ancestor of hexaploid *S. nigrum*[40]. To test if *Rpi-amr1* also contributes to late blight resistance in *S. nigrum*, we designed nested PCR primers on the basis of the *Rpi-amr1-2273* sequence and amplified and sequenced the coding sequence of *Rpi-amr1* homologues from two resistant and one reported susceptible *S. nigrum* accessions[39]. From two resistant accessions (SP1088 and SP1097; Supplementary Table 4), 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 to Q) change compared to Rpi-amr1-2273 (Supplementary Fig. 6a). 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* (Supplementary Fig. 6b). 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 (Supplementary Fig. 6c). 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

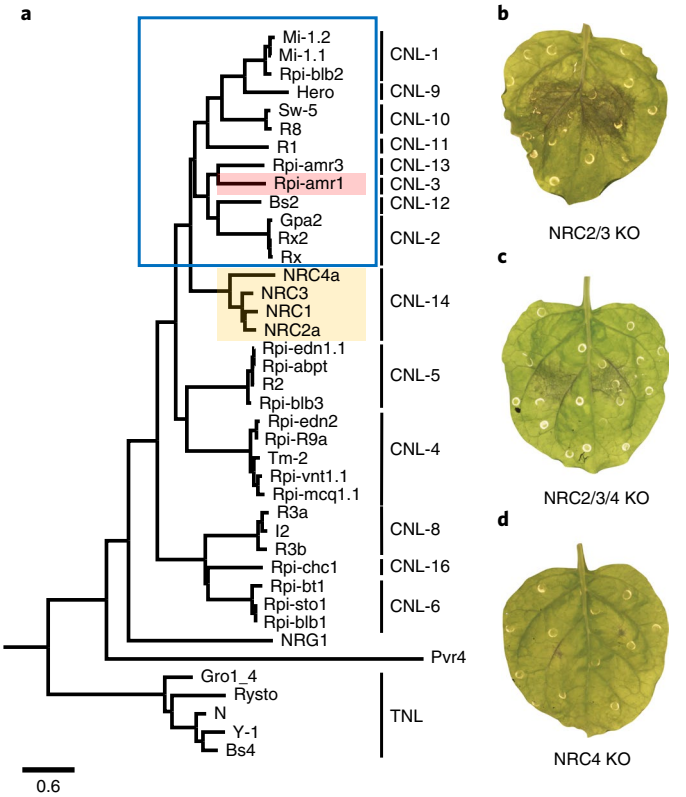

Fig. 4 | Rpi-amr1 is NRC2- or NRC3-dependent. a, Phylogenetic analysis of Rpi-amr1 protein and other functional Solanaceae NLR proteins. The NLR clades shown here are as described previously[30]; the NRC-dependent sensor clades are marked by the blue box. The scale bar represents the number of amino acid substitutions per site. **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.

determined by the *Rpi-amr1* homologues that were most likely inherited from *S. americanum*.

High allelic diversity at *Rpi-amr1* was generated through inter-paralogue and orthologue sequence exchange. *Rpi-amr1* alleles show relatively high nucleotide diversity ($\pi = 0.04$), which could be an indication of balancing or diversifying selection (Supplementary Table 5). In addition, *Rpi-amr1* alleles differ in their recognition of the *Avramr1* homologues (Fig. 3) which is also consistent with selection in a host–parasite coevolutionary 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* homologues. As expected, *Avramr1* homologues show a signature consistent with balancing selection (Tajima's $D = 2.27$) (Supplementary Table 5). Remarkably, despite the high nucleotide diversity, no clear signals of balancing or diversifying selection were detected for *Rpi-amr1* (Tajima's $D = 0.09083$) (Supplementary Table 5). 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* homologues (Fig. 2a and Supplementary Table 3); this pattern is consistent with occasional recombination between highly diverged alleles or paralogues.

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* orthologues from different *S. americanum* accessions and *Rpi-amr1* paralogues from SP2273 (Supplementary Table 6). Some sequence exchanges were visualized using HybridCheck (Supplementary Fig. 7)[41] and these data suggest that sequence exchange occurred between functional *Rpi-amr1* alleles and paralogues. To confirm these findings, we mapped all cloned *Rpi-amr1* coding sequence (CDS) back to the BAC_5G sequence from accession SP2273 (Supplementary Fig. 8). As expected, some *Rpi-amr1* homologues (for example, 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 homologues (for example, SP2271), 5' end sequences match different NLR sequences on the BAC_5G and for others (for example, SP2275) 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 paralogues, and not just through mutation accumulation.

Discussion

Achieving complete and durable resistance is the ultimate goal of resistance breeding. Here, we report substantial 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* strains[42]. 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[38,43]. *Rpi* genes from plant species that only rarely support pathogen growth have probably 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 plant[44].

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* homologues 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 cultivar 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 flax[45], and multiple and diverse recognized effectors from barley powdery mildew and *Mla* from barley. Similarly, *Avramr1* and its homologues from several *P. infestans* races[31] 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 distinctly diverged alleles and paralogues. 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 on the basis of 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 *Rp1* resistance genes in grasses[46] and in the vertebrate immune genes of the major histocompatibility complex (MHC)[47,48]. 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* paralogues from a single *P. infestans* strain, a scenario that might elevate durability of resistance. Since the *S. americanum* population recognizes multiple *Avramr1* alleles and paralogues, 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*[49], indicating *Avramr1* contributes an important function for the virulence of *P. infestans*. We hypothesize 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* homologues from *P. parasitica* and *P. cactorum* are recognized by all *Rpi-amr1* homologues. 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 paralogues, 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.

Methods

Development of mapping populations. Fourteen *P.* infestans-resistant diploid *S. americanum* and relatives were used in this study (Table 1). The F_1 populations were generated by crossing with a susceptible *S. americanum* accession 954750186 (SP2271) as a female parent. Heterozygous F_1 progeny was allowed to self-pollinate to generate F_2 segregating populations or further back-crossed to the susceptible parent and allowed to self-pollinate until resistance to *P. infestans* cosegregated as a monogenic trait.

***P. infestans* infection assay.** *P. infestans* isolates were cultured on rye and sucrose agar medium at 18 °C for 10 d. 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 ml^{-1}). 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 5–9 d after infection.

DNA and RNA extraction. RenSeq experiments (both short- and long-reads protocols) were conducted on genomic DNA 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) and Direct-zol RNA MiniPrep Kit (Zymo Research), following the manufacturer's recommendations.

Mapping of *Rpi-amr1*. To map the underlying resistance gene from the resistant parent 954750184 (SP2273), we generated an F_2 segregating population that was phenotyped with *P. infestans* isolates EC1_3626 and 06_3928A. Selected resistant plants were self-pollinated and up to 100 plants from F_3 populations were screened for resistance and susceptibility with *P. infestans* isolates EC1_3626 and 06_3928A. Genomic DNA from susceptible F_2 and F_3 plants (bulked susceptible pool), as well as genomic DNA from the resistant (R) and susceptible parent (S) were subjected to RenSeq using Solanaceae bait library[27] and sequenced with Illumina MiSeq 250 bp paired-end reads. Preprocessing, assembly, mapping and single nucleotide polymorphism (SNP) calling were performed as described earlier[27,30].

The same genomic DNA samples were used in a RAD-seq experiment using PstI digestion and Illumina HiSeq sequencing, which was outsourced to Floragenex. Bioinformatic analysis was also performed by Floragenex using *Solanum lycopersicum* genome as a reference[50]. SNP calling resulted in 16 polymorphic sites with 11 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 WGS of R and S samples to BGI for ~30 deep Illumina HiSeq sequencing with 100PE. Reads from the resistant parent were assembled as described in ref. [30] 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[35] to identify their position.

Screening a set of markers derived from these three approaches on genomic DNA of 94 susceptible F_2 and F_3 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; Supplementary Table 1). Four of these markers were found to cosegregate 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 (Fig. 1). Both 1 cM markers were subsequently used to genotype 1,793 F_2 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 (Fig. 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 cosegregating with the resistance locus (Fig. 1b).

Comparison of the linkage map (Fig. 1) with the potato reference genome[35] identified the homogeneous CNL-3 NLR gene subfamily to be within the cosegregating locus. This cluster comprises 18 members on potato reference chromosome 11.

BAC clones identification and analysis. Construction and screening of 5× BAC library from resistant parent SP2273 was outsourced to BioS&T. 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; both BACs were assembled into single contigs with length of 117,865 bp (5G) and 117,666 bp (12H). While the cosegregating marker WGS_2 was present on both BAC clones, a further cosegregating 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-annotator[51] and Geneious v.8.1.2 build-in open reading frame 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 the manufacturer's instructions. First-strand cDNA was synthesized from total RNA using SuperScript First-Strand Synthesis System for RT–PCR (Invitrogen) 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) 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).

RenSeq and GenSeq. SMRT RenSeq, short-read RenSeq and cDNA RenSeq were performed as described previously[27,30] and enriched libraries were sequenced at Earlham Institute.

Illumina GenSeq was performed as described above (Illumina RenSeq), except GenSeq baits[34] were used instead of RenSeq baits.

PacBio reads were processed and assembled using Geneious R v.8.1.8 (ref. [52]) as described in ref. [30]. NLR coding sequences were predicted with Geneious and AUGUSTUS[53] and annotated with NLR-parser[51].

To infer linked polymorphisms, the quality control for Illumina paired-end reads was performed using Trimmomatic[54] 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 mapper[55] with default settings. PCR duplicates and unmapped reads were removed and Mpileup files to find out potential linked SNPs were created using SAMtools[56]. Mpileup files were processed with VarScan[57] 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 browser[58]. TopHat[59] 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 genomic DNA with their own promoters (1–2 kilobases (kb) upstream of start codon) and up to 1-kb terminator elements, and cloned into USER vector as described in ref. [30]. Transient complementation assays followed by *P. infestans* inoculation were performed as described in ref. [30].

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 previously[60]. At least ten 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[36,37]. Knocking out of *NRC2*/*NRC3* in *N. benthamiana* were performed according to the methods described previously[36]. Forward primers CHW_sgNbNRCs and reverse primer JC_sgrna_R[36] were used to clone sgRNA2.1-4, sgRNA3.1-4 into Golden Gate level 1 vectors for different positions. Constructs of single guide RNAs (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::Cas9 (ref. [61]). 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*. T_0 transgenic plants were selected in the medium with phosphinothricin (2 mg l^{-1}) and then transferred into the soil. The progeny of the transformants were genotyped using amplicon sequencing as described previously[36] (Supplementary Fig. 6a). T_3 populations from the selected T_2 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 (Supplementary Fig. 6b).

Cloning *Rpi-amr1* homologues from resistant *S. 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; Supplementary Table 4), we amplified sequences with >99% nucleotide identity to *S. americanum Rpi-amr1-2273*.

To clone the full CDS of these *Rpi-amr1* homologues, we designed nested PCR primers on the basis of *Rpi-amr1-2273* sequence (first PCR: Rpi-amr1-77bp_F: 5′ATGTGGACATAAATCATTTAGACAA3′ and Rpi-amr1-USER_R: 5′GGTTTAAUTTACACAGTGCCGTCCTGCTT3′ ; second PCR: Rpi-amr1-UF: 5′GGCTTAAUATGGCATATGCTGCTCTTTCTT3′ and Rpi-amr1-USER_R: 5′GGTTTAAUTTACACAGTGCCGTCCTGCTT3′). The amplicons were cloned into USER vector with 35S promoter as described in ref. [30], 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 v.1.74 (ref. [62]) and the alignments were imported to the MEGA7 (ref. [63]) to build a maximum-likelihood phylogenetic tree with Jones-Taylor-Thornton (JTT) substitution model and 100 bootstraps.

Evolutionary analyses of *Rpi-amr1* and *Avramr1* homologues. CDS were aligned using MUSCLE[64] as implemented in seaview[65] with and without outgroup (the closest homologues from *S. lycopersicum* and *P. cactorum* for *Rpi-amr1* and *Avramr1*, respectively). Calculations of diversity statistics and the MacDonald–Kreitmann test were executed through DNAsp5.0 (ref. [66]); DAMBE[67] was used to rule out saturation. For *Rpi-amr1* homologues, the calculations were preformed separately on annotated full-length sequences as well as the individual domains.

We used 3SEQ (ref. [68]) 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, before visualization using the R package Sushi[70].

HybridCheck. For each accession, FASTA files of all *Rpi-amr1e* orthologues or *Rpi-amr1* paralogues in combinations of three (triplets) were generated and aligned using MUSCLE v.3.8.31 (ref. [64]). The sequence triplets were analysed using HybridCheck[41] to detect and date recombination blocks between *Rpi-amr1* orthologues (sliding windows = 200 bp) or paralogues (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 v.3.2.0 (https://www.r-project.org). The colour of each sequence window was calculated on the basis of the proportion of SNPs shared between pairwise sequences at informative sites.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Supporting raw reads were deposited in European Nucleotide Archive under project number PRJEB38240. BAC and *Rpi-amr1* allele sequences were deposited in GenBank under accession numbers MW345286-95 and MW348763. Detailed accession information is shown in Supplementary Table 7. 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. All authors approved the manuscript.

Competing interests

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.

Additional information

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Correspondence and requests for materials should be addressed to J.D.G.J.

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