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

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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 positioned 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 worldwide. *Phytophthora infestans*, an oomycete pathogen, causes late blight disease in potato and can result in complete crop failure. Disease management is primarily based on fungicide-resistant races have emerged.

To elevate late blight resistance, *Resistance to P. infestans* (*Rpi*) genes were identified in wild relatives of potato and used for resistance breeding. More than 20 *Rpi* genes have been mapped and cloned from different *Solanum* species, for example *R2* (*Rpi-bhl3*), *R3a, R8, Rpi-bhl1, Rpi-bhl2* and *Rpi-vnt1* (refs. 1,4). All encode coiled-coil (CC), nucleotide binding (NB), leucine-rich repeat (LRR) (NLR) proteins and some require helper NLR proteins of the NRC family. However, most cloned *Rpi* genes can be overcome by at least one strain of *P. infestans*. Provision of durable late blight resistance for potato remains a major challenge.

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

Technological advances like RenSeq (resistance gene enrichment sequencing) and PenSeq (pathogen enrichment sequencing) enable rapid definition of allelic variation and mapping of plant NLRs or discovery of variation in pathogen effectors. Combined with single-molecule real-time (SMRT) sequencing, SMRT RenSeq enabled cloning of *Rpi-amr3* from *Solanum americanum*. Similarly, long-read and complementary DNA PenSeq enabled us to identify *AvrAmr1* from *P. infestans*.

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.

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Results

Rpi-amr1 maps to the short arm of chromosome 11. We previously investigated S. americanum and isolated Rpi-amr3 from accession 944750095 (SP1102)\(^\text{99}\). 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) line (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\(_1\) progenies that derived from each self-pollinated F\(_1\) using a resistant parent were always used as a pollen donor. Around 60–100 F\(_2\) progeny derived from each self-pollinated F\(_1\) plant were phenotyped by DLA (Table 1 and Supplementary Table 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 late blight assay (DLA) (Table 1 and Supplementary Table 1). We expanded the mapping population and developed a PCR marker WGS_2 that cosegregated with resistance in 3,586 gametes (Fig. 1c). To identify Rpi genes from these resistant S. americanum accessions, we prioritized an F\(_1\) 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). The 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—are expressed 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 P. infestans isolate 88069 as described previously\(^{99}\). P. infestans growth was observed 6 dpi 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. 1c). 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

### 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            | Rpi-amr1-2272       | 100%       | Map-based cloning |
| 954750184 | SP2273       | S. americanum var. | Unknown         | RU     | Resistant              | Rpi-amr1-2273       | 92.8%      | Association genomics |
| sn27      | SP1032       | S. americanum sensu | China           | BGS    | Resistant              | Rpi-amr1-1032       | 90.4%      | Association genomics |
| Veg422    | SP1034       | S. americanum sensu | Unknown         | NN     | Resistant              | Rpi-amr1-2273       | 100%       | Association genomics |
| A54750014 | SP1101       | S. americanum sensu | Unknown         | RU     | Resistant              | Rpi-amr1-1101       | 89.4%      | SMRT RenSeq       |
| A14750006 | SP1123       | S. americanum sensu | 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-amr1-2273       | 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, Rodbard University, Nijmegen, the Netherlands; IPK, IPK Gatersleben, Germany; NHM, Natural History Museum, London, United Kingdom; BGS, Shanghai Botanical Garden, Shanghai, China; NN, Nobby’s Nursery Ltd, Kent, United Kingdom.
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 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 F1 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.
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-amr1-1101, 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-amr1-1123 and Rpi-amr1-2300, were identified and verified in transient assays as above (Fig. 2b).

Similarly, we inspected an F1 population derived from S. nigrescens accession SP3409 (Table 1). We applied BSA RenSeq and SMRT RenSeq to the resistant parents and F1 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 and found that Rpi-amr1-3409 locates on chromosome 1, based on the potato DM reference genome. This result suggests that a fragment of DNA that locates on the distal end of the short arm of chromosome 1 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
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.

**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* |
|---------|---------------|---------------|-------------|--------|------|
| NL00228 | R             | R             | S           | the Netherlands | 1.2.4.7 |
| US23    | R             | R             | S           | United States   | NA    |
| 3928A   | R             | R             | S           | United Kingdom  | 1.2.3.4.5.6.7.10.11 |
| EC3626  | R             | R             | S           | Ecuador        | NA    |
| NL14538 | R             | R             | S           | the Netherlands | NA    |
| NR47U   | R             | R             | S           | United Kingdom  | 1.3.4.7.10.11 |
| 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 |
| NL1179  | R             | R             | S           | the Netherlands | NA    |
| EC⁵     | R             | R             | S           | Ecuador        | 1.3.4.7.10.11 |
| NL01096 | R             | R             | S           | the Netherlands | 1.3.4.7.8.10.11 |

*Clonal lineage EU_13_A2 commonly known as Blue13. *Overcomes Rpi-vnt1. *Overcomes Rpi-vnt1 and partially Rpi-bb1, Rpi-bb2. *Clonal lineage EU_6_A1, commonly known as Pink1. *Summarized in ref. 31. *See ref. 32. NA, information not available.

**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 subclades31. 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. cactorum31. 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 expressed32 and when expressed from 3SS 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 isolates33 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)32.

To test this hypothesis, we transiently expressed Rpi-amr1-2273 together with PpAvramr1 in NRC4, NRC2/3 or NRC2/3/4 knockout N. benthamiana leaves34,35 (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 or NRC2/3 knockout.
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\(^{39}\), 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

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**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.
High allelic diversity at \textit{Rpi-amr1} was generated through inter-paralogue and orthologue sequence exchange. \textit{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, \textit{Rpi-amr1} alleles differ in their recognition of the \textit{Avr} homologues (Fig. 3) which is also consistent with selection in a host–parasite coevolutionary arms race. To test the hypothesis that allelic polymorphism at \textit{Rpi-amr1} results from diversifying selection, we calculated diversity statistics and performed a McDonald–Kreitman test on both \textit{Rpi-amr1} alleles and \textit{Avr} homologues. As expected, \textit{Avr} 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 \textit{Rpi-amr1} (Tajima’s $D = 0.9080$) (Supplementary Table 5). Aligning the \textit{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 \textit{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 \textit{Rpi-amr1} alleles, we predicted the possible recombination events using 3SEQ. Several recombination events were detected between \textit{Rpi-amr1} orthologues from different \textit{S. americanum} accessions and \textit{Rpi-amr1} paralogues from SP2273 (Supplementary Table 6). Some sequence exchanges were visualized using HybridCheck (Supplementary Fig. 7) and these data suggest that sequence exchange occurred between functional \textit{Rpi-amr1} alleles and paralogues. To confirm these findings, we mapped all cloned \textit{Rpi-amr1} coding sequence (CDS) back to the BAC_5G sequence from accession SP2273 (Supplementary Fig. 8). As expected, some \textit{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 \textit{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 \textit{Solanum} and many have been overcome by one or more \textit{P. infestans} strains\(^a\). Conceivably, resistance to \textit{P. infestans} in nearly all \textit{S. americanum} and \textit{S. nigrum} accessions is due to multiple NLR genes, as zoospores from \textit{P. infestans} can germinate on \textit{S. nigrum} leaves but penetration is stopped by strong HR. \textit{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 \textit{P. infestans}, and hence, the pathogen’s effectors have not (yet) evolved to evade detection by these \textit{Rpi} genes. Under this scenario, a pre-existing standing variation in the pathogen for overcoming such \textit{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\(^b\).

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

Many plant \textit{R} genes and their corresponding \textit{Avr} genes evolved differential recognition specificities with extensive allelic series for both \textit{R} gene and \textit{Avr} genes. Examples include \textit{ATR1} and \textit{RPP1} or \textit{ATR13} and \textit{RPP13} from \textit{Hyaloperonospora arabidopsidis} and \textit{Arabidopsis}\(^a\), \textit{Avr567} and \textit{L} genes from the rust \textit{Melampsora lini} and \textit{flax}\(^a\), and multiple and diverse recognized effectors from barley powdery mildew and \textit{Mla} from barley. Similarly, \textit{Avramr1} and its homologues from several \textit{P. infestans} races\(^a\) were found to be differentially recognized by alleles of the \textit{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 _Rpl_ resistance genes in grasses and in the vertebrate immune genes of the major histocompatibility complex (MHC).

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_, 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 _F1_ populations were generated by crossing with a susceptible _S. americanum_ accession 954750186 (SP2273) 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_ cosegregated as a monogenic trait.

#### _P. infestans_ infection assay

_Infected_ isolates were cultured on yeast 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

_Infected_ experiments (both short- and long-read protocols) were conducted on genomic DNA freshly extracted from young leaves using the DNaseasy Plant Mini Kit (Qiagen) according to the manufacturer’s protocol. For the cDNA _Infected_ experiment, RNA was extracted using TRI-Reagnt (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 susceptible parent 954750184 (SP2273), we generated an _F1_, segregating population that was phenotyped with _P. infestans_ isolates ECI_3626 and _06_3928A. Selected resistant plants were self-pollinated and up to 100 plants from _F2_ populations were screened for resistance and susceptibility with _P. infestans_ isolates ECI_3626 and _06_3928A. Genomic DNA from susceptible _F1_ and _F2_ 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 and sequenced with Illumina MiSeq 250 bp paired-end reads. Preprocessing, assembly, mapping and single nucleotide polymorphism (SNP) calling were performed as described earlier.

The same genomic DNA samples were used in a RAD-seq experiment using PET digestion and Illumina HiSeq sequencing, which was outsourced to Earlham Institute. Bioinformatic analysis was also performed by Florigenex using _Solanum lycopersicum_ genome as a reference. SNP calling resulted in 16 polymorphic sites with 11 of them located at the top of chromosome 11 (Supplementary Table 1). The remaining ones were randomly distributed on chromosomes 4 and 1.

Additional outsource 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. 18 and we used our previously published in silico trait mapping pipelines to perform SNP calling and detection of polymorphisms linked to disease resistance.

Screening a set of markers derived from these three approaches on genomic DNA of 94 susceptible _F1_ and _F2_ plants identified 12 markers linked to resistance response that flank the _R_ locus between 7.5 cm and 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 _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 (Fig. 1b) and tested in detached leaf assays for their response to _P. infestans_ isolates ECI_3626 and _06_3928A. This revealed that markers _CLC_3 (_WGS_3) and _RAD_1 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 identified the homogenous _CNL-3_ NLR gene subfamily to be within the cosegregating locus. This cluster comprises 13 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. Two candidate BAC clones (_5G_ and _12H_) were identified in PCR screen with WGS_2 marker-specific primers. BAC sequencing with RS1 PacBio platform and bioinformatic analysis was outsourced to Earlham Institute. Both BACs were assembled into single contigs with length of 117,865 bp and _NRC3-dependent_ expression (Fig. 1c).

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 MW348765). NLRs on the contig sequence were annotated using NLR-annotator and Genious v.8.1.2 build-in open reading frame prediction tool. Gene models were annotated manually using DNA _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 Kit 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.
RenSeq and GenSeq. SMRT RenSeq, short-read RenSeq and cDNA RenSeq were performed as described previously [3,5] and enriched libraries were sequenced at Earlham Institute. Illumina GenSeq was performed as described above (Illumina RenSeq), except GenSeq baits were used instead of RenSeq baits. PacBio reads were processed and assembled using Geneious R v8.1.8 (ref. [5]) as described in ref. [5]. NLR coding sequences were predicted with Geneious and AUGUSTUS [6] and annotated with NLR parser [6].

To infer linked polymorphisms, the quality control for Illumina paired-end reads was performed using Trimmomatic [7] with standard settings. For the RenSeq, the paired reads were mapped to PacBio-assembled contigs from the resistant parent, with Geneious, and were mapped to the reference DM genome (PGSC_DM_v4.03_pseudomolecules.fasta), using BWA mapper [8] with default settings. PCR duplicates and unmapped reads were removed and Mpileup files to find out potential linked SNPs were created using SAMtools [9]. Mpileup files were processed with VarScan – 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 [10]. TopHat [11] 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. [12]. Transient complementation assays followed by *P. infestans* inoculation were performed as described in ref. [12].

Stable transformation of susceptible potato cultivar Maris Piper. Stable transgenic plants with constructs carrying *Rpi-amr1-2272, Rpi-amr1-2273* or *Rpi-amr1α* under the control of their native regulatory elements were created in the background of potato cultivar Maris Piper as described previously [13]. 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 [14,15]. 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 [16]. Knockout out of NRC2/NRC3 in *N. benthamiana* were performed according to the methods described previously [17]. Forward primers CHW_sgNbNRCs and reverse primer JC_srgna_1A 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 pCL4723 together with pCL51107 (pCL47732: NOsp:BAR, Addgene no. 51145) and pCL47742:55S:Cas9 (ref. [18]). Leaf discs of *N. benthamiana* were transformed with the binary vector pCL4723 containing the BAR selection marker gene, Cas9 expression cassette and sgRNAs targeting NRC2 and NRC3. Transgenic plants were selected in the medium with phosphinothricin (2 mg l⁻¹) and then transferred into the soil. The progeny of the transformatants were genotyped using amplicon sequencing as described previously [18] (Supplementary Fig. 6a). T₃ populations from the selected T₃ plants were used for further experiments. NRC2/3 knockout line (nrc2_1,1,3,1) 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 first 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 SP2060), the selected T₂ plants (2060) did not exhibit any growth defects when compared to the wild type *E. coli*.

Evolutionary analyses of *Rpi-amr1* and *AvrAmr1* homologues. CDS were aligned using MUSCLE [20] as implemented in seaview [21] 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 DNApS5.0 (ref. [5]). DAMBE [22] was used to rule out saturation. For *Rpi-amr1* homologues, the calculations were performed separately on annotated full-length sequences as well as the individual domains. We used 3SEQ (ref. [23]) 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%) [24]. The resulting .psl files were converted into .bed files using a custom R script, before visualization using the R package Sushi [25].

HybridCheck. For each accession, FASTA files of all *Rpi-amr1* orthologues or *Rpi-amr1* paralogues in combinations of three (triplets) were generated and aligned using MUSCLE v3.8.31 (ref. [26]). The sequence triplets were analysed using HybridCheck [27] 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 v3.2.0 (https://www.ccr-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.

**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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**References**
1. World Food and Agriculture: Statistical Pocketbook 2019 (FAO, 2019).
2. Saville, A. et al. Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Dis. 99, 659–666 (2015).
3. Malcolmson, J. F. & Black, W. New *R* genes in *Solanum demissum* Indml. And their complementary racismes of *Phytophthora infestans* (Mont.) de Bary. *Euphytica* 15, 199–203 (1974).
4. Park, T.-H. et al. The late blight resistance locus *Rpi-blb3* from *Solanum bulbocastanum* belongs to a major late blight *R* gene cluster on chromosome 4 of potato. *Mol. Plant Microbe Interact.* 18, 722–729 (2005).
5. Huang, S. et al. Comparative genomics enabled the isolation of the *R3a* late blight resistance gene in potato. *Plant J.* 42, 251–261 (2005).
6. Vossen, J. H. et al. The *Solanum demissum* R8 late blight resistance gene is an *Sw-5* homologue that has been deployed worldwide in late blight resistant varieties. *Theor. Appl. Genet.* 129, 1785–1796 (2016).
7. Song, J. et al. *R* gene cloned from *Solanum bulbocastanum* confers broad spectrum resistance to potato late blight. *Proc. Natl Acad. Sci. USA* 100, 9128–9133 (2003).
8. van der Vossen, E. A. G. et al. The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mt-1* gene homologue conferring broad-spectrum late blight resistance in potato. *Plant J.* 44, 208–222 (2005).
9. Pel, M. A. et al. Mapping and cloning of late blight resistance genes from *Solanum venturi* using an interspecific candidate gene approach. *Mol. Plant Microbe Interact.* 22, 601–615 (2009).
10. Foster, S. J. et al. *Rpi-vent1*, a *Tm-22* homologue from *Solanum venturi*, confers resistance to potato late blight. *Mol. Plant Microbe Interact.* 22, 589–600 (2009).
11. Jones, D. G., Vance, R. E. & Bangl, J. L. Intracellular innate immune surveillance devices in plants and animals. *Science* 354, aaf6595 (2016).
12. Wu, C.-H. et al. NLR network mediates immunity to diverse plant pathogens. *Proc. Natl Acad. Sci. USA* 114, 8113–8118 (2017).
13. Fry, W. *Phytophthora infestans*: the plant (and *R* gene) destroyer. *Mol. Plant Pathol.* 9, 385–402 (2008).
14. Jones, J. D. G. & Bangl, J. L. The plant immune system. *Nature* 444, 323–329 (2006).
15. Rehmany, A. P. et al. Differential recognition of highly divergent downy mildew avirulence gene alleles by *RP11* resistance genes from two *Arabidopsis* lines. *Plant Cell* 17, 1839–1850 (2005).
resistance to
Colon, L. T. e hypersensitive response is associated with host and nonhost
resistance in the age of e
sequence data.

Poczai, P. & Hyvönen, J. On the origin of Solanum nigrum
Lebecka, R. Host–pathogen interaction between
S. tuberosum
Govers, F. Resistance of
Phytophthora infestans
mediated by the recognition of the elicitor protein INF1.

Jouet, A. et al. Multiple pairs of allelic MLA immune receptor-powdery mildew AVRA effectors reveal sites of positive selection.

Kearse, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data.

Siebenegger, B. et al. NLR parsing: rapid annotation of plant NLR complements. Bioinformatics 10, 1665–1667 (2015).

Kearse, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649 (2012).

Stein, M. et al. Multiple enrichment of functional NLR alleles in cultivated barley reveals sites of positive selection. Mol. Plant Microbe Interact 23, 497–509 (2010).

Camoun, S., van West, P., Vleeshouwers, V. G. A. A., de Groot, K. E. & Govers, F. Resistance of Nicotiana benthamiana to Phytophthora infestans is mediated by the recognition of the elicitor protein INF1. Plant Cell Physiol 10, 1413–1425 (1998).

Grund, E., Tremousaygue, D. & Deslandes, L. Plant NLRs with integrated de novo synthetase activity in the cytoplasm.

Saur, I. M. et al. Multiple pairs of allelic MLA immune receptor-powdery mildew AVRA effectors argue for a direct recognition mechanism. eLife 8, 1957 (2019).

Anderson, C. et al. Genome analysis and avirulence gene cloning using a high-density RADseq linkage map of the flax rust fungus, Melampsora lini. BMC Genomics 17, 667 (2016).

Juge, F. et al. Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. Plant J. 76, 530–544 (2013).

Thilliez, G. J. A. et al. Pathogen enrichment sequencing (PenSeq) enables population genomics in model species. New Phytol. 190, 999 (2018).

Jouet, A. et al. Albego candida race diversity, ploidy and host-associated miRNAs reveal microRNA sequence capture on diseased plants in the field. New Phytol. 93, 999 (2018).

Witek, K. et al. Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. Nat. Biotechnol. 34, 656–660 (2016).

Lin, X. et al. Identification of Avr3 from Phytophthora infestans using long read and cdDNA pathogen-enrichment sequencing (PenSeq). Mol. Plant Pathol. 21, 1502–1512 (2020).

Kamoun, S., van West, P., Vleeshouwers, V. G. A. A., de Groot, K. E. & Govers, F. Resistance of Solanum lycopersicum to Phytophthora infestans is mediated by the recognition of the elicitor protein INF1. Plant Cell Physiol 10, 1413–1425 (1998).

Grund, E., Tremousaygue, D. & Deslandes, L. Plant NLRs with integrated de novo synthetase activity in the cytoplasm.

Saur, I. M. et al. Multiple pairs of allelic MLA immune receptor-powdery mildew AVRA effectors reveal sites of positive selection.

Kearse, M. et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649 (2012).

Stein, M. et al. Multiple enrichment of functional NLR alleles in cultivated barley reveals sites of positive selection. Mol. Plant Microbe Interact 23, 497–509 (2010).

Camoun, S., van West, P., Vleeshouwers, V. G. A. A., de Groot, K. E. & Govers, F. Resistance of Nicotiana benthamiana to Phytophthora infestans is mediated by the recognition of the elicitor protein INF1. Plant Cell Physiol 10, 1413–1425 (1998).

Grund, E., Tremousaygue, D. & Deslandes, L. Plant NLRs with integrated domains: unity makes strength. Plant Physiol. 179, 1227–1235 (2019).

Chen, X. et al. Identification and rapid mapping of a gene conferring broad-spectrum late blight resistance in the diploid potato species Solanum verrucosum through DNA capture technologies. Theor. Appl. Genet. 131, 1287–1297 (2018).

Xu, X. et al. Genome sequence and analysis of the tuber crop potato. Nature 475, 189–195 (2011).

Wu, C.-H. et al. Genome sequence and analysis of the tuber crop potato. Nature 528, 1287–1297 (2018).

Phytophthora infestans
Mol. Plant Pathol. 21, 853–864 (2000).
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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., I.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., 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.

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