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# Identification of *I-7* expands the repertoire of genes for resistance to Fusarium wilt in tomato to three resistance gene classes

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## SUMMARY

The tomato *I-3* and *I-7* genes confer resistance to *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*) race 3 and were introgressed into the cultivated tomato, *Solanum lycopersicum*, from the wild relative *Solanum pennellii*. *I-3* has been identified previously on chromosome 7 and encodes an S-receptor-like kinase, but little is known about *I-7*. Molecular markers have been developed for the marker-assisted breeding of *I-3*, but none are available for *I-7*. We used an RNA-seq and single nucleotide polymorphism (SNP) analysis approach to map *I-7* to a small introgression of *S. pennellii* DNA (*c.* 210 kb) on chromosome 8, and identified *I-7* as a gene encoding a leucine-rich repeat receptor-like protein (LRR-RLP), thereby expanding the repertoire of resistance protein classes conferring resistance to *Fol*. Using an *eds1* mutant of tomato, we showed that I-7, like many other LRR-RLPs conferring pathogen resistance in tomato, is EDS1 (Enhanced Disease Susceptibility 1) dependent. Using transgenic tomato plants carrying only the *I-7* gene for *Fol* resistance, we found that *I-7* also confers resistance to *Fol* races 1 and 2. Given that *Fol* race 1 carries *Avr1*, resistance to *Fol* race 1 indicates that I-7-mediated resistance, unlike I-2- or I-3-mediated resistance, is not suppressed by Avr1. This suggests that Avr1 is not a general suppressor of *Fol* resistance in tomato, leading us to hypothesize that Avr1 may be acting against an EDS1-independent pathway for resistance activation. The identification of *I-7* has allowed us to develop molecular markers for marker-assisted breeding of both genes currently known to confer *Fol* race 3 resistance (*I-3* and *I-7*). Given that I-7-mediated resistance is not suppressed by Avr1, *I-7* may be a useful addition to *I-3* in the tomato breeder's toolbox.

**Keywords:** *Fusarium oxysporum* f. sp. *lycopersici*, Fusarium wilt, leucine-rich repeat, plant disease resistance gene, receptor-like protein, *Solanum lycopersicum*, *Solanum pennellii*.

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## INTRODUCTION

The fungus *Fusarium oxysporum* causes devastating wilt diseases of many important crop plants, including banana/plantain, cotton, potato, tomato, capsicum, beans, peas, chickpeas and melons. However, individual pathogenic isolates of *F. oxysporum* are highly specific for a particular species of host plant. For example, *F. oxysporum* f. sp. *cubense* (*Foc*) is specific for banana/plantain, *F. oxysporum* f. sp. *vasinfectum* (*Fov*) is specific for cotton and *F. oxysporum* f. sp. *lycopersici* (*Fol*) is specific for tomato. In contrast with Fusarium wilt of banana or cotton, Fusarium wilt of tomato (caused by *Fol*) has been managed for many years by the breeding of disease-resistant cultivars, but this approach is not possible for all crop plants. Either breeding for resistance is very difficult, as is the case for banana, or no major genes for resistance have so far been identified, as is the case for cotton. Therefore, in addition to its importance as a crop pathosystem, the tomato–*Fol* pathosystem has become an important, well-established model system for the study of plant–*F. oxysporum* interactions at a genetic and molecular level.

*Fol* infection involves the germination of soil-borne spores in the vicinity of growing roots, attachment to the root surface, penetration of the root cortex and proliferation of hyphae within the root vasculature. Eventually, *Fol* invades and colonizes the parenchyma of the dying tomato plant and sporulates on the plant surface (Michielse and Rep, 2009). The disease is characterized by yellowing, wilting and browning of the leaves, stunted growth and, eventually, death of the plant. These symptoms result from the obstruction of water and nutrient flow caused by hyphae within the xylem vessels, as well as tyloses, callose, gums and gels produced by the host plant.

Three races of *Fol* have been reported and named races 1, 2 and 3 in order of appearance. *Fol* races 1 and 2 were discovered over 70 years ago (Alexander and Tucker, 1945; Bohn and Tucker, 1939) and are distributed almost worldwide. *Fol* race 3 was first discovered in Queensland (Australia) in 1978 (Grattidge and O'Brien, 1982) and by the 1980s had caused great yield losses (McGrath *et al*., 1987). *Fol* race 3 was later reported in the USA (Volin and Jones, 1982) and subsequently Mexico (Valenzuela-Ureta *et al*., 1996), Brazil (Reis *et al*., 2005) and many other parts of the world.

The use of resistant cultivars remains the most practical, cost-effective and environmentally safe method for the control of Fusarium wilt in tomato.

Wilt-resistant cultivars have been generated by the introgression of genes for *Fol* resistance, designated *Immunity* genes, into the cultivated tomato *Solanum lycopersicum*, from wild relatives. The *I* gene was identified in the wild tomato *Solanum pimpinellifolium* (accession PI79532) and confers resistance against *Fol* race 1 (Bohn and Tucker, 1939). However, the rapid emergence of race 2, virulent on tomato cultivars carrying the *I* gene, soon necessitated the search for a new source of resistance. The *I-2* gene was identified in an *S. lycopersicum* × *S. pimpinellifolium* hybrid (accession PI126915; Stall and Walter, 1965) and confers resistance to *Fol* race 2. *I-2* encodes a coiled-coil, nucleotide-binding, leucine-rich repeat (CC-NB-LRR) resistance protein (Simons *et al*., 1998). The eventual emergence of race 3, virulent on tomato cultivars carrying the *I-2* gene, necessitated another search for a new source of resistance. Two genes for resistance to *Fol* race 3 were identified in *Solanum pennellii*, one from accession LA716 (Scott and Jones, 1989) and the other from accession PI414773 (McGrath *et al*., 1987). Originally, both genes were designated *I-3*, but gene-mapping work by Lim *et al*. (2006) revealed that the two genes were not the same. The *I-3* gene from *S. pennellii* LA716 encodes an S-receptor-like kinase (SRLK) protein (Catanzariti *et al*., 2015; Lim *et al*., 2008) and, together with the rice *Pi-d2* gene for resistance to *Magnaporthe grisea* (Chen *et al*., 2006) and the *Arabidopsis thaliana RFO3* gene for resistance to *F. oxysporum* (Cole and Diener, 2013), forms the basis for a new class of plant disease resistance genes. However, at the time of commencing the work described here, nothing was known about the resistance gene from *S. pennellii* PI414773, renamed *I-7*, except that it was not located in the *I-3* region on chromosome 7 (Lim *et al*., 2006).

Here, we describe an RNA-seq analysis of tomato cultivars, differing by the presence or absence of *I-7*, which not only enabled us to identify genes expressed in roots (where *I-7* would be expected to act), but also the introgressed region of *S. pennellii* DNA carrying *I-7*. This enabled a candidate gene to be identified and complementation tests to be carried out confirming the identification of *I-7*.

## RESULTS

### Single nucleotide polymorphism (SNP) analyses of Tristar and M82 RNA-seq data reveal a cluster of SNPs on Tristar chromosome 8

An RNA-seq analysis of root transcripts was carried out on the tomato cultivars Tristar (carrying *I-7*) and M82 (lacking *I-7*). More than 169 million reads for Tristar and 152 million reads for M82 (Table S1, see Supporting Information) were mapped against the tomato reference transcriptome and analysed for SNPs, and, in both cases, more than 55,000 SNPs were identified (Table S1). SNPs supported by at least 75% of the mapped reads were extracted: 30,069 for Tristar and 31,391 for M82. Then, 20,450 SNPs common to Tristar and M82 were discarded. The remaining unique polymorphisms were used to calculate the SNP frequency for each transcript (number of SNPs per transcript divided by the length of the transcript). The SNP frequency was then plotted against the corresponding gene positions on each tomato chromosome. We reasoned that an introgressed region of DNA from a tomato relative should generate a cluster of genes showing a higher frequency of unique SNPs compared with the corresponding region of *S. lycopersicum* DNA or surrounding *S. lycopersicum* sequences. We also reasoned that the *S. pennellii* introgression carrying *I-7* should be relatively small, given the failure of an extensive genome-wide marker survey that attempted to identify the *I-7* introgression (Gonzalez-Cendales *et al*., 2014). Consistent with these expectations, a small cluster of genes with a higher frequency of SNPs in Tristar relative to M82 and to adjacent Tristar genes was identified on chromosome 8 (Fig. 1).

### Identification of *I-7* as the Tristar allele of *Solyc08g77740*

Cleaved amplified polymorphic sequence (CAPS) markers were designed for several genes in the SNP cluster found on chromosome 8 (Table S2, see Supporting Information) based on restriction site polymorphisms between Tristar and M82. To determine whether these markers were linked to *I-7*, marker analysis was performed on 15 Tristar × M82 F3 or F4 lines scored as homozygous resistant and 16 scored as homozygous susceptible. The results showed complete correspondence between marker genotype and resistance (Fig. S1, see Supporting Information), indicating that we had correctly identified the *S. pennellii* introgression carrying *I-7*.

The extent of the introgressed region was determined by looking for root-expressed genes showing a higher frequency of SNPs in Tristar than in M82 relative to the reference transcriptome, and for flanking root-expressed genes lacking SNPs relative to the reference transcriptome (Table S3, see Supporting Information). Based on this analysis, the introgressed region was found to be about 210 kb and to carry 29 genes (*Solyc08g077520* to *Solyc08g077800*). The Tristar RNA-seq data showed that 11 of these 29 genes were not expressed in roots, as determined by no or very few mapped reads (Table S3). *I-7* should be expressed in roots because that is where it acts to limit *Fol* infection. Therefore, annotations of the 18 root-expressed genes (Table S3) were examined for members of the major plant disease resistance gene classes. One gene, *Solyc08g077740*, was found to encode an extracellular LRR receptor-like protein (LRR-RLP), thus resembling a resistance gene. Of the remaining 17 root-expressed genes, 16

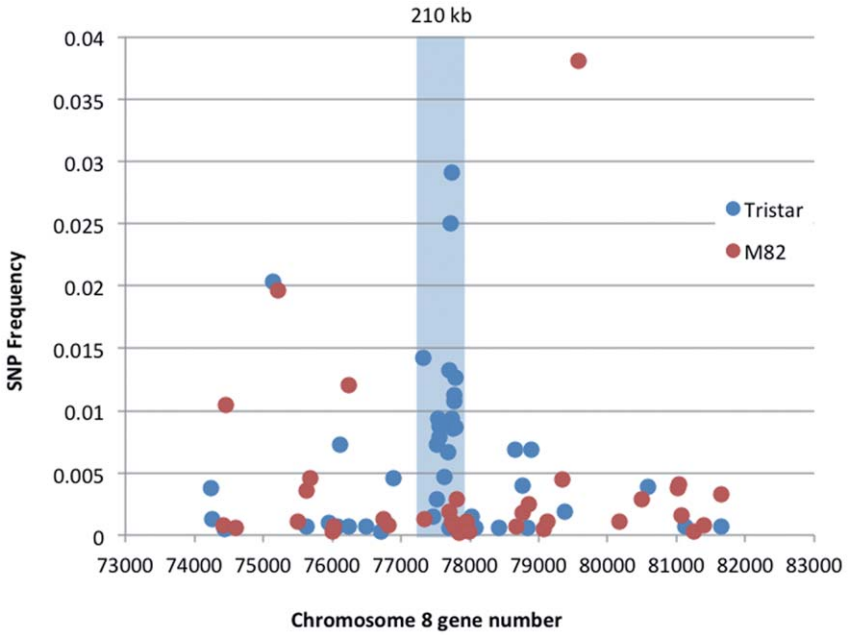



**Fig. 1** Plot of single nucleotide polymorphism (SNP) frequencies for Tristar and M82 transcripts relative to the tomato reference transcriptome for genes on the long arm of chromosome 8. The SNP cluster corresponding to the introgressed region of *Solanum pennellii* DNA on Tristar chromosome 8 is highlighted in blue. SNP frequencies were calculated as the number of SNPs per transcript unique to Tristar or M82 divided by the length of the transcript.

encoded proteins with no resemblance to known resistance proteins and one encoded a protein of unknown function, leaving *Solyc08g077740* as the sole candidate for *I-7*.

To test this possibility, the *Solyc08g077740* genes from Tristar and M82 were first polymerase chain reaction (PCR) amplified from genomic DNA using cultivar-specific LIC7774 primers designed using Tristar and M82 consensus sequences for *Solyc08g077740* assembled from the RNA-seq data (Table S4, see Supporting Information). The resulting PCR products of approximately 2.9 kb were cloned via ligation-independent cloning (LIC) into the binary LIC vector pL2. The resulting plasmids pL2-Tristar and pL2-M82 were used for the transformation of tomato cultivars Moneymaker and M82 in the case of pL2-Tristar and Moneymaker alone in the case of pL2-M82. Six independent transgenic Moneymaker T1 plants (MM-7774$^{Tristar}$) and 12 independent transgenic M82 T1 plants (M82-7774$^{Tristar}$) were obtained with the Tristar gene. Seven independent transgenic Moneymaker T1 plants (MM-7774$^{M82}$) were obtained with the M82 gene. The presence of the transgene in T1 plants was confirmed by PCR as described below.

T2 seedlings from two independent MM-7774$^{Tristar}$ lines and one M82-7774$^{Tristar}$ line were screened for the presence of the transgene by multiplex PCR using the CAPS7774 marker (Table S2, Fig. S2, see Supporting Information), which amplified a product from the endogenous *Solyc08g077740* gene, and 7774F4 (Table S4) and M13 reverse primers, which amplified a product from the *Solyc08g077740* transgene (Fig. S2). Seedlings carrying the transgene from each line were inoculated with *Fol* race 3. All were found to show little or no sign of disease, like Tristar their transgene donor, but unlike Moneymaker or M82, their progenitors, which were severely diseased (Fig. 2). Pathogen tests were also carried out on two additional MM-7774$^{Tristar}$ lines and four MM-7774$^{M82}$ lines without first screening for seedlings carrying the transgene, i.e. on plants segregating for the transgene. Both MM-7774$^{Tristar}$ lines segregated for plants showing little or no sign of disease and a small number of severely diseased plants (Fig. S3, see Supporting Information), whereas the MM-7774$^{M82}$ lines showed diseased plants with symptoms ranging from severe to mild (Fig. 3), the latter indicating that overexpression of the M82 gene may reduce susceptibility. Together, these data indicate that the *Solyc8g077740* gene from Tristar confers resistance to *Fol* race 3 and corresponds to *I-7*, whereas the *Solyc8g077740* gene from M82 confers no or little resistance to *Fol* race 3 and is here designated *i-7*.

### *I-7* confers resistance to *Fol* races 1 and 2

Although *I-7* confers resistance to *Fol* race 3, it was not known whether it also confers resistance to *Fol* races 1 and 2 because Tristar also carries the *I* gene for race 1 resistance and the *I-2* gene for race 2 resistance. Therefore, T2 seedlings from MM-7774$^{Tristar}$ line #1, which lacks *I* and *I-2*, were screened by multiplex PCR as described above to identify seedlings carrying the *I-7* transgene, and then inoculated with *Fol* races 1 or 2. These seedlings showed little or no sign of disease with either race, like Tristar, but unlike Moneymaker, which was severely diseased (Figs S4 and S5, see Supporting Information). These results show that *I-7* confers resistance to *Fol* races 1, 2 and 3.

### *I-7* resistance is dependent on *EDS1*

Some LRR-RLPs, including Cf-9 and Ve1, have been shown to depend on EDS1 (Enhanced Disease Susceptibility 1), a lipase-like

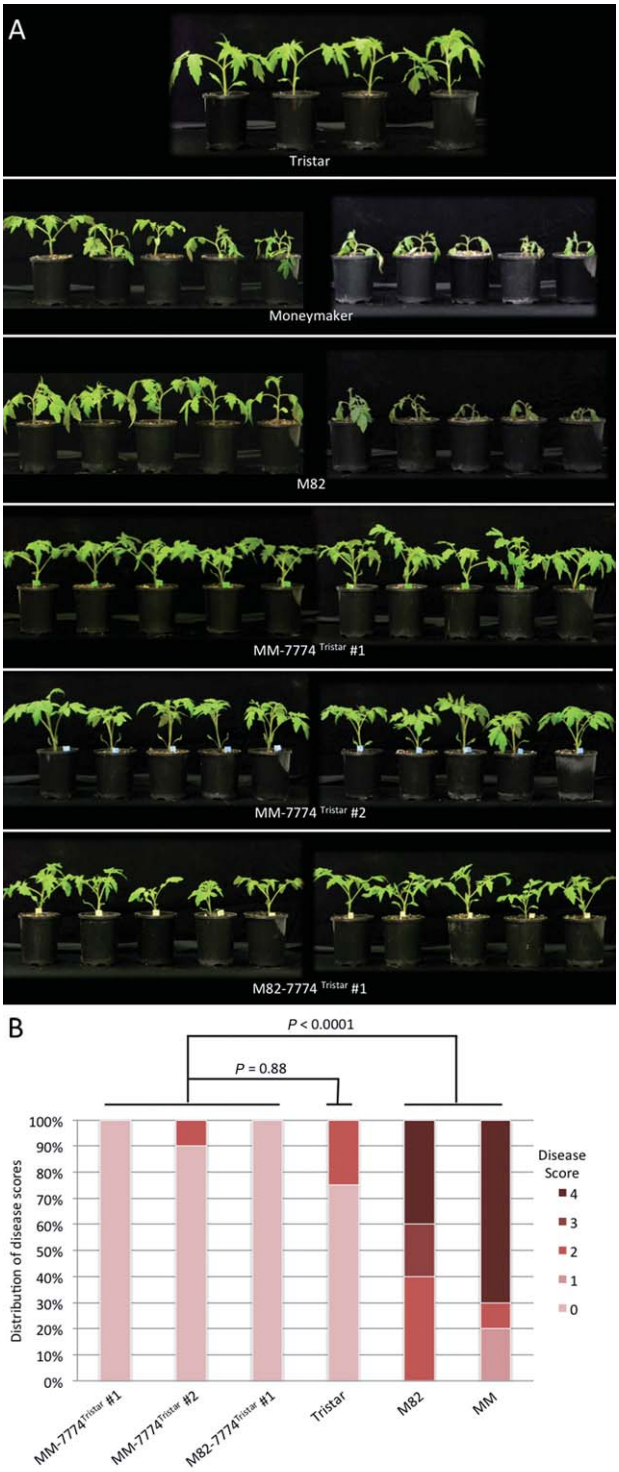



**Fig. 2** (A) Disease assays on T2 plants carrying the *Solyc08g077740* gene from Tristar in a Moneymaker (MM) or M82 background. T2 plants, together with Tristar (resistant), Moneymaker (susceptible) and M82 (susceptible) control plants, were inoculated with *Fol* race 3. Photographs were taken at 18 days post-inoculation. (B) Disease scores for the T2 plants shown in (A). Probability values were obtained using the non-parametric Kruskal–Wallis test to determine significant differences in disease scores between plant lines

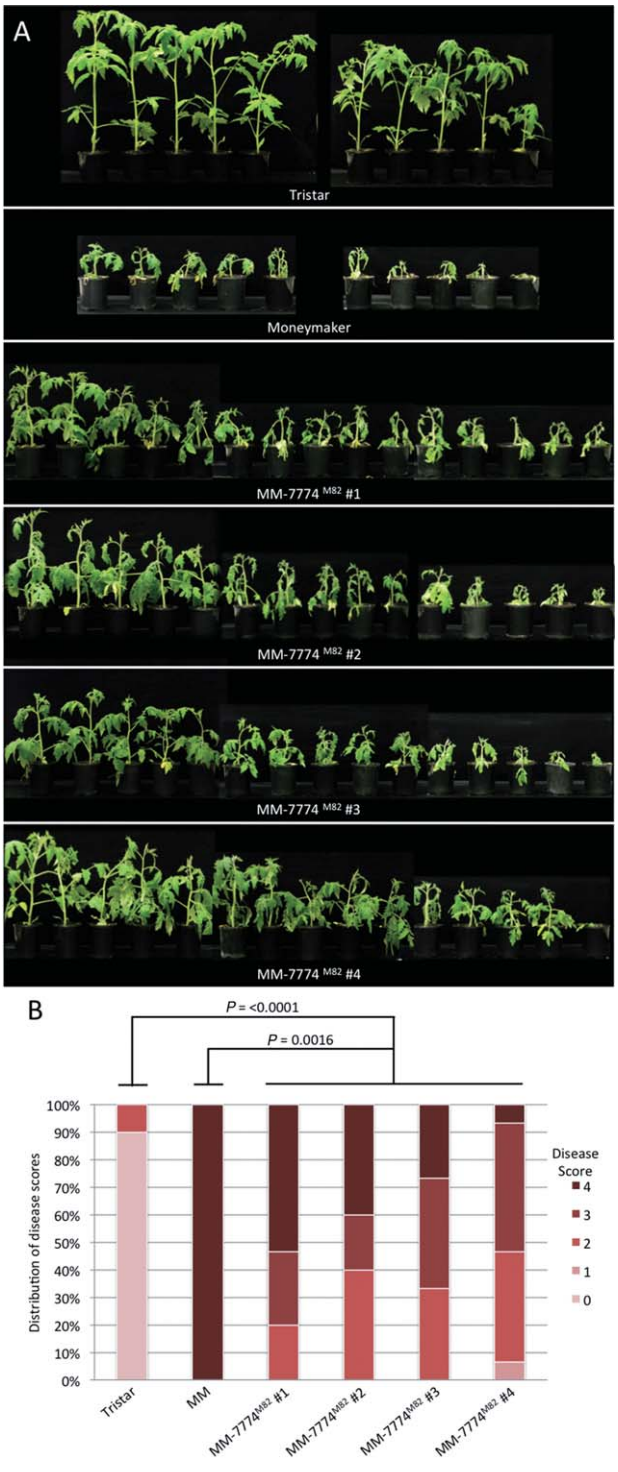



**Fig. 3** (A) Disease assays on T2 plants carrying the *Solyc08g077740* gene from M82 in a Moneymaker (MM) background. T2 plants, together with Tristar (resistant) and Moneymaker (susceptible) control plants, were inoculated with *Fol* race 3. Photographs were taken at 21 days post-inoculation. (B) Disease scores for the T2 plants shown in (A). Probability values were obtained using the non-parametric Mann–Whitney test to determine significant differences in disease scores between plant lines

protein involved in defence activation (Hu *et al*., 2005). To assess whether *I-7*-mediated resistance is dependent on *EDS1*, an F2 family segregating for both *I-7* versus *i-7* and *EDS1* versus *eds1* was obtained from a cross between Tristar and the sun1-1 (*eds1*) mutant of tomato (Hu *et al*., 2005). Around 50 Tristar × sun1-1 F2 seedlings were genotyped for *EDS1* and *eds1* by multiplex PCR using a single reverse primer (CCTGCTGCACGAAGACACAG; Hu *et al*., 2005), annealing downstream of the deletion in sun1-1, and two forward primers, one (TGCTTGCTCCTCTTTCATCA) annealing to the region of EDS1 deleted in sun1-1 plants and the other (GTAGGGGTGTTCGTGATTCGATTTG; Hu *et al*., 2005) annealing upstream of the deletion in sun1-1 (Fig. S6A, see Supporting Information). Based on these results, two sets of F2 seedlings were selected for a disease assay: those homozygous for the *eds1* mutation (*eds1/eds1*) and those that were heterozygous (*EDS1/eds1*). These plants, together with the parent lines, Tristar and sun1-1, were inoculated with *Fol* race 3, and DNA samples used to screen for the presence of *eds1* were screened for the presence of *I-7* using the CAPS7774 marker (Fig. S6B). Although resistance was slightly reduced in some *EDS1/eds1* plants carrying *I-7* compared with Tristar, resistance was lost altogether in *eds1/eds1* plants carrying *I-7* (Fig. 4). These results show clearly that *EDS1* is required for the resistance response mediated by *I-7*.

## Analysis of the protein sequence encoded by I-7

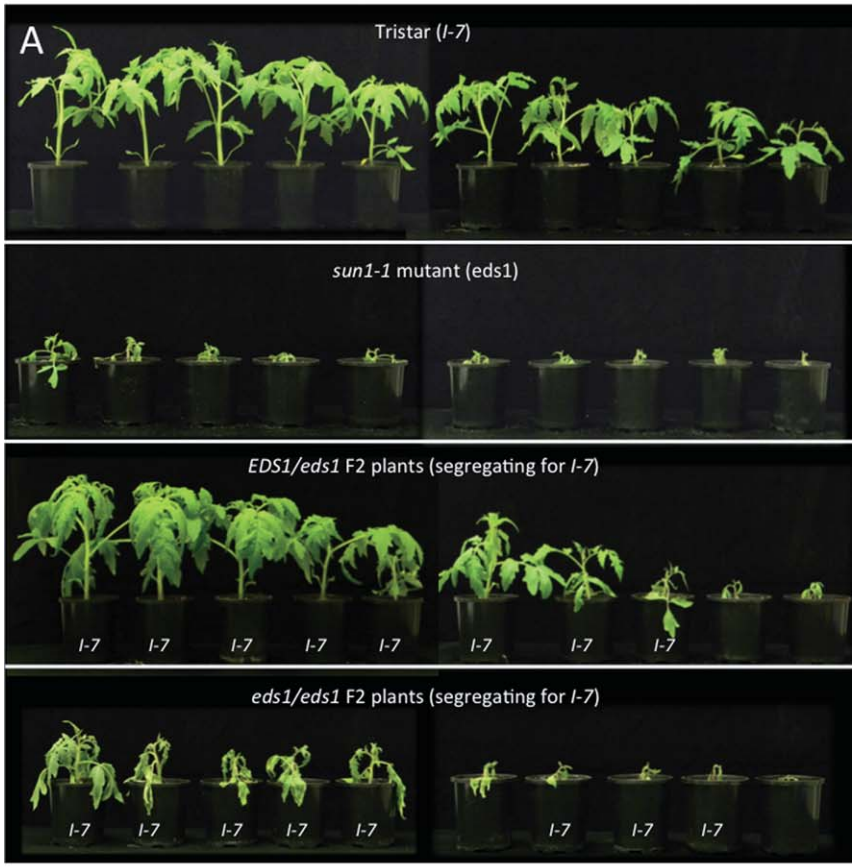
The *I-7* gene [National Center for Biotechnology Information (NCBI) accession KT185194] encodes a typical LRR-RLP of 966 amino acids (Fig. 5). The first 25 amino acids are predicted to function as a signal peptide directing protein secretion (von Heijne, 1986). Amino acids 26–85 constitute the mature N-terminus comprising an N-terminal LRR-flanking domain containing four conserved cysteines likely to be involved in disulfide bond formation. The N-terminal end is followed by a typical extracellular LRR region consisting of 30 repeats (from amino acids 86 to 851) separated into two blocks (26 and four repeats) by a loop out region (amino acids 709–755). Amino acids 852–875 constitute a C-terminal LRR-flanking region containing two conserved cysteines potentially involved in disulfide bond formation. Amino acids 876–906 comprise a very acidic domain with 13 negatively charged residues and only one positively charged residue. Amino acids 907–929 comprise a predicted transmembrane domain. Amino acids 930–966 comprise a very basic cytosolic C-terminal domain, with 13 positively charged residues and only two negatively charged residues. The cytosolic domain also includes a putative YXXø endocytosis motif (YVKF), where ø represents an amino acid with a bulky hydrophobic side chain (Bonifacino and Traub, 2003). The predicted LRR region contains 21 potential *N*-glycosylation sites matching the consensus motif N*x*(S/T)*x* (where *x* is any amino acid except P; Gavel and von Heijne, 1990).

Compared with *I-7*, *i-7* encodes a slightly shorter 963-amino-acid protein with 93.4% identity to I-7. I-7 and i-7 differ by 68 polymorphic residues (Fig. 5). The N-terminal flanking domain contains a high proportion of the polymorphisms comprising only 6.6% of the protein, but containing 23.5% (16) of the polymorphisms. The acidic domain is also fairly polymorphic; it comprises only 3.2% of the protein, but possesses 13.2% (nine) of the polymorphisms. The LRR domain is relatively conserved between the two proteins compared with the non-LRR regions. Surprisingly, the predicted solvent-exposed residues in the β-sheet region of the LRR domain, thought to determine recognitional specificity in other LRR-RLPs, show very few polymorphisms between I-7 and i-7, with only five polymorphic residues out of 150 (3.3%).

## *I-7* is a member of a small gene family

A BLASTN search (http://www.sgn.cornell.edu/tools/blast/; default settings) of the *S. lycopersicum* Heinz 1706 genome sequence reveals an *I-7* orthologue (i.e. *i-7*) on chromosome 8 (*Solyc08g077740*; 96% nucleotide identity) and a paralogue on chromosome 6 (*Solyc06g033920*; 86% nucleotide identity). Similarly, a BLASTN search of the *S. pennellii* LA716 genome sequence reveals an orthologue on chromosome 8 (*Sopen08g026260*; 99% nucleotide identity) and a paralogue on chromosome 6 (*Sopen06g014090*; 86% nucleotide identity). However, this search also revealed a paralogue on chromosome 8 (*Sopen08g026250*; 89% nucleotide identity) adjacent to the *I-7* orthologue. This raised the possibility that the *S. pennellii* introgression in Tristar also carried a paralogue adjacent to *I-7*. Further examination of the RNA-seq data described above revealed transcripts corresponding to a homologue present in Tristar, but absent in M82. The coding sequence for this homologue, apart from the first 52 bases, was assembled from the Tristar RNA-seq data (NCBI accession KT185195). This homologue showed higher nucleotide identity to the *I-7* paralogue on chromosome 8 of LA716 (99% nucleotide identity) than *I-7* (88% nucleotide identity), the *I-7* orthologue on chromosome 8 of LA716 (89% nucleotide identity) or the paralogue on chromosome 6 of LA716 (90% nucleotide identity). From these data, we infer the presence of an *I-7* paralogue adjacent to the *I-7* gene in Tristar.

Alignment of the predicted protein sequences (Fig. 6) clearly shows that the chromosome 6 and 8 paralogues are more closely related to one another than they are to *I-7* or its orthologues. The alignment also shows that there are 78 variable positions with three or more different amino acids represented among the seven sequences: 56 within the LRR domain and 21 at predicted solvent-exposed positions in the β-sheet region of the LRR domain. Almost one-half (25) of these positions and two-thirds of those predicted to be solvent exposed are clustered within LRRs 14–19. In contrast, only seven of the variable posi-



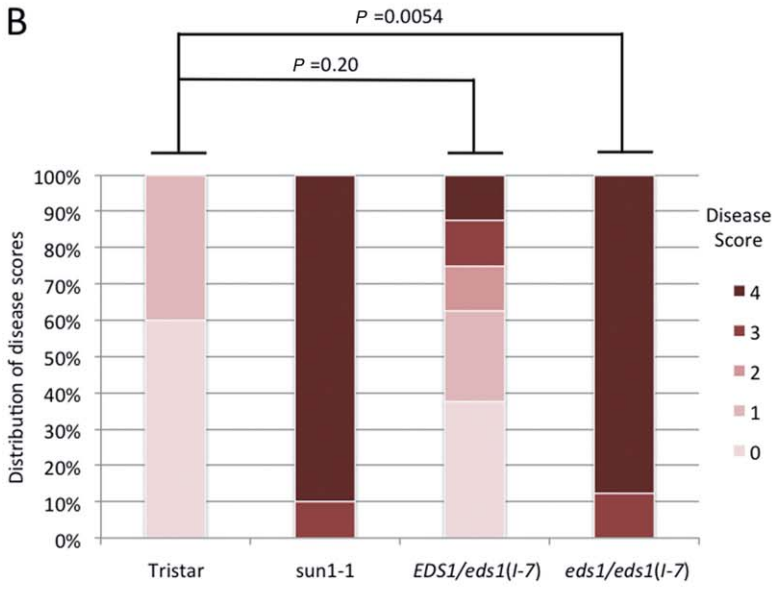




**Fig. 4** (A) Disease assays on Tristar × sun1-1 *EDS1/eds1* and *eds1/eds1* F2 plants (segregating for *I-7*) and parent lines inoculated with *Fol* race 3. F2 plants were genotyped for *I-7* by cleaved amplified polymorphic sequence (CAPS) marker analysis and plants carrying *I-7* are indicated. Photographs were taken at 21 days post-inoculation. (B) Disease scores for the T2 plants shown in (A), excluding F2 plants not carrying *I-7*. Probability values were obtained using the non-parametric Mann–Whitney test to determine significant differences in disease scores between plant lines.

tions are present in LRRs 20–30 and none of these is predicted to be solvent exposed. The alignment also shows extensive polymorphism for the presence/absence of putative *N*-glycosylation sites among the gene family, with 18 of 33 positions being polymorphic, and one site unique to I-7. This alignment also shows that there are only 21 amino acid differences between I-7 and its orthologue in LA716.

To test whether the LA716 orthologue of *I-7* also confers *Fol* resistance, three LA716 introgression lines, IL8-1, IL8-2 and IL8-3, which together span *S. pennellii* chromosome 8 (Eshed

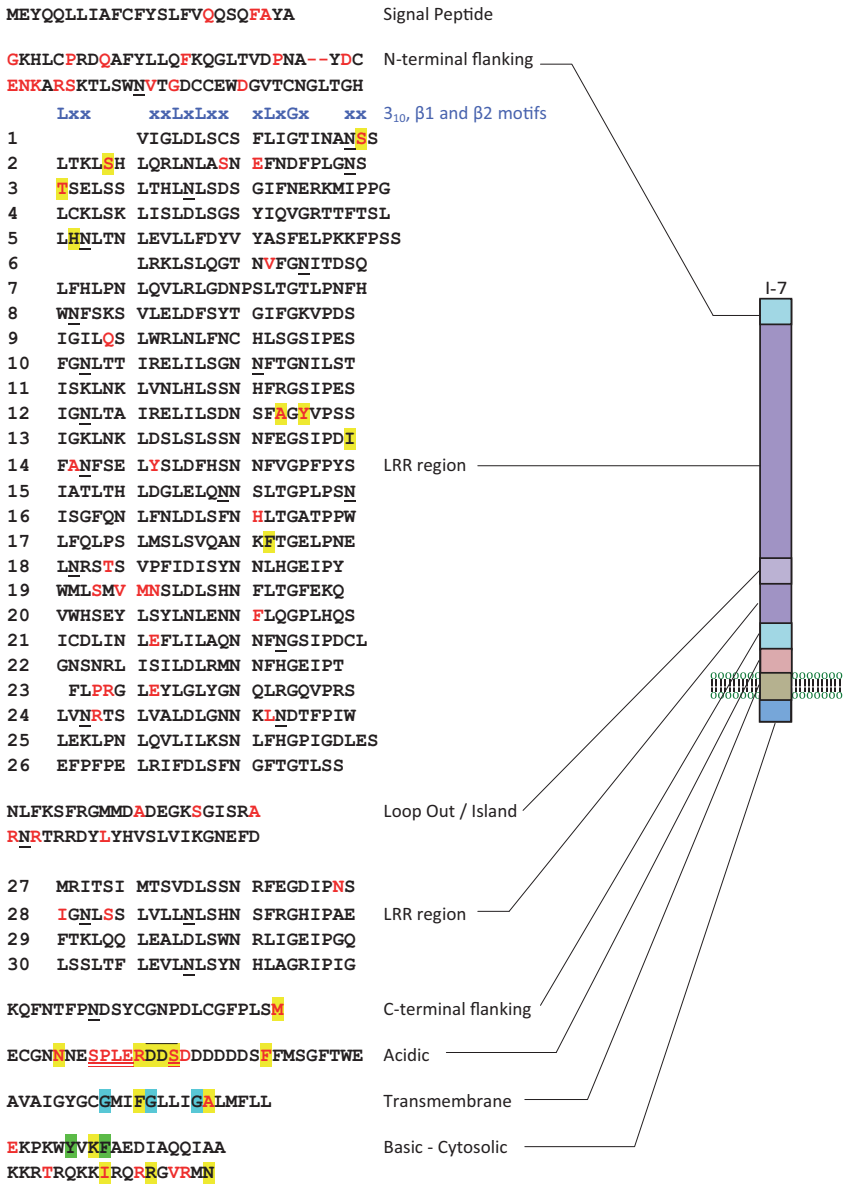



**Fig. 5** Sequence and graphic representation of the predicted I-7 protein divided into eight domains: signal peptide, N-terminal flanking region, typical extracellular leucine-rich repeat (LRR) region (with LRRs numbered 1–30), divided into two blocks by a loop out region or island domain, C-terminal LRR-flanking region, acidic domain, transmembrane domain and basic cytosolic domain. The locations of predicted $3_{10}$ (*xxLxx*), concave β1 (*xxLxLxx*) and convex β2 (*xLxGx*) motifs are shown in blue above the LRR domain. The positions of amino acid differences between I-7 and i-7$^{M82}$ are shown in red, whereas those between I-7 and i-7$^{LA716}$ are highlighted in yellow. Putative *N*-glycosylation sites are underlined and a putative endocytosis motif is highlighted in green. A possible SOBIR1-dimerization motif (G*xxx*G*xxx*G; Bi *et al*., 2015) is highlighted in light blue. Amino acids present in I-7, but missing from i-7$^{M82}$, owing to deletions in *i-7* relative to *I-7*, are double underlined, whereas those missing from i-7$^{LA716}$ are overlined.

and Zamir, 1995), were tested for resistance to *Fol* race 3. None of the three lines, including IL8-2, which carries the LA716 orthologue of *I-7*, showed resistance to *Fol* race 3 (Fig. S7, see Supporting Information). This result is consistent with previous work showing that the IL8 lines do not confer resistance to *Fol* races 1 or 2 (Sela-Buurlage *et al*., 2001). Thus, the LA716 orthologue of *I-7* is also an *i-7* allele. We have designated this gene *i-7*$^{LA716}$ to distinguish it from the M82 allele, which we have now designated *i-7*$^{M82}$. Nine of the amino acid differences between I-7 and i-7$^{M82}$ or i-7$^{LA716}$ are unique to I-7 (Fig. 6) and, although four of these nine (S110, T131, A171 and Y173) occur in the LRR region, none are at solvent-exposed positions within the β-sheet region of the LRR domain (Fig. 5).

## DISCUSSION

Prior to this work, the only genes for resistance to Fusarium wilt that had been cloned from tomato were *I-2*, encoding a CC-NB-LRR protein (Simons *et al*., 1998), and *I-3*, encoding an SRLK protein (Catanzariti *et al*., 2015). Now, through the identification of *I-7*, which encodes an LRR-RLP, another class of R gene has been added to this repertoire. The existence of a diverse group of resistance genes against just one pathogen is unusual. In most plant–pathogen systems, genes conferring resistance to different isolates of the same pathogen belong to just one or two classes. The fact that resistance to *Fol* is provided by genes in three different classes suggests that pathogen recognition and defence

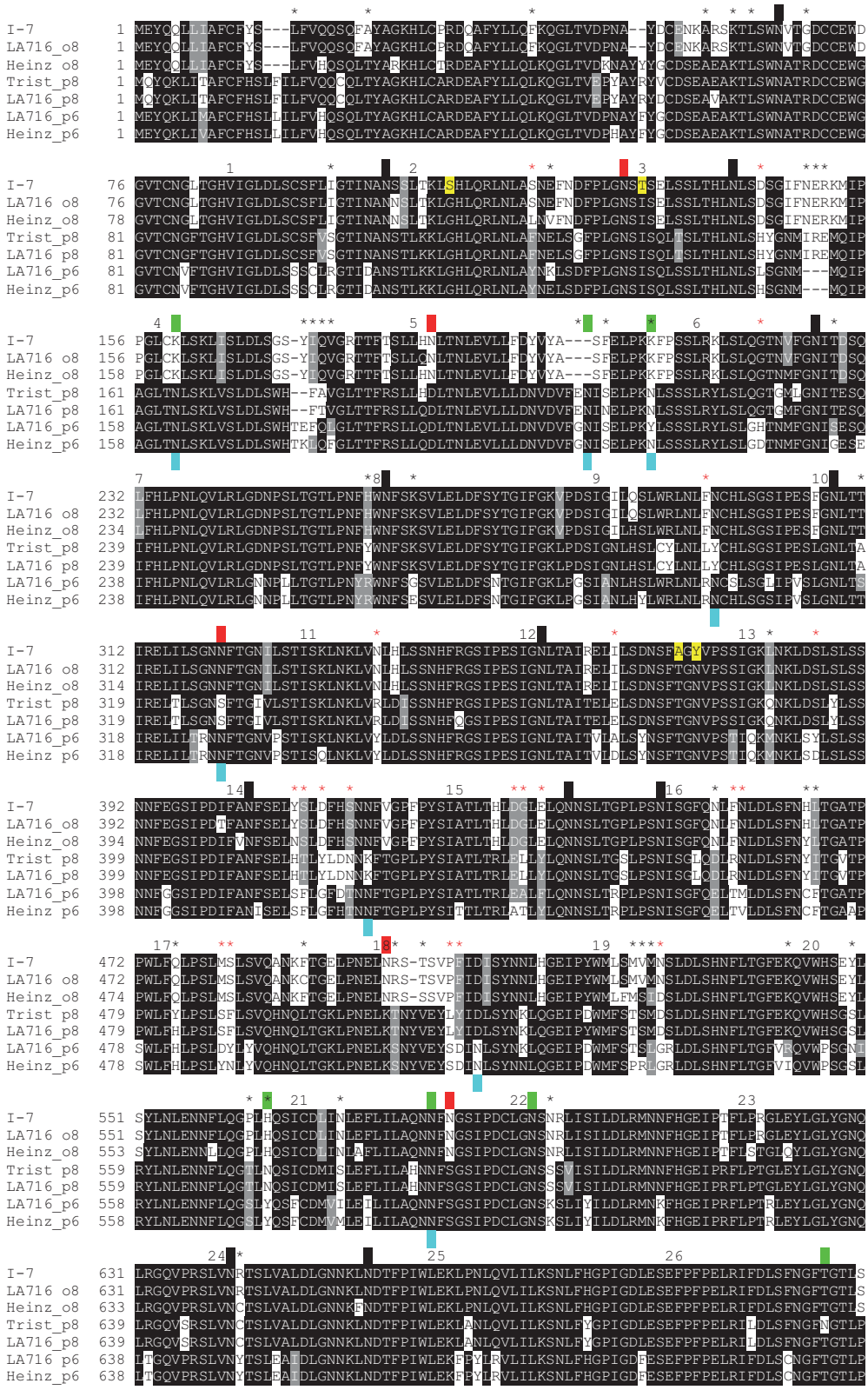


**Fig. 6** Continued.

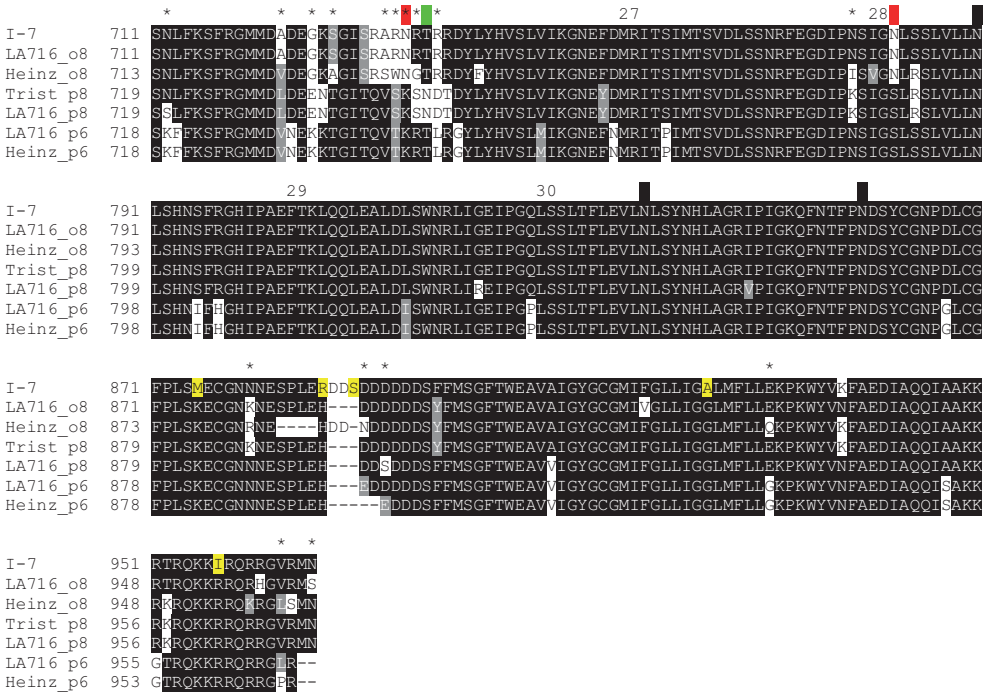



**Fig. 6** Alignment of I-7 and orthologous and paralogous proteins from Tristar, Heinz 1706 and LA716. Sequences were aligned using the EMBL-EBI MAFFT server at http://www.ebi.ac.uk/Tools/msa/mafft/, and the alignment was shaded using the ExPASy BoxShade server at http://www.ch.embnet.org/software/BOX_form.html. Amino acid identities are highlighted in black. Amino acid similarities are highlighted in grey. Putative *N*-glycosylation sites encoded by orthologous and paralogous genes on chromosome 8 (labelled o8 and p8, respectively) and paralogous genes on chromosome 6 (labelled p6) are indicated by black boxes above the sequence alignment. Putative *N*-glycosylation sites encoded by genes at only one or two of these three loci are indicated by red boxes (o8) or green boxes (p8) above the alignment or blue boxes (p6) below the alignment. Residues unique to I-7 are highlighted in yellow. Numbers above the alignment indicate the leucine-rich repeat (LRR) number, and asterisks indicate variable sites with three or more different residues, with red asterisks indicating those in predicted solvent-exposed residues in the β-strand motif of the LRR. It should be noted that the first 18 amino acids of the Trist_p8 sequence are inferred from the LA716_p8 sequence.

activation are more complex in the tomato–*Fol* pathosystem than in other pathosystems. Such complexity may also be a feature of resistance in other plant–*F. oxysporum* pathosystems, given that Arabidopsis genes for quantitative resistance to *F. oxysporum* show similar diversity, with *RFO1* encoding a wall-associated kinase (a non-LRR receptor-like kinase; Diener and Ausubel, 2005), *RFO2* encoding an LRR-RLP (Shen and Diener, 2013), like I-7, and *RFO3* encoding an SRLK (Cole and Diener, 2013), like I-3. It is possible that the complexity of the *F. oxysporum* infection process (penetration of the root epidermis, growth across the root cortex, colonization of the root xylem and a switch from biotrophy to necrotrophy) provides multifarious opportunities for recognition that might result in a more complex array of plant resistance mechanisms that counter infection. Moreover, the fact that tomato uses both cytosolic (I-2) and surface (I-3 and, most likely, I-7) receptors to detect *Fol* effectors suggests that *Fol* secretes effectors that act both inside and outside the plant cell.

Many resistance genes occur in gene clusters. For example, the *I-2* gene is one of seven homologous genes clustered at the *I-2* locus (Simons *et al*., 1998), and *I-3* is one of six homologous genes clustered at the *I-3* locus. *I-7* belongs to a small gene family in tomato that includes a homologue on chromosome 6 and a homologue on chromosome 8 adjacent to *I-7*. Although it is clear that the homologue on chromosome 6 does not confer resistance to *Fol*, we cannot rule out the possibility that the chromosome 8 homologue in Tristar also confers resistance to *Fol*. However, given its amino acid sequence divergence from I-7, it would be unlikely to confer the same specificity and, given that I-7 can function in its absence, it is unlikely to be a co-receptor.

*I-7* encodes a protein with a domain architecture typical of LRR-RLPs (Fig. 5). Other LRR-RLPs identified in tomato and tobacco include disease resistance proteins, such as the tomato Cf-2 and Cf-9 proteins, conferring resistance to *Cladosporium fulvum* (Dixon *et al*., 1996; Jones *et al*., 1994), and pattern recognition receptors (PRRs), including the tomato Ve1 protein, which mediates resistance against *Verticillium dahliae* (Kawchuk *et al*., 2001) through recognition of the Ave1 protein present in various pathogenic fungi (de Jonge *et al*., 2012), the tomato Eix1 and Eix2 receptors, which mediate perception of the cell wall-derived ethylene-inducing xylanase (EIX) from *Trichoderma* species (Ron and Avni, 2004), and the tobacco EILP protein, which responds to a cell wall elicitor from *Phytophthora infestans* (Takemoto *et al*.,

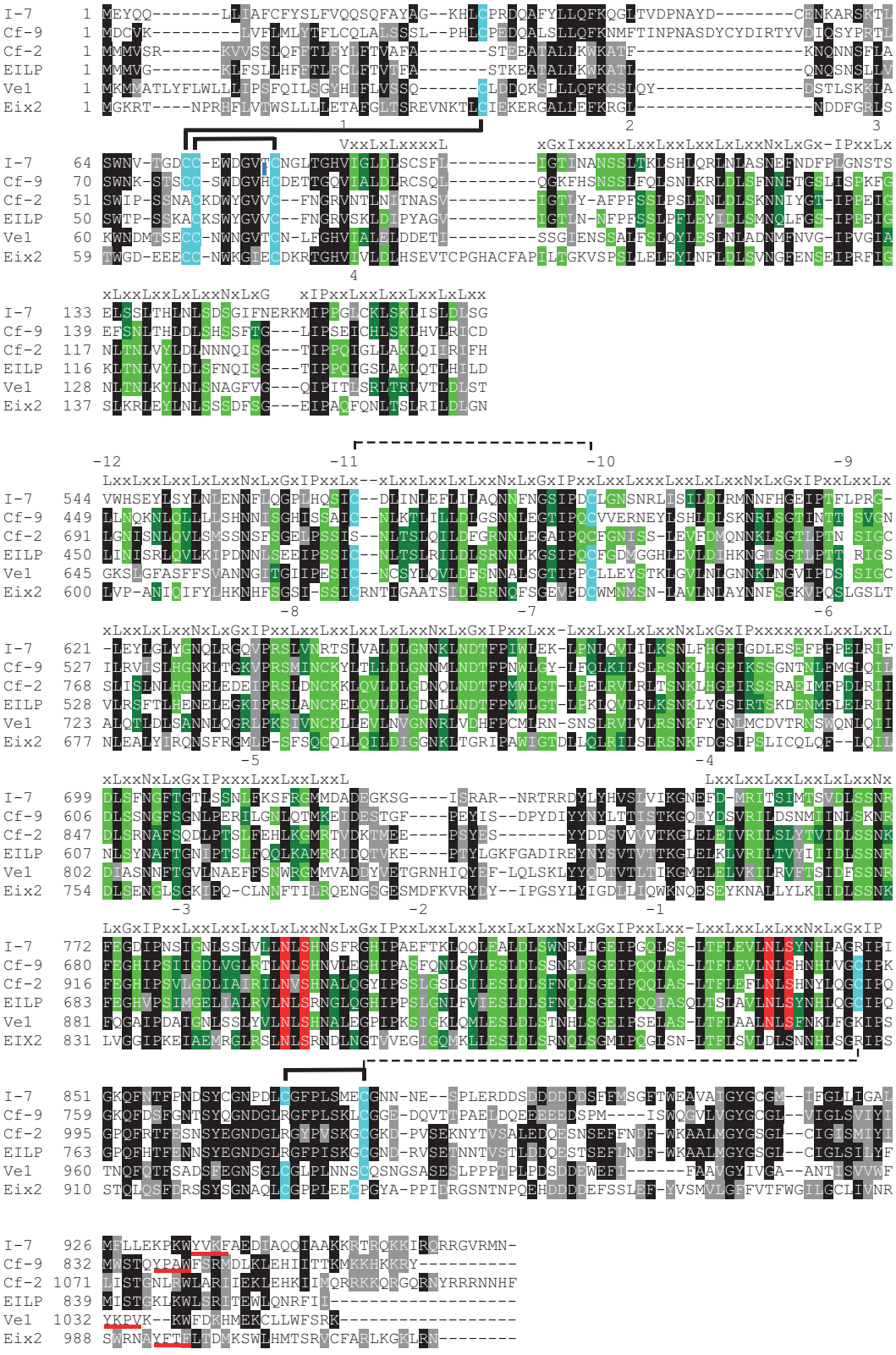
**Fig. 7** Alignment of N- and C-terminal regions conserved between I-7 and other tomato and tobacco leucine-rich repeat receptor-like proteins (LRR-RLPs). Sequences were aligned using the EMBL-EBI MAFFT server at http://www.ebi.ac.uk/Tools/msa/mafft/, and the alignment was shaded using the ExPASy BoxShade server at http://www.ch.embnet.org/software/BOX_form.html. Amino acid identities are highlighted in black. Amino acid similarities are highlighted in grey. In LRR regions, the consensus sequence is shown above the alignment, and amino acid identities for non-consensus residues are highlighted in bright green and amino acid similarities for non-consensus residues are highlighted in dark green. Conserved cysteine residues likely to be involved (full connecting lines) or potentially involved (broken connecting lines) in disulfide bonding, based on structural studies of other plant extracellular LRR proteins (Di Matteo *et al*., 2003; Hothorn *et al*., 2011), are highlighted in blue. Conserved *N*-glycosylation motifs are highlighted in red. LRR-RLP sequences were derived from GenBank accessions AAA65235 (Cf-9), AAC15779 (Cf-2), BAA88636 (EILP), ACR33106 (Ve1) and AAR28378 (Eix2).

2000). The LRR domains of these proteins differ in repeat number and the sequence of non-LRR residues within the LRR motif. Conversely, these proteins show conserved N- and C-terminal regions encompassing three N- and 11 C-terminal LRRs that contain conserved cysteine residues likely to be involved in disulfide bonds, two or four at the N-terminus, two in adjacent LRRs and two in the C-terminal LRR-flanking domain (Fig. 7). C-terminal conservation, including the 11 C-terminal LRRs, C-terminal LRR-flanking domain and transmembrane G*xxx*G*xxx*G motif (Fig. 5), is thought to reflect interaction with a downstream signalling partner, possibly SOBIR1 (Bi *et al*., 2015; Liebrand *et al*., 2013) and/or BAK1 (Postma *et al*., 2015). The intervening LRRs, which vary in number and sequence between LRR-RLPs, are often involved in the recognition of microbial proteins, including specific avirulence proteins (Chakrabarti *et al*., 2009; van der Hoorn *et al*., 2001; Thomas *et al*., 1997, 1998; Wulff *et al*., 2009). Accordingly, I-7 might recognize an Avr7 effector protein yet to be identified, possibly corresponding to one of the SIX (secreted in xylem) proteins produced by *Fol* during tomato root colonization (Rep, 2005; Schmidt *et al*., 2013).

Most of the amino acid differences between I-7 and its homologues are among predicted solvent-exposed β-strand residues clustered within LRRs 14–19 (Fig. 6). Plant extracellular LRR domains generally fold into superhelical structures with parallel β-sheets on the concave side and parallel β-sheets and α- or $3_{10}$-helices on the convex side (Di Matteo *et al*., 2003; Hothorn *et al*., 2011; She *et al*., 2011; Sun *et al*., 2013), providing a structural framework for the presentation of amino acids involved in protein–protein interactions (Kobe and Kajava, 2001). Solvent-exposed residues on the conserved concave surface β-strand/β-turn structural motif are often determinants of recognitional specificity in plant extracellular LRR proteins (Benedetti *et al*., 2011; Dunning *et al*., 2007; van der Hoorn *et al*., 2001; Leckie *et al*., 1999; Spinelli *et al*., 2009; Wulff *et al*., 2009). However, none of the amino acid substitutions that distinguish I-7 from i-$7^{LA716}$ occur in the concave β-strand/β-turn region of I-7. Interestingly, the only residues in central LRRs that distinguish I-7 from both i-$7^{M82}$ and i-$7^{LA716}$ are two solvent-exposed residues (A171 and Y173) in the predicted convex β-strand region (*x*L*x*G*x*) of LRR12 (Fig. 5), suggesting that I-7 could function differently from other LRR-RLPs involved in microbial protein recognition. Other differences that distinguish I-7 from both i-$7^{M82}$ and i-$7^{LA716}$ include three residues (S104, S110 and T131) in or near the predicted $3_{10}$-helix region (*xx*L*xx*) of LRRs 1, 2 and 3 (Fig. 5), which might also be involved in recognition. T131 replaces the conserved leucine of the *xx*L*xx* motif, suggesting that it could potentially disrupt $3_{10}$-helix formation and therefore also affect protein structure and stability. The remaining differences that distinguish I-7 from both i-$7^{M82}$ and i-$7^{LA716}$ are clustered in the non-LRR C-terminus of the protein (Fig. 5), suggesting that they could play a role in protein stability or signalling rather than recognition.

Protein sequence analysis also revealed that more than 50% of the putative *N*-glycosylation sites showed polymorphisms for presence/absence among I-7 and its orthologues and paralogues (Fig. 6). Plant LRR domains are often characterized by large numbers of putative *N*-glycosylation sites. For example, Cf-9 contains 22 putative *N*-linked glycosylation sites in its extracellular domain, 19 of which are evenly distributed over different LRRs (van der Hoorn *et al*., 2003). Functional and structural studies have shown that putative *N*-linked glycosylation sites not only contribute to Cf-9 activity, but are essential when located in the putative helical regions of the LRR domain (van der Hoorn *et al*., 2005). More recently, a functional study of the interactions between *N*-glycosylation mutants of the Arabidopsis LRR receptor kinases (EFR and FLS2) and their bacterial interactors (elf18 from elongation factor Tu and flg22 from flagellin) has shown that a single *N*-glycan plays a crucial role in receptor abundance and ligand recognition (Haweker *et al*., 2010). Given the important role of *N*-glycosylation sites in the function of various LRR-containing proteins, it is possible that polymorphisms for the presence/absence of putative *N*-glycosylation sites among I-7 family members might have an impact on the abundance and activity of these proteins. In addition to its possible disruptive effect on $3_{10}$-helix formation, the T131 residue also converts N129 into a putative glycosylation site unique to I-7, so that this residue could potentially have a significant impact on I-7 structure and function.

Several LRR-RLPs contain a potential endocytosis motif, e.g. a di-leucine E/DXXXLø (in Ve1) or tyrosine YXXø (in Ve1, Eix1 and Eix2) motif, where ø is any amino acid with a bulky hydrophobic side chain (Fradin *et al*., 2014; Kawchuk *et al*., 2001; Ron and Avni, 2004) (Fig. 7). Like Ve1, Eix1 and Eix2, I-7 possesses a potential endocytosis motif (YVKF) in its cytosolic tail. However, the motif is not well conserved in sequence or position among LRR-



RLPs, and mutation experiments have shown that the E/DXXXLø and YXXø motifs of Ve1 are not required for function (Zhang *et al*., 2014). Similarly, A. Chakrabarti *et al*. (unpublished results) have shown that mutation of the Y or W of the YXXø motif (YAPW) of Cf-9 does not affect Avr9-dependent necrosis. In contrast, mutation of the Y in the YXXø motif of Eix2 abolishes the EIX response (Ron and Avni, 2004). The role of the YVKF motif in I-7 resistance remains to be determined experimentally.

Avr1, present in *Fol* race 1, suppresses both I-2- and I-3-mediated resistance (Houterman *et al*., 2008). We have shown that I-7 confers resistance against *Fol* race 1, indicating that Avr1 does not suppress I-7-mediated resistance and is therefore not a general suppressor of resistance. It is possible that the action of Avr1 is limited to Avr2 and Avr3 through a functional relationship between the three effectors, e.g. Avr1 might direct the degradation of a host protein (or proteins) targeted for modification by Avr2 and Avr3, but not by Avr7, to circumvent recognition of the modified host protein(s) by I-2 and I-3. Alternatively, the failure of Avr1 to suppress resistance triggered by Avr7 could indicate that Avr1 is a pathway-specific effector, acting on or after a point of convergence in I-2 (CC-NB-LRR) and I-3 (SRLK) signalling, but not I-7 (LRR-RLP) signalling. EDS1 is required for resistance mediated by LRR-RLPs and Toll-Interleukin 1 Receptor (TIR)-NB-LRRs, basal defences and systemic acquired resistance, but not resistance mediated by CC-NB-LRRs (Aarts *et al*., 1998; Breitenbach *et al*., 2014; Fradin *et al*., 2009; Hu *et al*., 2005; Parker *et al*., 1996). We have shown that EDS1 is required for I-7 resistance, and it can be assumed that EDS1 is not required for I-2 resistance because I-2 is a CC-NB-LRR protein. If I-3 is found to be EDS1 independent, it is plausible that I-7 operates through a different signalling pathway to I-2 and I-3. Significantly, Avr1 triggers defence signalling through recognition by I, which is also EDS1 dependent (Hu *et al*., 2005), indicating that the *I* gene most probably encodes a TIR-NB-LRR or LRR-RLP that signals through the same pathway as I-7. Together, these observations are consistent with the idea that Avr1 might act to suppress signalling through a non-EDS1-dependent pathway.

The identification of *I-7* has allowed us to develop a CAPS marker to the gene itself (CAPS7774; see Table S2 and Fig. S2), thereby providing plant breeders with perfect genetic markers for the selection and breeding of elite tomato cultivars carrying either or both genes currently known to confer *Fol* race 3 resistance (see Catanzariti *et al*., 2015 for *I-3* markers). Given that I-7-mediated resistance is not suppressed by Avr1, *I-7* may be a useful backup to *I-3* in the plant breeder's toolbox.

## EXPERIMENTAL PROCEDURES

### Plant and fungal material

Tomato cultivars Tristar (carrying *I-7;* resistant to *Fol* race 3; McGrath, 1988) and M82 (lacking *I-7*; susceptible to *Fol* race 3) were crossed, and F3 and F4 lines were produced by recurrent selfing. Homozygous resistant or susceptible F3 or F4 lines were identified by screening with *Fol* race 3, as described below. Tristar was also crossed to the sun1-1 mutant (homozygous for a mutation of the tomato *EDS1* gene; Hu *et al*., 2005) of the tomato cultivar VF36 (lacking *I-7*; susceptible to *Fol* race 3) and an F2 line produced by selfing. F2 plants were scored for *I-7*-dependent resistance by screening with *Fol* race 3, as described below. Tomato cultivars Moneymaker (no resistance to *Fol*) and M82 (lacking *I-7*, but carrying the *I* gene conferring resistance to *Fol* race 1) were used as *Fol* race 3-susceptible lines for plant transformation.

*Fol* race 1 (Fol004 carrying *Avr1*, *Avr2* and *Avr3*) and race 2 (Fol007 carrying *Avr2* and *Avr3*) were provided by Dr Martijn Rep (University of Amsterdam, the Netherlands). *Fol* race 3 isolate #1943 (carrying *Avr3*) was provided by Mr Des McGrath (Agri-Science Queensland, Australia).

### Pathogen tests for Fusarium wilt resistance

Tomato seeds were sown in seed-raising mix (Martins Fertilizers, Yass, NSW, Australia), and seedlings were grown for 10–13 days in a temperature-controlled glasshouse with a maximum day temperature of 25 °C and minimum night temperature of 18 °C prior to fungal inoculation. *Fol* cultures were grown in potato dextrose broth (Difco, Detroit, MI, USA) for 5 days on a rotary shaker (200 rpm) at 25 °C, and conidia were collected by filtering the cultures through four layers of miracloth. Conidia were washed twice by centrifugation at 6000 ***g*** for 10 min and resuspension in deionized water. Conidial concentrations were adjusted to $5 \times 10^6$ or $1 \times 10^7$ conidia/mL after the final resuspension. Seedlings were removed from the seed-raising mix and their roots were washed with deionized water, trimmed and then dipped in a conidial suspension for 3 min before replanting in potting mix (Martins Fertilizers). Roots of control plants were dipped in deionized water for the purposes of mock inoculation to ensure that disease phenotypes were a consequence of *Fol* infection, rather than the inoculation process *per se* (results not shown apart from the example in Fig. S7). After inoculation, plants were kept in a controlled-environment growth room with a 16 h, 25 °C day/8 h, 20 °C night cycle. After 18 or 21 days, wilt symptoms and vascular browning were recorded and used to calculate disease scores according to the following criteria described by Rep *et al*. (2005): 0, no reaction, healthy plant; 1, slightly swollen or bent hypocotyl; 2, one or two brown vascular bundles in hypocotyl; 3, at least two brown vascular bundles and growth distortion; 4, all vascular bundles are brown; plant either dead or very small and wilted.

### RNA-seq analysis

Ten-day-old seedlings of tomato cultivars Tristar and M82 were inoculated with *Fol* race 3 or mock inoculated for the purposes of conducting an RNA-seq experiment looking at transcriptional responses to *Fol* infection. Only the data obtained for the mock-inoculated plants were used for the work described in this paper. Roots of 15 plants from each cultivar were collected 2 days after mock inoculation, washed with sterile deionized water, pooled and frozen in liquid nitrogen for RNA extraction.

Frozen root samples were ground in liquid nitrogen, and total RNA was isolated using a Plant RNeasy kit (Qiagen, Hilden, Germany) following the

manufacturer's instructions. Extracted RNA (5 μg per sample) was sent to The Ramaciotti Centre (University of New South Wales, Sydney, Australia) for cDNA synthesis, library preparation and the generation of 100-base paired-end reads using the HiSeq2000 sequencing platform (Illumina, San Diego, CA, USA). The RNA-seq experiment was repeated three times to generate biological replicates, but, for the purposes of the analysis described in this paper, data from the replicates were pooled.

The RNA-seq data were analysed using CLC Genomics Workbench 4.0 (http://www.clcbio.com/). Sequence reads were trimmed using default parameters and then mapped against the tomato (cv. Heinz 1706) reference transcriptome (file ITAG2.3_cdna_alignments.gff3) obtained from the SOL genomics network (http://solgenomics.net/organism/Solanum_lycopersicum/genome). Mapping was performed using the read mapper tool with global alignment (mismatch cost = 2; insertion cost = 3; deletion cost = 3; length fraction = 0.5; similarity = 0.8). The RNA-seq analysis tool was used to analyse the transcript data (maximum pair distance = 500; minimum paired distance = 1; maximum mismatches = 2; minimum length fraction = 0.97; minimum similarity fraction = 0.8) to determine which genes were expressed in roots. Expression levels were recorded as reads per kilobase per million (RPKM) mapped reads (Mortazavi *et al*., 2008).

### SNP analysis

To identify SNPs between mapped reads and the reference transcriptome, the CLC Genomics Workbench 4.0 SNP detection tool was used with default parameters, and a minimum coverage of 10 and a minimum variant frequency of 35. The SNP data were then transferred into an Excel spreadsheet, and SNPs with a read frequency of ≥75% were selected for further analysis using the Filter function in Excel. SNPs that were common to Tristar and M82 relative to the reference transcriptome were identified using the VLOOKUP function in Excel, and discarded. The remaining polymorphisms were used to calculate the frequency of SNPs in each transcript (number of SNPs per transcript divided by the length of transcript) that were unique to Tristar or M82. The SNP frequency was plotted against the corresponding tomato gene positions on each tomato chromosome to identify genes with a higher frequency of SNPs in Tristar compared with M82 or with surrounding Tristar genes.

Once such cluster of genes was identified. CAPS markers were designed targeting restriction site polymorphisms associated with the SNPs in these genes. Markers were then used to screen plants from 15 homozygous resistant and 16 homozygous susceptible Tristar × M82 F3 or F4 lines to identify correlation between marker genotypes and resistance or susceptibility to *Fol* race 3.

### Generation of transgenic tomato lines for complementation testing

The *I-7* candidate gene from Tristar and the corresponding gene from M82 were PCR amplified from genomic DNA using LIC7774 forward and reverse primers (Table S4), designed for ligation-independent cloning (LIC), and iProof high-fidelity DNA polymerase (BioRad Laboratories, Hercules, CA, USA), according to the manufacturer's instructions. PCR products were cloned via LIC into pL2, a binary vector based on pGreenII (Hellens *et al*., 2000), containing an *nptII* gene for the selection of transformed plants and a LIC site flanked by a cauliflower mosaic virus (CaMV) 35S promoter and terminator (Fig. S8, see Supporting Information). Purified PCR product was treated with T4 DNA polymerase (NEB, Ipswich, MA, USA) in 1 × Buffer 2 (NEB) and 2.5 mM dCTP at 22 °C for 30 min. To prepare the vector, pL2 was digested with *Sna*BI, purified and treated with T4 DNA polymerase in 1 × Buffer 2 and 2.5 mM dGTP at 22 °C for 30 min. PCR products (75 ng) and vector (50 ng) were incubated for 20 min at 75 °C, cooled at 4 °C, mixed together, the volume adjusted to 10 μL with water, and incubated at 65 °C for 1 min, and then 22 °C for 20 min, to anneal the complementary vector and insert overhangs prior to heat shock transformation of *Escherichia coli* Mach1 cells. Transformants were screened by colony PCR using insert-specific primers, and insert integrity was confirmed by sequencing.

Binary vectors were extracted from *E. coli* and transformed together with pSoup, required for the replication of pGreenII-based vectors in *Agrobacterium* (Hellens *et al*., 2000), into *Agrobacterium tumefaciens* GV3101 (pMP90) by electroporation. Transformants were screened by colony PCR using insert-specific primers, and positive transformants were confirmed by plasmid rescue (plasmid extraction and re-transformation of *E. coli*). *Agrobacterium* transformants were then used to transform tomato cultivars M82 (susceptible to *Fol* races 2 and 3, but resistant to race 1) and Moneymaker (susceptible to all three races) using the standard *Agrobacterium* co-cultivation technique, essentially as described by McCormick (1991). Transgenic plants were selected using 100 mg/L kanamycin and 200 mg/L timentin, and the presence of the transgene was confirmed by PCR analysis of plant DNA extracts using a transgene-specific forward primer and a T-DNA-specific (M13) reverse primer.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

**Fig. S1** Marker analysis on Tristar, M82 and 15 homozygous-resistant and 16 homozygous-susceptible Tristar × M82 F3 or F4 lines using CAPS7774 primers (Table S2) targeting the *Solyc08g077740* gene. Polymerase chain reaction (PCR) amplification generates an 808-bp product with all genomic DNA templates (not shown) and, after digestion with *Age*I, Tristar shows digested products of 612 and 196 bp, whereas M82 products remain undigested. Resistant lines all show the Tristar banding pattern, whereas susceptible lines all show the M82 banding pattern, indicating complete linkage between *I-7* and the CAPS7774 marker.

**Fig. S2** Polymerase chain reaction (PCR) screening of MM-7774$^{Tristar}$ T2 plants from two lines for the presence of the *Solyc08g077740* transgene from Tristar using 7774F4 (Table S4) and M13 reverse primers to detect the transgene (top band) and CAPS7774 primers (Table S2) to detect the transgene and the endogenous *Solyc08g077740* gene in transgenic plants, or the endogenous *Solyc08g077740* gene alone in non-transgenic sibs (bottom band). Asterisks indicate plants carrying the transgene. Controls included a genomic DNA sample from Moneymaker (MM), a no tempate control (Water) and plasmid DNA containing the transgene (pL2-Tristar).

**Fig. S3** (A) Disease assays on T2 plants from two additional lines segregating for the *Solyc08g077740* gene from Tristar in a Moneymaker (MM) background. T2 plants, together with Tristar (resistant) and Moneymaker (susceptible) control plants, were inoculated with *Fol* race 3. Photographs were taken at 21 days post-inoculation (dpi). (B) Disease scores for the plants shown in (A).

**Fig. S4** (A) Disease assays on MM-7774$^{Tristar}$ #1 T2 plants carrying the *Solyc08g077740* gene from Tristar in a Moneymaker (MM) background. T2 plants, together with Tristar (resistant) and Moneymaker (susceptible) control plants, were inoculated with *Fol* race 1. Photographs were taken at 20 days post-inoculation (dpi). (B) Disease scores for the T2 plants shown in (A).

**Fig. S5** (A) Disease assays on MM-7774$^{Tristar}$ #1 T2 plants carrying the *Solyc08g077740* gene from Tristar in a Moneymaker (MM) background. T2 plants, together with Tristar (resistant) and Moneymaker (susceptible) control plants, were inoculated with *Fol* race 2. Photographs were taken at 20 days post-inoculation (dpi). (B) Disease scores for the T2 plants shown in (A).

**Fig. S6** (A) Polymerase chain reaction (PCR) screening for the presence of *EDS1* and the *eds1* mutation in Tristar × sun1-1 F2 plants using the primers as described in the main text. Plants containing the *EDS1* gene produce a PCR product of 644 bp, whereas plants carrying the *eds1* mutation produce a PCR product of about 850 bp. The presence of the *EDS1* gene is represented by a capital E and the presence of the *eds1* allele by a lower case e. Plants heterozygous for *eds1* (with an Ee genotype shown in white) and homozygous for *eds1* (with an ee genotype shown in red) were inoculated with *Fol* race 3 and screened for the presence of *I-7* as shown in (B). (B) PCR screening for the presence of *I-7* among the Ee and ee plant DNA samples shown in (A) using the CAPS7774 primers (Table S2). *I-7* generates 612- and 196-bp

products after digestion with *Age*I, whereas the *i-7* allele from sun1-1 generates an 808-bp product that remains undigested. Plants carrying *I-7* are indicated. In both (A) and (B), the PCR products amplified from genomic DNA of the parent lines (Tristar and sun1-1) are shown (boxed).

**Fig. S7** (A) Authentication of IL8-1, IL8-2 and IL8-3 lines using chromosome 8 cleaved amplified polymorphic sequence (CAPS) and sequence characterised amplified region (SCAR) markers diagnostic for each line (Table S5, see Supporting Information). (B) Analysis of the Tristar, M82, IL8-1, IL8-2 and IL8-3 lines using the CAPS7774 marker (Table S2), showing that IL8-2 carries the LA716 allele ($i\text{-}7^{LA716}$) of the *I-7* gene. (C) Disease assays on IL8-1, IL8-2 and IL8-3 plants inoculated with *Fol* race 3 or mock inoculated. Photographs were taken at 21 days post-inoculation (dpi).

**Fig. S8** Ligation-independent cloning (LIC) using the in-house binary vector pL2 derived from pGreenII showing: (A) steps in the LIC process; (B) a map of pL2.

**Table S1** RNA-seq output, mapping and single nucleotide polymorphism (SNP) analysis data

**Table S2** Cleaved amplified polymorphic sequence (CAPS) markers designed for genes within the cluster of Tristar single nucleotide polymorphisms (SNPs) on chromosome 8

**Table S3** Genes present in the introgressed and neighbouring regions of Tristar chromosome 8. The introgressed region encompasses genes *Solyc08g077520* to *Solyc08g077800*, genes which show a high frequency of single nucleotide polymorphisms (SNPs) unique to Tristar.

**Table S4** Primers used for amplification, cloning and sequencing of *Solyc08g077740* genes from Tristar and M82.

**Table S5** Cleaved amplified polymorphic sequence (CAPS) and sequence characterised amplified region (SCAR) markers used to authenticate *Solanum pennellii* LA716 chromosome 8 introgression lines IL8-1, IL8-2 and IL8-3.