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Elicitin recognition confers enhanced resistance to *Phytophthora infestans* in potato

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Potato late blight, caused by the destructive Irish famine pathogen *Phytophthora infestans*, is a major threat to global food security[1,2]. All late blight resistance genes identified to date belong to the coiled-coil, nucleotide-binding, leucine-rich repeat class of intracellular immune receptors[3]. However, virulent races of the pathogen quickly evolved to evade recognition by these cytoplasmic immune receptors[4]. Here we demonstrate that the receptor-like protein ELR (elicitin response) from the wild potato *Solanum microdontum* mediates extracellular recognition of the elicitin domain, a molecular pattern that is conserved in *Phytophthora* species. ELR associates with the immune co-receptor BAK1/SERK3 and mediates broad-spectrum recognition of elicitin proteins from several *Phytophthora* species, including four diverse elicitins from *P. infestans*. Transfer of ELR into cultivated potato resulted in enhanced resistance to *P. infestans*. Pyramiding cell surface pattern recognition receptors with intracellular immune receptors could maximize the potential of generating a broader and potentially more durable resistance to this devastating plant pathogen.

Potato (*Solanum tuberosum* L.) is the most important non-grain food crop and a major source of calories for the world's poor[5]. Increasing potato production is critical to prevent global malnutrition and hunger in an era of expanding world population. Unfortunately, potato suffers from the devastating late blight disease, which is caused by the notorious oomycete pathogen *Phytophthora infestans*. To limit losses to late blight, potato breeders rely on fungicide treatment and breeding of disease resistance (*R*) genes, all of which identified thus far belong to the coiled-coil, nucleotide-binding, leucine-rich repeat (CC-NB-LRR) class of immune receptors. These intracellular proteins recognize pathogen avirulence (*Avr*) proteins of the RxLR class of effectors to mount defence responses. However, RxLR effectors display high evolutionary rates[4], and as a result, *P. infestans* can rapidly circumvent recognition by intracellular R immune receptors, thereby limiting the development of sustainable and durable genetic resistance. Therefore, novel types of immune receptors that recognize a broader spectrum of pathogen molecules are needed.

To fend off pathogens, plants rely on two classes of immune receptors that either reside inside the plant cell (NB-LRRs) or on the cell surface. The first line of defence is initiated by surface receptors, also called pattern recognition receptors (PRRs). PRRs are characteristically receptor-like proteins (RLPs), such as Ve1/Ve2[6], Cfs[7,8] and LeEIX1[9], or receptor-like kinases (RLKs), such as FLS2, EFR and XA21[10]. PRRs typically recognize conserved pathogen-associated molecular patterns (PAMPs)[11]. So far, only a relatively few cell surface receptors against agronomically important pathogens have been identified.

Elicitins are structurally conserved extracellular proteins in *Phytophthora* and *Pythium* pathogen species (Pfam PF00964)[12–14]. *P. infestans* contains six elicitin genes that are conserved among different strains[13,15]. Elicitins are recognized as oomycete PAMPs but their intrinsic function in oomycetes is to bind lipids. Some elicitins sequester sterols from plants, thereby fulfilling an important biological function in *Phytophthora* and *Pythium* species that cannot synthesize sterols[12]. Targeting such conserved 'Achilles heel' proteins of pathogens is expected to lead to a more broad-spectrum resistance.

To identify novel types of potential immune receptors against the potato late blight pathogen, we initiated the cloning of *ELR*, a gene that determines response to elicitins[16]. We screened a collection of wild *Solanum* germplasm by *Potato virus X* (PVX) agroinfection for responses to INF1, a secreted elicitin of *P. infestans* (Fig. 1a). *Solanum microdontum* genotype mcd360-1 consistently responded to INF1 with a cell death response (Fig. 1b). We crossed mcd360-1 with *S. microdontum* ssp. *gigantophyllum* gig714-1 that does not respond to INF1. The F1 population segregated for response to INF1 in a 1:1 ratio, which suggests that ELR is a single dominant gene.

Genetic mapping placed ELR on the south arm of chromosome 12 of potato, in a region corresponding to contig 167 in the reference potato genotype RH (Supplementary Fig. 1a and Supplementary Table 1). BAC end sequences were used to develop CAPS markers, Rhl8 and Rhr0 of RH[18,19], and a co-localizing tomato BAC clone sequence led to marker LBC (Fig. 1c and Supplementary Fig. 1b). The RH contig 167 spans the ELR locus and contains sequences with homology to RLPs (Supplementary Fig. 1c). By screening a BAC library of mcd360-1 with Rhl8, Rhr0 and LBC, four BAC clones that cover a major part of the Rhl8-Rhr0 interval were identified (Supplementary Fig. 1d). Sequencing of these BACs revealed a cluster of 13 RLPs. Further fine mapping

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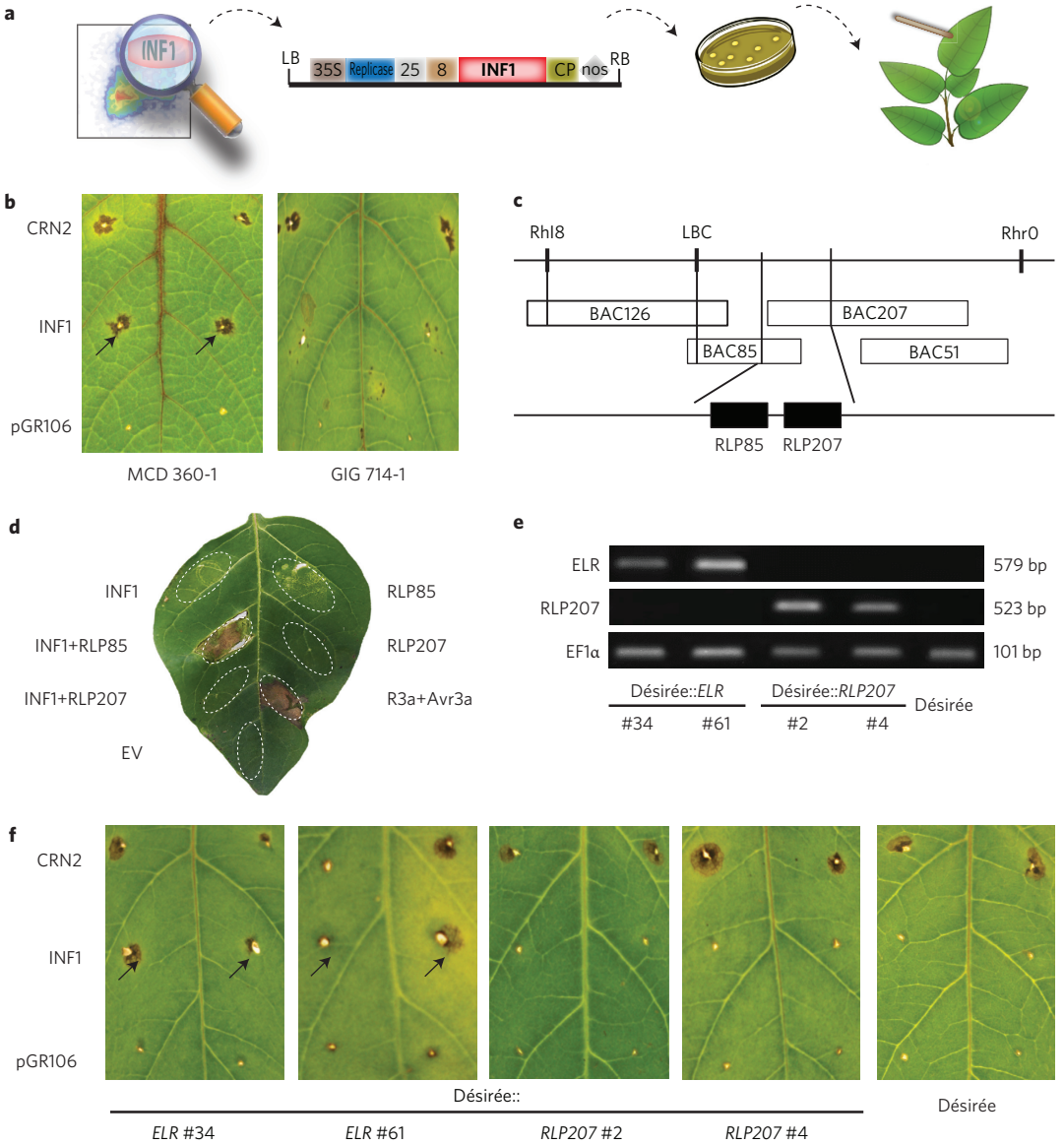

Figure 1 | *Solanum microdontum* ELR confers response to elicitins. a, ELR was identified using effector-based phenotyping[16]. *Inf1* elicitin of *Phytophthora infestans* was transiently expressed by PVX agroinfection in potato germplasm. **b**, *Solanum microdontum* mcd360-1 shows cell death response to INF1 (arrows), *S. microdontum* ssp. *gigantophyllum* gig714-1 does not. CRN2[17], a common cell death inducing gene from *P. infestans*, and the empty pGR106 vector were included as positive and negative controls, respectively. **c**, Genetic mapping places the gene conferring elicitin response (ELR) on chromosome 12 between flanking markers RhI8/LBC and Rhr0. RLP85 and RLP207 (see Supplementary Fig. 1 for more details) are identified as candidate genes. **d**, Complementation test in *Solanum hjertingii*. Agro-co-infiltration of INF1 with 35S-RLP85 or with 35S-RLP207 shows that RLP85 induces a specific response to INF1. Photo was taken at 3 days post inoculation. **e**, Expression of ELR (RLP85) and RLP207 in transgenic potato cv Désirée. RT-PCR analysis shows that *ELR* is specifically expressed in the Désirée-ELR transformants ELR#34 and ELR#61, and *RLP207* in the Désirée-RLP207#2 and RLP270#4. **f**, Complementation test with PVX-INF1 on transgenic potato cv Désirée. Two Désirée transformants expressing 35S-ELR (ELR#34, ELR#61) show specific cell death responses to pGR106-INF1 (arrows), two 35S-RLP207 transformants (RLP207#2, #4) and control Désirée do not show responses to INF1.

ultimately resulted in two candidate genes, RLP85 and RLP207 (Fig. 1c and Supplementary Fig. 1e).

To determine whether RLP85 or RLP207 confer recognition of INF1, transient expression assays were performed in *Solanum hjertingii* genotype hjt349-3. *Agrobacterium tumefaciens*-mediated co-expression (agroinfiltration) of RLP85 with INF1 led to cell death, whereas RLP207 did not (Fig. 1d). We designated RLP85 as ELR. We generated stable transgenic potato cultivar Désirée plants expressing ELR (RLP85) and RLP207 (Fig. 1e) and subjected the regenerants to PVX agroinfection with INF1. Désirée-ELR transgenic plants, but not Désirée-RLP207, showed specific cell death to PVX-INF1 (Fig. 1f). The *ELR* gene has an ORF encoding a protein of 1,094 amino acids predicted to contain a signal peptide, 36 extracellular LRR domains, a transmembrane domain and a short cytoplasmic tail (Supplementary Fig. 2). The ELR protein shares between 28% and 36% amino acid sequence identity with tomato RLP proteins implicated in immunity to fungal pathogens, namely Cf4/Cf9, Cf2/Cf5, Ve1 and LeEix1. In the reference genome of potato[19], the *ELR* gene is not present but there are *ELR*-like homologues that lack LRR repeats (Supplementary Fig. 3). The extent to which these homologues affect response to INF1 is unknown, however reference potato DM1-3 516 R44 plants do not show cell death to INF1 similar to most potato cultivars tested so far[16].

To study the recognition spectrum of ELR, we explored its ability to induce cell death response to a broad range of elicitins of

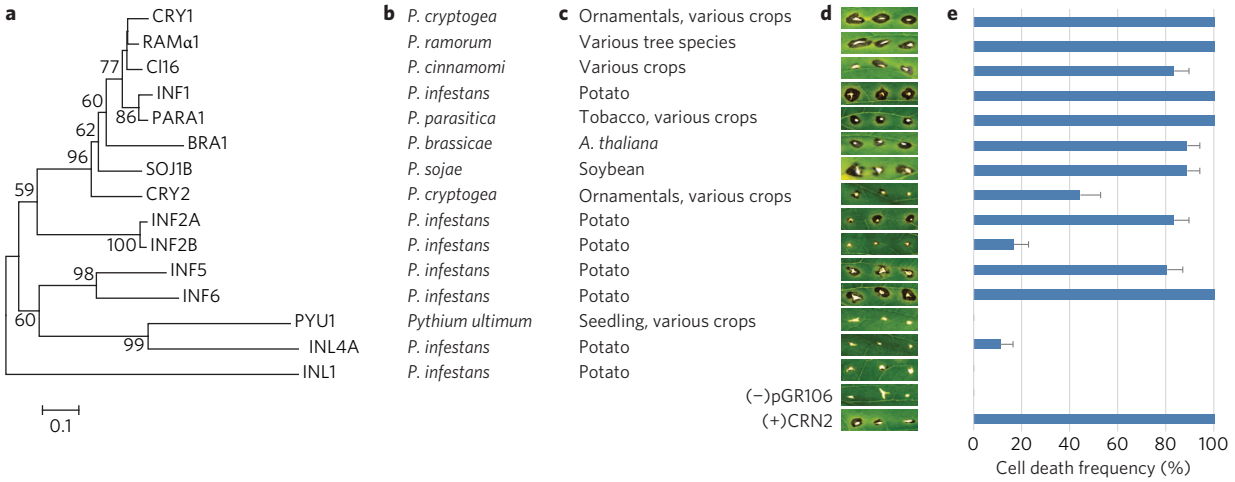

Figure 2 | ELR mediates broad-spectrum response to elicitins of oomycetes. a, Neighbour-joining tree of elicitins. The tree was based on the 98-amino-acid elicitin domain that is conserved in oomycete elicitins. Bootstrap values exceeding 50% are indicated at the nodes, branch lengths represent weighted amino acid substitutions. **b**, The tested elicitins belong to oomycete species, including various *Phytophthora* species and *Pythium ultimum*. **c**, Host plants. **d**, PVX agroinfection with pGR106-elicitins in potato transformant Désirée ELR#34, representative photographs of triplicate inoculations. The empty pGR106 vector and pGR016-CRN2 were used as negative and positive control, respectively. **e**, Frequency of cell death responses upon PVX agroinfection. Error bars represent s.e.m. ($n = 36$).

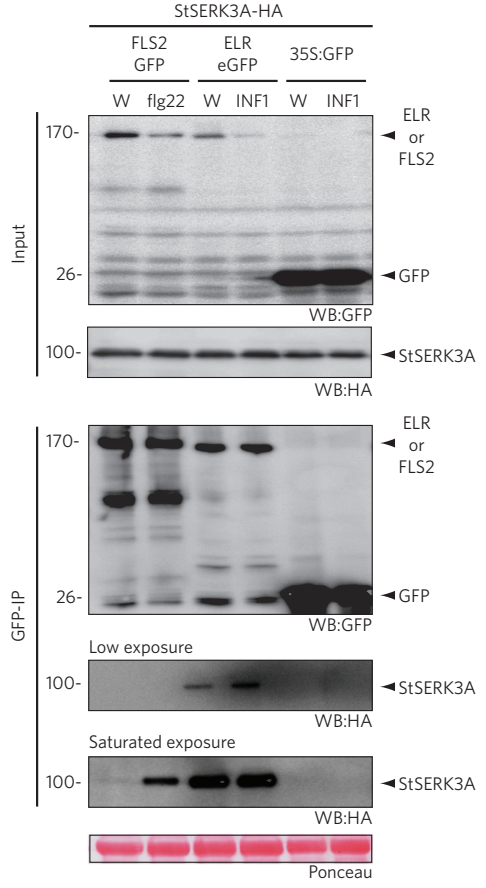

Figure 3 | ELR associates with the immune co-receptor BAK1/SERK3. ELR and FLS2 co-immunoprecipitate with StSERK3A and the association is enhanced upon elicitor treatment. FLS2-GFP or ELR-eGFP was transiently co-expressed with StSERK3A-HA in *N. benthamiana*. Leaves were treated with water (W), 100 nM flg22 or 10 μg/ml INF1 elicitin for 15 min. Proteins were extracted, enriched for membrane proteins, subjected to immunoprecipitation (IP) with anti-GFP and detected with anti-GFP or anti-HA antibodies. Ponceau stain of the total extract blot indicates equal loading (bottom).

oomycetes that infect various host plants (Fig. 2a–c). We tested six *Inf* elicitins of *P. infestans*, two elicitin-likes, seven elicitins from six other *Phytophthora* species and one from *Pythium ultimum* (Supplementary Table 2). PVX agroinfection in Désirée-ELR#34 showed high (>40%) frequencies of cell death for four elicitins, that is INF1, INF2A, INF5 and INF6 of *P. infestans* and to eleven elicitins of diverse other *Phytophthora* species (Fig. 2d). This broad spectrum of elicitin recognition by ELR was confirmed by agroinfiltration assays (Supplementary Fig. 4). Remarkably, the amino acid sequence of the four recognized elicitins of *P. infestans* is diverse, ranging from as low as 45–65% sequence identity (Supplementary Fig. 5). In addition, these four elicitins are all expressed during infection in all examined *P. infestans* strains, suggesting that they are potentially recognized by ELR during host colonization (Supplementary Fig. 6). These data show that ELR induces defence responses upon recognition of a broad spectrum of elicitins, even extending beyond the species of *P. infestans*. Although cell death is not characteristic for PAMP-triggered immunity, this strong phenotype might be caused by high expression in the transient assays. Altogether ELR seems to be a good candidate as a typical PRR, although the binding to elicitins still needs to be demonstrated.

A central regulator of cell surface-mediated immunity in plants is the BRI1 associated kinase 1 (BAK1), also known as SERK3 in solanaceous plants. This cell surface protein undergoes complex formation with PRRs on ligand binding[20,21]. Response to INF1 is dependent on BAK1/SERK3 in tobacco[22]. Co-immunoprecipitation experiments revealed that various GFP-tagged ELR fusion proteins associate with BAK1/SERK3 homologues from potato and Arabidopsis (Fig. 3 and Supplementary Fig. 7). In five experiments out of ten, the association of ELR with BAK1/SERK3 was mildly enhanced after elicitation with INF1, although not to the degree observed with FLS2 association with BAK1/SERK3 after treatment with flagellin flg22 (Supplementary Table 3)[20]. Control experiments showed that ELR-eGFP was still able to induce INF1-mediated cell death (Supplementary Fig. 8). eGFP- and GFP-tagged ELR localized to the plasma membrane *in planta* similar to other RLPs, such as LeEIX[23] (Supplementary Fig. 9). These experiments indicate that ELR most likely functions at the cell surface where it associates with BAK1/SERK3 to activate defence responses.

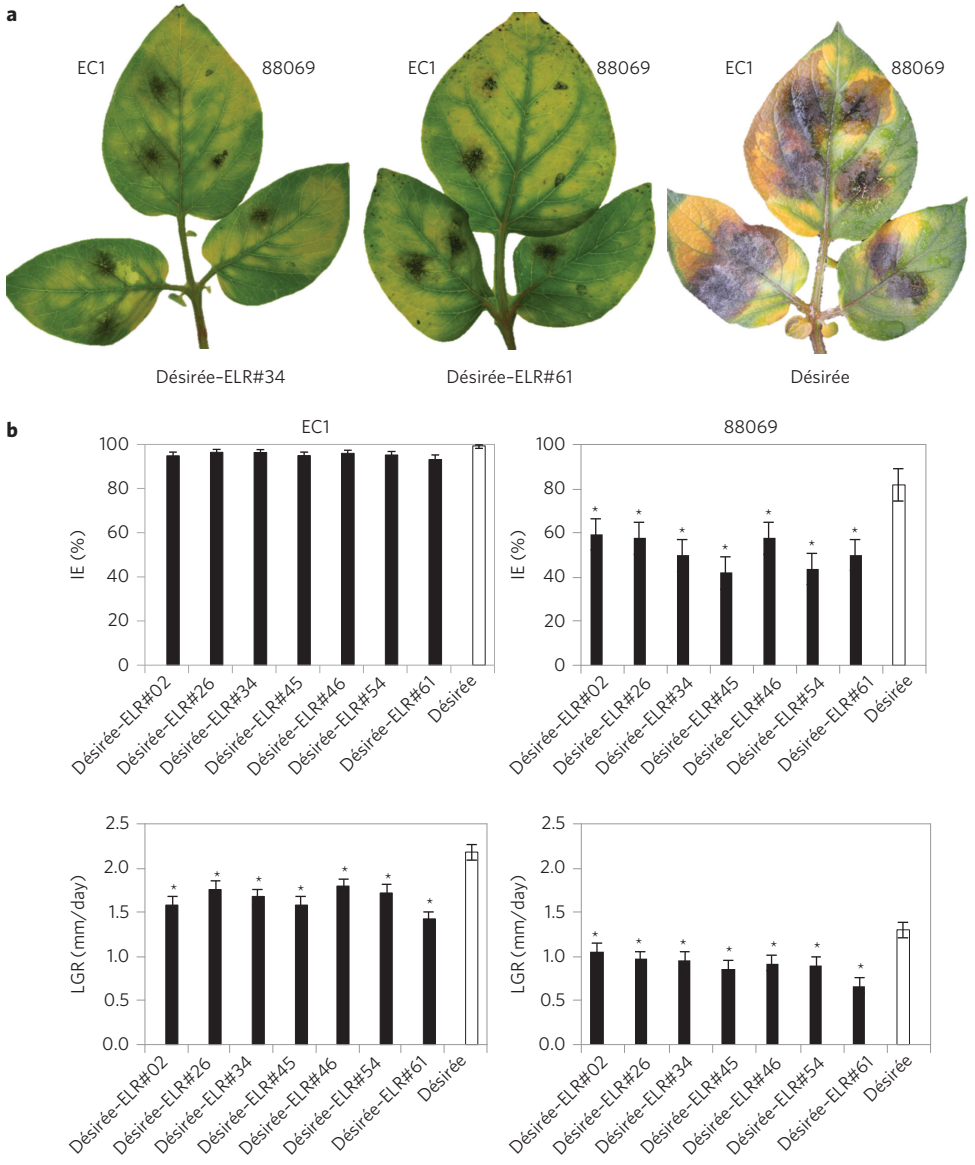

Figure 4 | ELR confers enhanced resistance to *P. infestans* in potato. Désirée-ELR transformants display lower lesion growth rates (LGRs) and lower infection efficiencies (IE) than Désirée control. Leaves were spot-inoculated with two *P. infestans* strains EC1_DC2005 (EC1) and 88069 on the left or right side of the mid vein, respectively. **a**, Representative photographs are shown for Désirée control, Désirée-ELR#34 and Désirée-ELR#61. **b**, LGR was significantly lower (marked with *) in all seven Désirée-ELR transformants (black bars), compared to Désirée controls (white bars), for both tested *P. infestans* strains (Supplementary Tables 4–6). The least aggressive *P. infestans* strain 88069 shows fewer lesions in Désirée-ELR transformants. The average data from three independent experiments, each based on 40 inoculation spots per plant/isolate combination, are presented. Error bars represent SE for LGR (Mixed Model, $n = 3$) and IE (Generalized Linear Mixed Model $n = 3$).

To test whether ELR enhances resistance to *P. infestans*, we subjected Désirée and seven Désirée-ELR transformants to disease tests. Detached leaves of potato plants were spot-inoculated with zoospore suspensions of two *P. infestans* strains, 88069 and EC1_DC2005. Lesion sizes were measured from three to six days after inoculation, and lesion growth rates (LGRs) were statistically analysed. For both *P. infestans* strains, lesions expanded at slower rates in the Désirée-ELR transformants compared to Désirée and Désirée-RLP207 control plants (Fig. 4 and Supplementary Tables 4–6). Independent assessment of pathogen colonization with a *P. infestans* strain 88069td, expressing a variant of the red fluorescent protein, confirmed that ELR confers enhanced resistance to *P. infestans* strains (Supplementary Fig. 10).

Our study demonstrates that ELR has the potential to contribute to disease resistance of agronomically important crops. ELR mediates the recognition of multiple elicitins, a family of evolutionary conserved proteins under purifying selection, which sharply contrast with the rapidly evolving RxLR effectors that are recognized by NB-LRR immune receptors[4,13,24]. Breeding potatoes with immune receptors that target conserved proteins, such as elicitins, is expected to maximize the potential for disease resistance durability. An attractive approach would be to pyramid the layer of broad-spectrum quantitative resistance conferred by surface proteins, such as ELR, with cytoplasmic NB-LRR receptors that provide a higher degree but narrower spectrum of resistance. In addition, given that ELR participates in the perception of the elicitin molecular pattern of a wide range of *Phytophthora* species, this receptor-like protein could potentially enhance disease resistance to a number of oomycete plant pathogens, many of which are severe threats to a variety of crops and to world food security[25].

Methods

Methods and any associated references are available in the Supplementary Information.

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Author contributions

J.D., E.V., A.C.G., G.B., L.C.P., J.Z. and V.G.A.A.V. performed experiments and analysed data; T.W.H.L. and F.G. contributed constructs; J.D., E.V., A.C.G., E.A.G.v.d.V., C.X., J.Z., S. R., S.K. and V.G.A.A.V. designed experiments; E.A.G.v.d.V., C.X., S.R., S.K., V.G.A.A.V., E. J. and R.G.F.V. supervised; J.D., E.V., A.C.G., S.K. and V.G.A.A.V. wrote the manuscript.

Additional information

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Competing interests

The authors declare no competing financial interests.