Plant Biotechnology Journal
Open Access

Plant Biotechnology Journal (2023) **21**, pp. 2157–2159 doi: 10.1111/pbi.14127

Brief Communication

Mutation of *PUB17* in tomato leads to reduced susceptibility to necrotrophic fungi

Miguel Ramirez Gaona[1], Ageeth van Tuinen[1], Danny Schipper[1], Akihito Kano[2], Pieter J. Wolters[1], Richard G. F. Visser[1], Jan A. L. van Kan[3], Anne-Marie A. Wolters[1] and Yuling Bai[1,*]

[1]*Plant Breeding, Wageningen University & Research, Wageningen, The Netherlands*
[2]*Plant Breeding and Experiment Station, Takii & Company Limited, Konan, Japan*
[3]*Laboratory of Phytopathology, Wageningen University & Research, Wageningen, The Netherlands*

Received 7 March 2023;
revised 8 June 2023;
accepted 7 July 2023.
**Correspondence* (Tel +31 317 482136; email bai.yuling@wur.nl)

Keywords: tomato, PUB17, susceptibility gene, necrotroph, *Botrytis cinerea*, *Alternaria solani*.

Necrotrophic fungi, such as *Botrytis cinerea* and *Alternaria solani*, cause severe damage in tomato production. There is no report of any single resistance (R) gene that can provide dominant resistance against necrotrophic fungi (Davis *et al*., 2009; Finkers *et al*., 2007). In this study, we demonstrate that breeding tomato for broad-spectrum resistance towards necrotrophic fungi can be achieved by editing host susceptibility (S) genes.

Screening a tomato Micro-Tom (MT) EMS population (Yan *et al*., 2021) we identified mutant M2042 showing a 20%–30% reduction in lesion diameter after *B. cinerea* infection when compared to the wild-type MT control in a detached leaf assay (DLA). A bulk segregant analysis and whole genome sequencing (BSA-seq) approach was applied in the F2 population derived from the cross of M2042 and Moneymaker (MM, susceptible to *B. cinerea*; Methods S1, Figures S1 and S2). Two potential non-synonymous mutations were identified. The first mutation (A → T SNP) was at position 1477 of the coding region of gene Solyc02g072080 resulting in a premature stop codon R493* (Figure 1a). This gene is the tomato orthologue of *PUB17*, a conserved U-box-containing E3 ubiquitin ligase (Trenner *et al*., 2022). The second mutation (G → A SNP) occurred in exon 5 of gene Solyc02g078920 resulting in an amino acid change G158D. This gene encodes an S1/P1 nuclease. Of the two candidate genes, only the expression of *PUB17* was altered in the mutant at 24 and 48 h after *Botrytis* inoculation (Figure 1b). Further fine mapping showed that the SNP in *PUB17* co-segregated with the resistance (Figure 1c).

To confirm that the *PUB17* gene is an S gene to *B. cinerea*, knock-down and out transformants were produced using RNAi and CRISPR (Table S1). In both DLA and stem assays, significantly reduced susceptibility was observed in T3 well-silenced plants of three independent RNAi transformants (Figures S3 and S4) generated by transforming MM with RNAi constructs (Figure 1a). For CRISPR analysis, a construct with 4 sgRNAs targeting the different protein domains were made (Figure 1a) to transform MM. Homozygous mutant T3 progeny could be obtained from T1 transformants 7 (mutant allele 1 and 2) and 36 (mutant allele 3 and 4; Figure 1a, Figures S5 and S6). For each mutant allele, two T3 families were tested with *B. cinerea* in both stem assay and a DLA. All T3 families exhibited significantly lower disease severity compared with MM and CRISPR T2 family TV33 containing wild-type *PUB17* alleles (Figure 1d, Figure S4). A multiple sequence alignment of the predicted PUB17 proteins (Figure S7) revealed that mutations of all the mutant alleles led to partial deletion of the Armadillo repeats (ARM; Figure 1e). Additional to *B. cinerea*, the *pub17* mutants (both EMS and CRISPR) showed significantly reduced lesion diameters after *A. solani* infection, when compared to the corresponding controls (MM or MT; Figure 1f). Meanwhile, no increased susceptibility was observed in these *pub17* mutants when tested with obligate biotrophic tomato powdery mildew pathogen *Pseudoidium neolycopersici* (Figure S8).

The original EMS-mutant plant M2042 had slightly smaller leaves than wild-type MT, and the leaves were slightly wrinkled. To assess the breeding value of the EMS *pub17* mutation, we introgressed the EMS-mutant allele into different tomato breeding lines (Figure S9). Results demonstrated that occurrence of autonecrosis of *pub17* mutants depends on the genetic background. We succeeded in producing F1 hybrids carrying homozygous *pub17* alleles with a plant phenotype and fruit setting indistinguishable from the F1 without the *pub17* mutation.

In this study, we report for the first time that *SlPUB17* operates as an S gene during plant interaction with necrotrophic pathogens. The Arabidopsis *PUB* family consists of 64 *PUB* genes classified into seven classes and their encoded proteins contain a highly conserved U-box domain along with various additional domains (Trenner *et al*., 2022). *PUB17* belongs to Class V containing a U-box N-terminal domain (UND) at the N-terminus and ARM repeats at the C-terminus (Figure 1a). The individual EMS- and CRISPR-induced mutations resulted in the loss of multiple ARM repeats for all mutant alleles (Figure 1e). ARM repeats participate in protein–protein interactions (Samuel *et al*., 2006). The loss of these repeats would hamper the interaction of proteins associated with PUB17 and consequently the ubiquitination of proteins targeted by PUB17.

Previous studies on *PUB17* homologues of *Arabidopsis thaliana*, *Nicotiana tabacum*, potato and cotton showed that PUB17 is a positive regulator of immunity against different diseases caused by biotrophic and hemibiotrophic pathogens, such as *Cladosporium fulvum*, *Phytophthora infestans* and *Verticillium dahliae* (Ni *et al*., 2010; Tian *et al*., 2007; Yang *et al*., 2006; Zhang *et al*., 2016). In contrast, our results revealed a role of PUB17 as an S gene for necrotrophic pathogens by acting as a negative regulator of immunity against *B. cinerea* and *A. solani*. Given that the *SlPUB17*

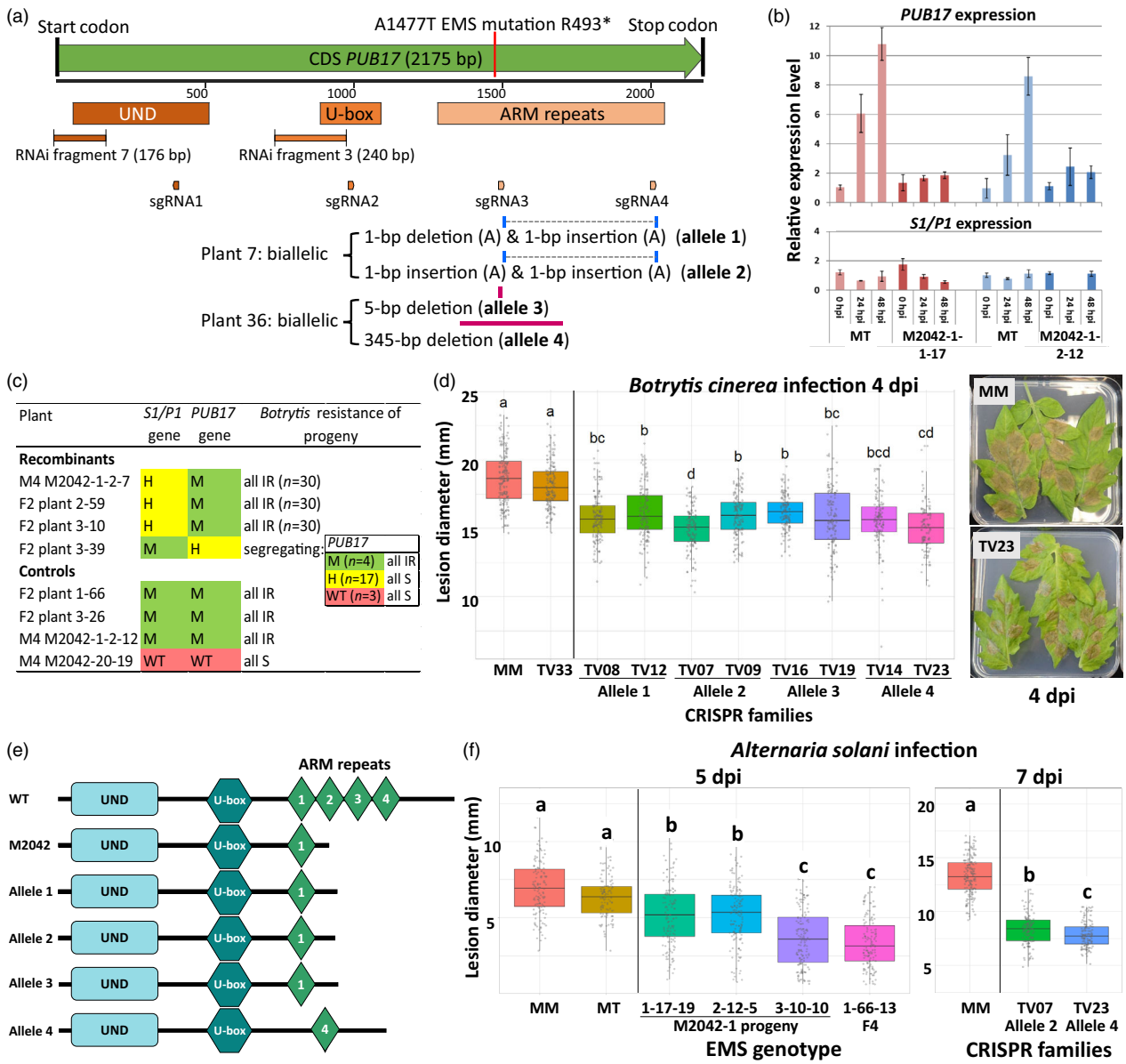

Figure 1 *PUB17* is a susceptibility gene for necrotrophic fungi. (a) Mutant candidate gene *PUB17*. Tomato *PUB17* (Solyc02g072080) has three domains: U-box N-terminal domain UND, U-box and ARM armadillo repeats. Positions of RNAi fragments and four sgRNAs for CRISPR editing are indicated. CRISPR-induced mutations are shown. (b) Relative expression levels of candidate genes *PUB17* and *S1/P1 nuclease* in wild-type Micro-Tom (MT) and mutant plants M2042-1-1-17 and M2042-1-2-12 at 0, 24 and 48 h post-inoculation (hpi) with *Botrytis cinerea*. (c) Resistance of progenies of M2042 M4 and F2 recombinant plants and controls. H, heterozygous with one WT allele and one mutant allele; IR, intermediate resistant; M, homozygous for the mutant allele; S, susceptible; WT, homozygous for the MT allele. (d) Boxplot of *B. cinerea* lesion diameters (in mm) on T3 CRISPR families at 4 dpi (days post-inoculation) on leaves from *PUB17* CRISPR mutant T3 families, compared with negative controls MM and non-mutant T2 family TV33. (e) Protein domains in mutant *PUB17* alleles. WT, wild-type tomato PUB17 protein; M2042, EMS *PUB17* mutant allele; allele 1–4, CRISPR *PUB17* mutant alleles. (f) Boxplots of *Alternaria solani* lesion diameters on tomato leaves. Left, 5 dpi on leaves of MM and MT and EMS-induced mutant *PUB17* M5 families M2042-1-1-17-19, M2042-1-2-12-5, M2042-1-3-10-10 and in F4 family 1–66-13 (Figure S2 for pedigree). Right, at 7 dpi on leaves from *PUB17* CRISPR mutant T3 families TV07 and TV23. Different letters in (d) and (f) above the boxplots indicate significant differences ($P < 0.05$, Tukey HSD method).

mutants exhibit lower susceptibility to necrotrophic pathogens manifested as smaller lesion diameters, we deduce that PUB17 operates as a positive regulator of programmed cell death (PCD). The role of PUB17 in the PCD pathway has yet to be confirmed, since the specific target(s) for degradation by the ubiquitin ligase PUB17 remain unknown. Yet, as several necrotrophic pathogens rely on (programmed) cell death to facilitate growth, it is plausible to propose that the resistance observed in *PUB17* mutants to *Botrytis* and *Alternaria* could be linked to interference with PCD.

Since S genes are shown to be conserved across different plant species (Koseoglu *et al.*, 2022), we expect that the identified *PUB17* gene might be explored in a range of crops for resistance to *B. cinerea* and *A. solani* by induced mutations via mutagenesis,

RNAi (easy for polypoid crops) and especially gene-editing with CRISPR.

Acknowledgements

This research was financially supported by grants from Foundation Topconsortium voor Kennis en Innovatie (TKI) Horticulture & Starting Materials projects EZ-2012-07 and TU-18015. We thank Fien Meijer-Dekens and Bertus van der Laan for taking care of the plants in the greenhouse. Thanks also to Alejandro Thérèse Navarro for his guidance in R programming.

Competing interests

None declared.

Author contributions

YB, AMAW, JALvK and RGFV conceived the study. AvT performed the screening of the EMS population. MRG, DS, AvT and PJW performed the experiments. PJW and JALvK provided disease testing resources and protocols. MRG, AMAW and YB analysed the results. AK participated in advanced material production. MRG wrote the manuscript with input from all co-authors.

Data availability

The data used to support the findings of this study are available in the main text and Supporting information of this article.

References

Davis, J., Yu, D., Evans, W., Gokirmak, T., Chetelat, R.T. and Stotz, H.U. (2009) Mapping of loci from *Solanum lycopersicoides* conferring resistance or susceptibility to *Botrytis cinerea* in tomato. *Theor. Appl. Genet.* **119**, 305–314.

Finkers, R., van den Berg, P., van Berloo, R., ten Have, A., van Heusden, A.W., van Kan, J.A.L. and Lindhout, P. (2007) Three QTLs for *Botrytis cinerea* resistance in tomato. *Theor. Appl. Genet.* **114**, 585–593.

Koseoglu, E., van der Wolf, J.M., Visser, R.G.F. and Bai, Y. (2022) Susceptibility reversed: modified plant susceptibility genes for resistance. *Trends Plant Sci.* **27**, 69–79.

Ni, X., Tian, Z., Liu, J., Song, B., Li, J., Shi, X. and Xie, C. (2010) StPUB17, a novel potato UND/PUB/ARM repeat type gene, is associated with late blight resistance and NaCl stress. *Plant Sci.* **178**, 158–169.

Samuel, M.A., Salt, J.N., Shiu, S.-H. and Goring, D.R. (2006) Multifunctional arm repeat domains in plants. *Int. Rev. Cytol.* **253**, 1–26.

Tian, M., Win, J., Song, J., van der Hoorn, R., van der Knaap, E. and Kamoun, S. (2007) A *Phytophthora infestans* cystatin-like protein targets a novel tomato papain-like apoplastic protease. *Plant Physiol.* **143**, 364–377.

Trenner, J., Monaghan, J., Saeed, B., Quint, M., Shabek, N. and Trujillo, M. (2022) Evolution and functions of plant U-box proteins: from protein quality control to signaling. *Annu. Rev. Plant Biol.* **73**, 93–121.

Yan, Z., Appiano, M., van Tuinen, A., Meijer-Dekens, F., Schipper, D., Gao, D., Huibers, R. *et al*. (2021) Discovery and characterization of a novel tomato *mlo* mutant from an EMS mutagenized Micro-Tom population. *Genes*, **12**, 719.

Yang, C.-W., González-Lamothe, R., Ewan, R.A., Rowland, O., Yoshioka, H., Shenton, M., Ye, H. *et al*. (2006) The E3 ubiquitin ligase activity of *Arabidopsis* PLANT U-BOX17 and its functional tobacco homolog ACRE276 are required for cell death and defense. *Plant Cell*, **18**, 1084–1098.

Zhang, H., Zhang, W., Jian, G., Qi, F. and Si, N. (2016) The genes involved in the protective effects of phytohormones in response to *Verticillium dahliae* infection in *Gossypium hirsutum*. *J. Plant Biol.* **59**, 194–202.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Methods S1 Materials and methods.
Figure S1 Pedigree of M2042 EMS mutant.
Figure S2 Results from the BSA-seq analysis.
Figure S3 Analysis of *PUB17* RNAi transformants.
Figure S4 *Botrytris cinerea* stem assay results of *PUB17* transformants.
Figure S5 Analysis of *PUB17* CRISPR transformants.
Figure S6 Multiple genomic sequence alignment of *Solanum lycopersicum* wild-type and mutant *PUB17* alleles.
Figure S7 Multiple protein sequence alignment of *Solanum lycopersicum* wild-type and mutant *PUB17* alleles.
Figure S8 Powdery mildew disease scoring results of *PUB17* CRISPR mutants.
Figure S9 EMS-mutant p*ub17* pre-breeding results.
Table S1 List of primers.