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## Brief Communication

## Immune-enhancing miPEPs reduce plant diseases and offer new solutions in agriculture

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\*Correspondence (Tel +33 (0)5 34 32 38 11; fax +33 (0)5 34 32 38 02; email [jean-philippe.combier@univ-tlse3.fr](mailto:jean-philippe.combier@univ-tlse3.fr))<sup>a</sup>Present address: Epigenetics and Plant Development, Centre for Research in Agricultural Genomics (CRAG), UAB, Barcelona, Spain<sup>b</sup>Present address: The Department of Biology, The Center for Genomics and Systems Biology, New York University, New York, USA<sup>†</sup>These authors contributed equally to the work.**Keywords:** microRNAs, miPEPs, peptides, plant defence, pathology.

The biggest challenge for global agriculture today is to combat pathogens, especially fungi, with fewer and fewer active molecules available. In fact, fungi are responsible for 10%–20% of crop losses, equivalent to a loss of up to \$200 billion per year ([www.ars.usda.gov](http://www.ars.usda.gov)). Moreover, the use of current fungicides (mainly chemicals) is often associated with health and environmental issues ([Fungicide\\_Uses\\_and\\_Risks](#)). Finally, fungi are becoming increasingly resistant to the fungicides currently used ([Fungicide\\_Resistance\\_Management](#)). In this context, the identification of new compound families controlling fungi in agriculture is of critical importance to maintain or increase global crop yields. Diseases can be managed in different ways: by direct toxicity to fungi to control fungal growth and reproduction ([Fungicides](#)) or by enhancing the plant defence response using elicitors.

MiPEPs are short peptides encoded by primary transcripts of miRNAs and activate their transcription (Lauressergues *et al.*, 2015). Due to their straightforward application through irrigation or spraying of plants with synthetic peptides, miPEPs appear as a credible alternative to chemicals in agronomy by improving crop growth and reducing weed growth (Ormancey *et al.*, 2021).

Here, we used the full set of miPEPs corresponding to conserved miRNAs (from miR156 to miR399) to identify those that can enhance *PR1* expression, a gene induced in response to several pathogens, and commonly used as a marker of plant defence responses (Venegas-Molina *et al.*, 2020). We treated *Arabidopsis thaliana* seedlings expressing *PR1* promoter fused to the  $\beta$ -glucuronidase (*GUS*) with each of the 89 miPEPs (Lauressergues *et al.*, 2022), in the absence of any elicitor or pathogen, and quantified *GUS* activity. Out of the 89 miPEPs tested, 17 miPEPs were able to significantly decrease *PR1*

expression, and three, miPEP169c, miPEP169h and miPEP396b, were able to increase *PR1* expression (Figure 1a; Figure S1a). In order to know whether this ability to induce *PR1* expression was correlated with a better resistance to pathogens, we inoculated *A. thaliana* plants with the necrotrophic fungus *Botrytis cinerea* and treated them in parallel with the three active miPEPs. Interestingly, the different miPEPs were able to decrease fungal colonization, without any direct toxicity of the peptides on fungal spores (Figure 1b; Figures S1b and S2a). To go further, we validated that each miPEP was able to up-regulate the expression of their pri-miRNA (Figures S1c–e). Then, we measured the expression of several plant defence marker genes in response to each of the miPEPs using qRT-PCR (Table S1). The expression of four marker genes was significantly induced by the three miPEPs (Figure 1c; Figure S2): *PDF1.1* and *PDF1.2* for miPEP169c, *RD20* for miPEP396b and *OSM34* for miPEP169h. Interestingly, these four genes are known to be involved in antifungal responses. *PDF1.1* and *PDF1.2* are defensins involved in resistance to pathogens (De Coninck *et al.*, 2010), *RD20* is a peroxxygenase controlling cell death (Hanano *et al.*, 2015) and *OSM34* belongs to the osmotin family involved in cell death (Hakim *et al.*, 2018). While the direct target genes of miR169 and miR396 are well known, the signalling pathways controlled by them are not well-characterized (Figure S3).

To know whether the functions of these miPEPs are conserved among plants, we identified the entire miR169 and miR396 families in three crops: strawberry, bean and tomato, and identified their miPEPs (Table S2). Treatment of plants with these peptides allowed us to identify miPEPs reducing the incidence of *B. cinerea* infection in the three plant species (Figure 1d; Figures S4 and S5).

Our analysis reveals very distinct roles of the different miR169 and miR396 members in these plant species (Figure S4). While some members reduce fungal infection, some others increase plant susceptibility. This explains the conflicting roles of miR169 across different species, promoting (Song *et al.*, 2018), or decreasing plant resistance (Soto-Suárez *et al.*, 2017). Hence, our analysis highlights the requirement to study each member of miRNA families to fully understand miRNA biological roles.

We then focussed on the most active miPEP in tomato, that is SlmiPEP169d, which is potentially the closest functional homologue of AtmiPEP169c-h. We tested whether this miPEP could protect tomato against other pathogens: *Alternaria solani*, a fungus responsible for early blight, and *Pseudomonas syringae* pv.

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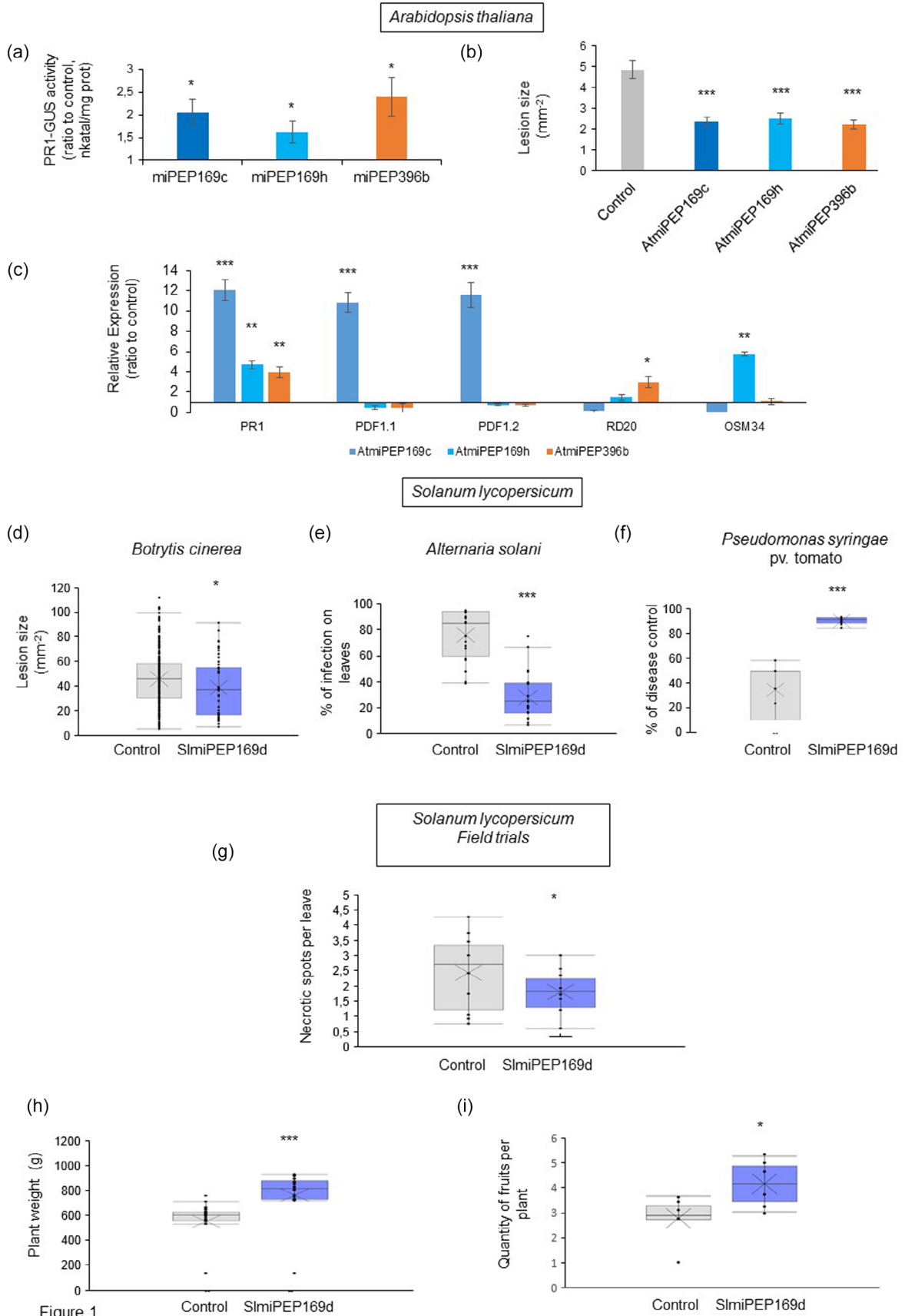


Figure 1

**Figure 1** Identification of miPEPs eliciting plant defence. (a) PR1-GUS activity of *Arabidopsis thaliana* seedlings treated with the indicated miPEP compared with seedlings treated with a control (Figure S1a). (b) Lesion size of *A. thaliana* plants inoculated with *Botrytis cinerea* spores and treated with the indicated peptide. (c) Ratio of relative expression of marker genes in *A. thaliana* plants treated with the indicated peptide compared with the expression in plants treated with a control. (d) Lesion size of tomato plants inoculated with *B. cinerea* spores and treated with the indicated miPEP compared with lesion size in plants treated with a control (Figure S3). (e) Percentage of infection of *Alternaria solani* on leaves treated with the indicated peptide. (f) Percentage of disease control of *Pseudomonas syringae* pv. tomato inoculated tomato plants treated with the indicated peptide (100% indicates no disease symptoms). (g) Quantification of necrotic spots, caused by *Xanthomonas* sp. and mildew, per leaves on plants treated with SImiPEP169d or a control during field assay. (h) Total plant weight of tomato plants treated with SImiPEP169d or a control during field assay. (i) Total number of fruits per plant treated with SImiPEP169d or a control during field assay. Error bars represent SEMs, asterisks indicate a significant difference between the test condition (miPEP-treated plants) and the control (irrelevant peptide-treated plants) (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$ ) according to Student's *t*-test (b, d–i) or the Wilcoxon test (a, c) (a, c,  $n = 5$ ; b, d,  $n = 60$ ; e, f,  $n = 30$ ; g–i,  $n = 24$ ).

tomato, a bacterium responsible for bacterial speck. We performed infection assays on tomato plants, treated independently with the active miPEP. In each case, a decrease in disease severity was observed when plants were treated with SImiPEP169d, correlated with a decreased quantity of bacteria in leaves (Figures 1e,f; Figure S6), confirming its activity at promoting the general resistance against pathogens.

Plant diseases are responsible for huge yield losses in crop productions. To know whether the protective effect of the tomato SImiPEP169d against different pathogens impinge on the final yield, we carried out field trials with this peptide. Interestingly, the plants treated with SImiPEP169d showed less necrotrophic spots on leaves than untreated plants (Figure 1g). We then measured the total plant weight and the total number of fruits obtained at a time point of the crop cycle. Interestingly, SImiPEP169d was able to improve tomato crop yields by 35% (Figures 1h,i), confirming the relevance of our approach to identify miPEPs active in crop protection through appropriate molecular screening.

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## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

JPC and MB designed the research; MO, BG, CB, CM and NJ performed the molecular biology and plant experiments; HSC performed bioinformatics; JPC, MB, PT and SP wrote the paper.

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## Supporting information

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

**Data S1** Material and Methods.

**Figure S1** Three *A. thaliana* miPEPs increase plant defense.

**Figure S2** miPEPs modulate plant defense and are not toxic against *B. cinerea*.

**Figure S3** Discussion.

**Figure S4** Infection assays of miPEPs on strawberry, bean and tomato.

**Figure S5** Pictures of *B. cinerea* infection assays on strawberry, bean and tomato.

**Figure S6** Tomato infection assays with *P. syringae* pv. tomato.

**Table S1** List of primers used.

**Table S2** Sequences of peptides.