

Transient expression of anti-HrpE scFv antibody reduces the hypersensitive response in non-host plant against bacterial phytopathogen *Xanthomonas citri* subsp. *citri*

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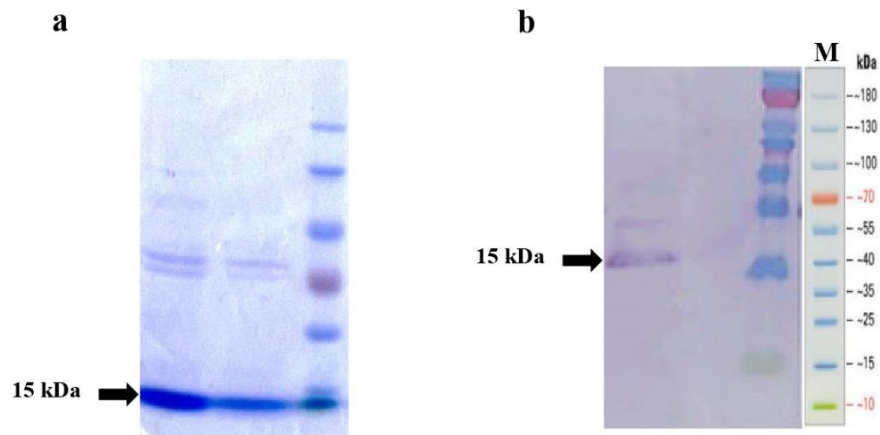
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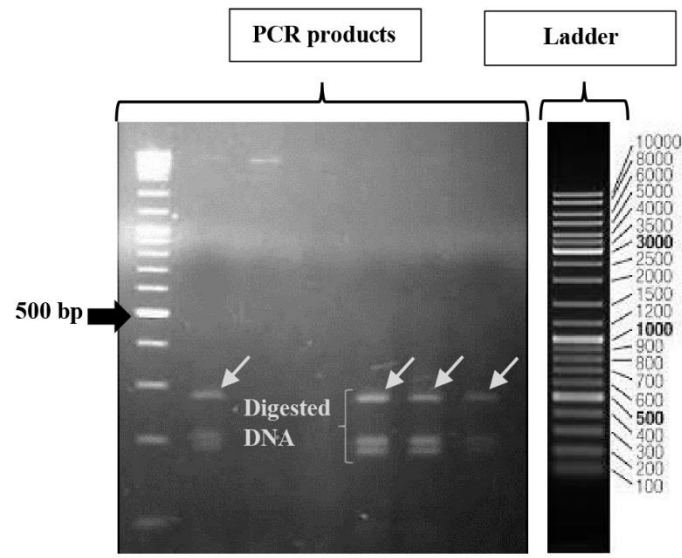
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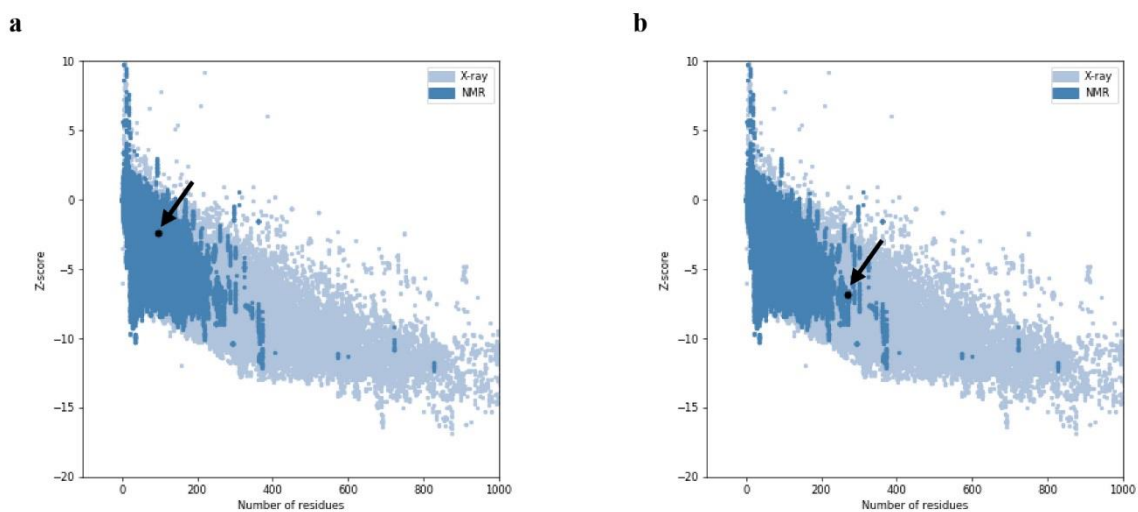
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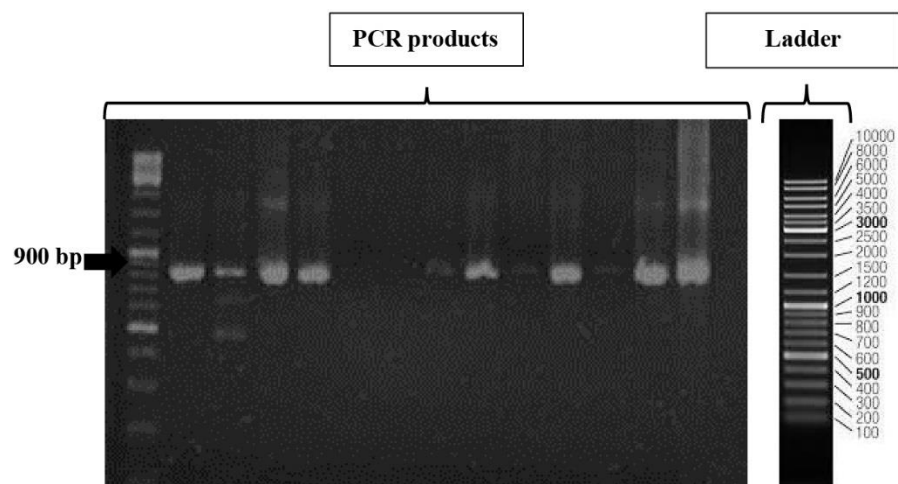
Supplementary Figure 1. (a) SDS-PAGE analysis of the expressed recombinant HrpE (rHrpE) gene in *Escherichia coli* Rosetta strain (DE3). Proteins were separated on 12% polyacrilamide gels and stained with Coomassie brilliant blue. **(b)** Western blot analysis to assess rHrpE purification with dilution 1:10000 of anti-His tag antibody. M: Marker PageRuler Prestained Protein Ladder (Thermo Scientific, USA).



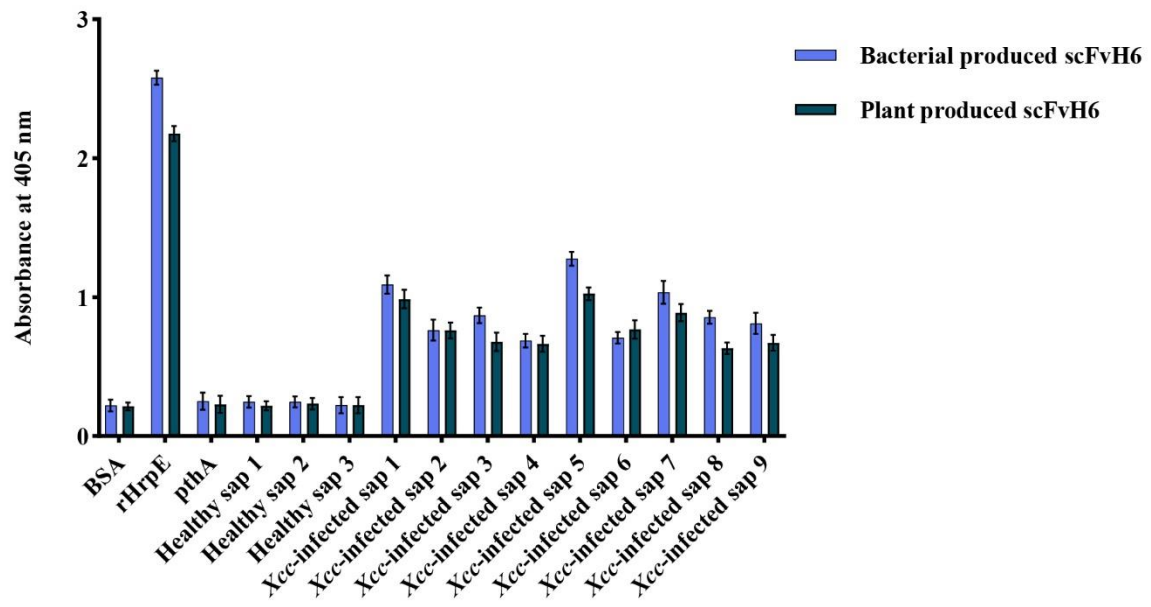
Supplementary Figure 2. Finger printing analysis of PCR product of selected monoclonal scFvs from Tomlinson I phage display library against recombinant HrpE (rHrpE) using *Bst*NI digestion. Ladder: GeneRuler DNA Ladder Mix (Thermo Scientific, USA).



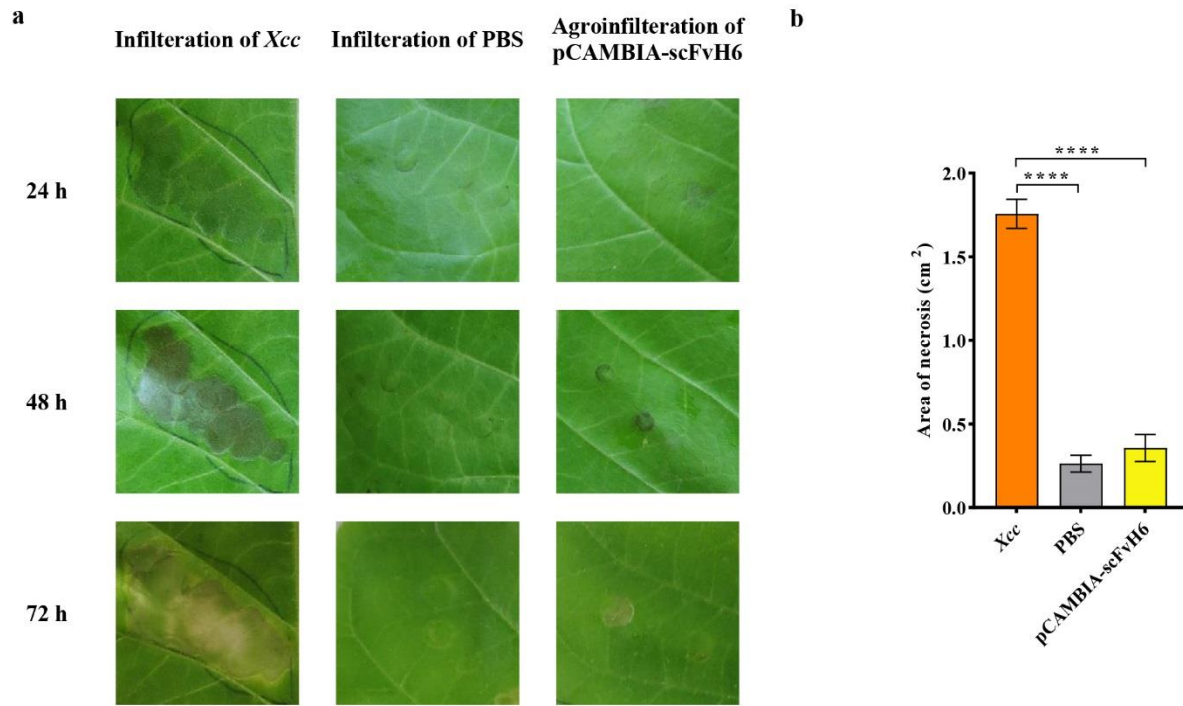
Supplementary Figure 3. ProSA Z-score plot of three-dimensional (3D) modeled **(a)** HrpE and **(b)** scFvH6. The bold black circle shows the position of each model among experimentally solved protein structures.



Supplementary Figure 4. PCR amplification of scFvs cDNA in *Agrobacterium radiobacter* transformed with pCAMBIA-scFvH6 using specific scFv primers. Ladder: GeneRuler DNA Ladder Mix (Thermo Scientific, USA).



Supplementary Figure 5. The characterization of *in vitro* binding activity of bacterial-produced and plant-produced scFvH6 against recombinant HrpE (rHrpE) and native HrpE in *Xcc*-infected samples using indirect ELISA. Data shown are means $\pm$ SD of three independent experiments.

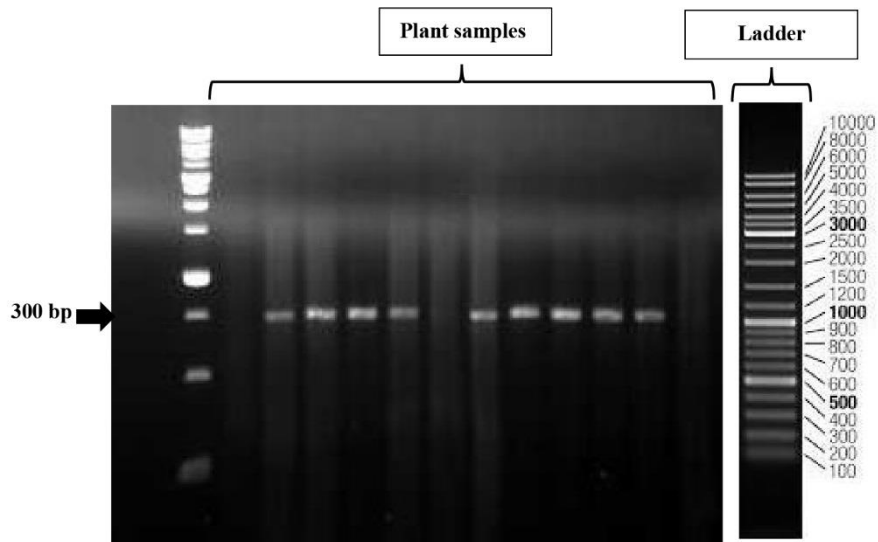


Supplementary Figure 6. (A) Appearance of untransformed *N. tabacum* leaves inoculated with suspension of *Xanthomonas citri* subsp. *citri* (*Xcc*) (10^8 CFU/mL). The leaves were infiltrated agrobacterium bearing pCAMBIA-scFvH6 and PBS were used as negative controls. (B) Comparison of necrosis area in terms of mean lesion size (2 dpi). Data shown are means $\pm$ SD of the average lesion size of three independent experiments with six plants in each replicate. *P* value of <0.05 was considered significant (*****P* <0.0001) by unpaired student's *t* test and one-way ANOVA statistical analysis.

a



b



Supplementary Figure 7. (a) Canker symptom, including brown and corky round spots sunken in center with water-soaked margins surrounded by yellow chlorotic halos, in collected citrus samples. **(b)** PCR Amplification of *Xanthomonas citri* subsp. *citri* (*Xcc*) using HrpE-specific primers. Ladder: GeneRuler DNA Ladder Mix (Thermo Scientific, USA).

Original uncropped Western blotting images

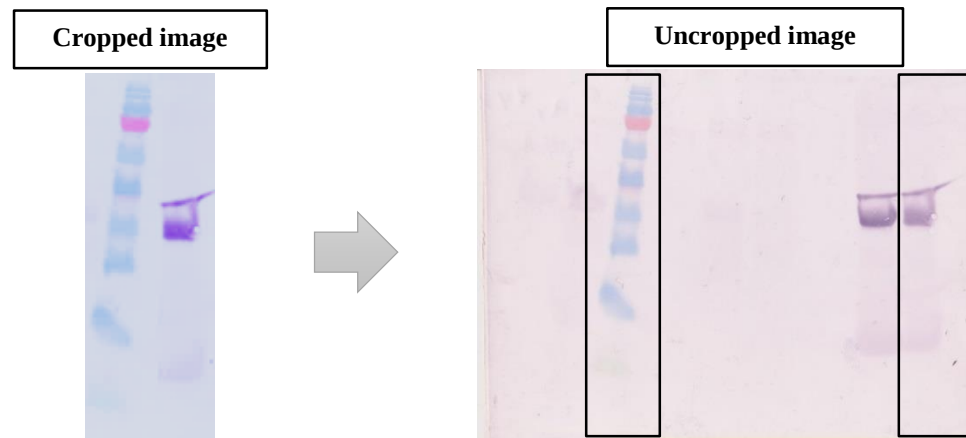


Figure 2. Western blot analysis to assay purification of scFH6 by using anti-His tag antibody (1:10000).

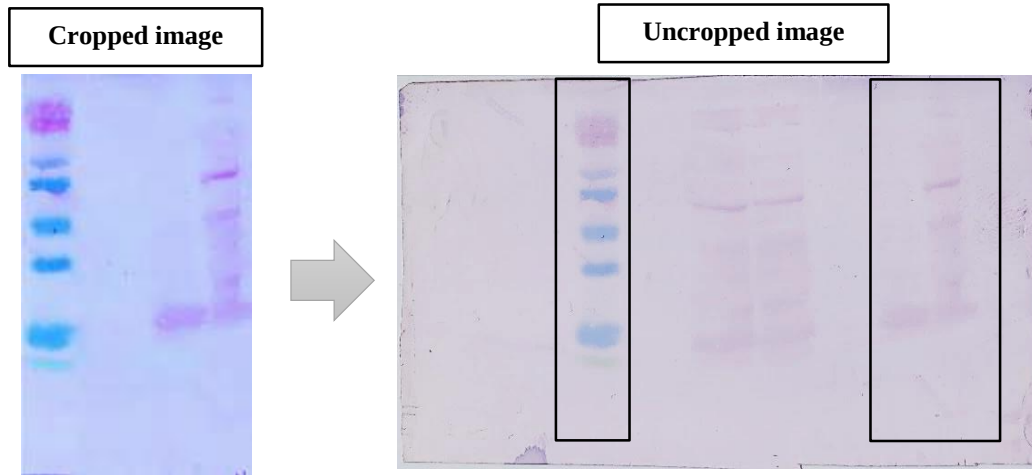


Figure 3. The characterization of *in vitro* binding activity of scFvH6 against rHrpE and native HrpE in *Xcc*-infected samples using Western blot analysis.

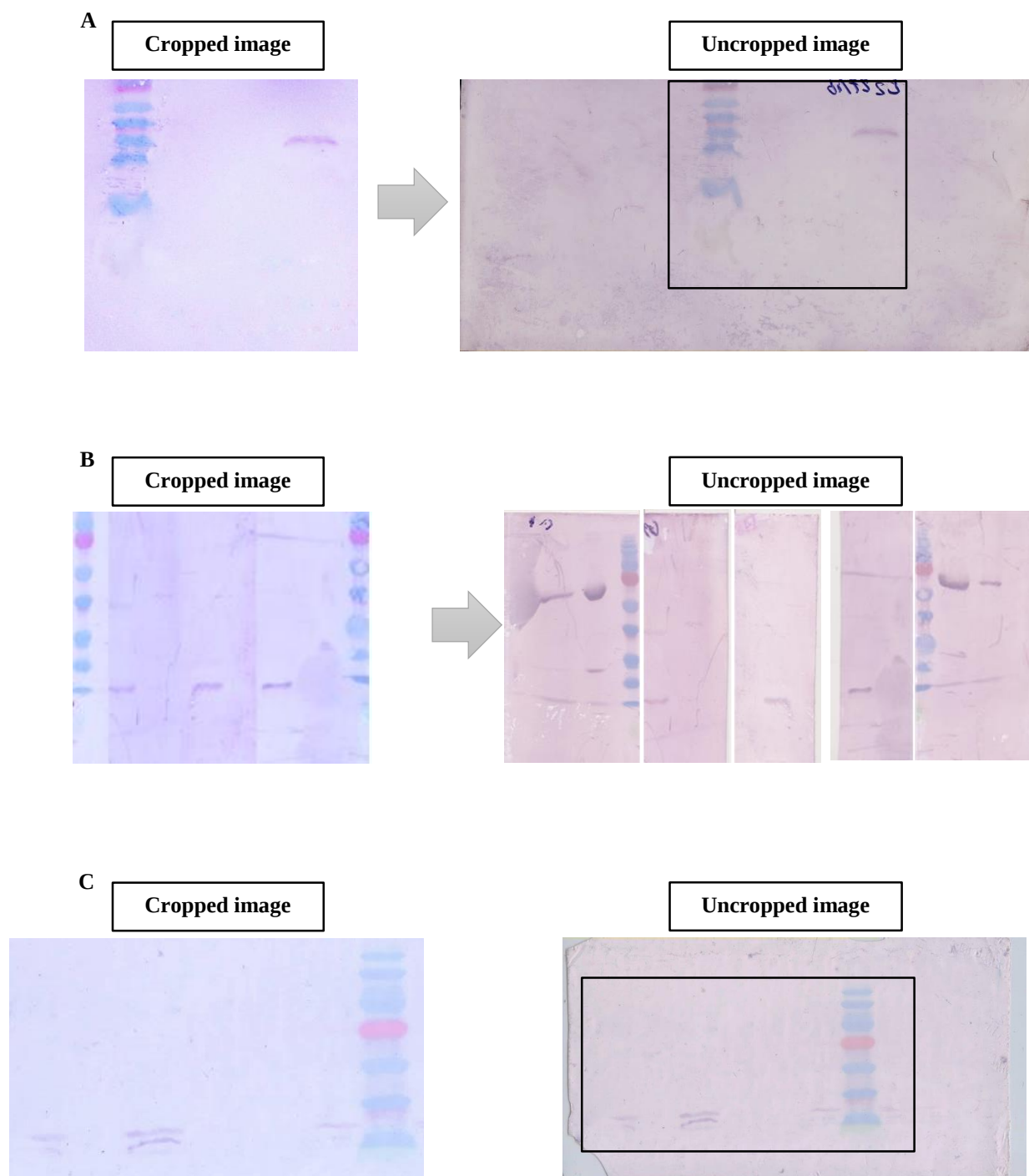


Figure 5. (a) The detection and characterization of expression of scFvH6 in leaves of *Nicotiana tabacum* cv. *Samson* during 1-3 day post inoculation (dpi) using Western blotting. **(b)** The binding activity of plant-produced scFvH6 against rHrpE and **(c)** native HrpE in *Xcc*-infected samples using Western blotting.