Exploring the implications of ligno-suberin barriers and related metabolites for resistance against bacterial wilt in tomato

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Background

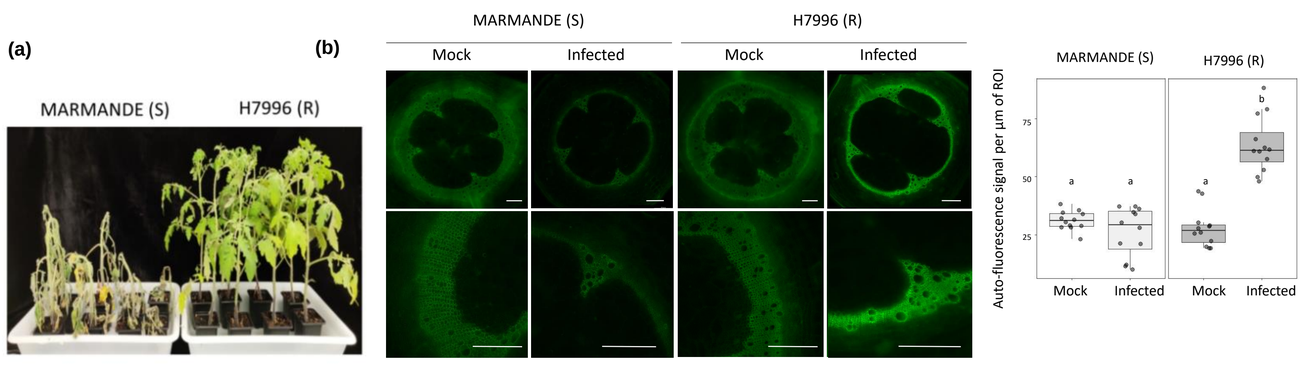
The soil borne pathogen *Ralstonia solanacearum* is the causative agent of bacterial wilt, a devastating disease for major crops of agronomic interest. This bacterium enters the plants through wounding points at the roots, reaching the vasculature, where it proliferates massively in the xylem, provoking rapid wilting.

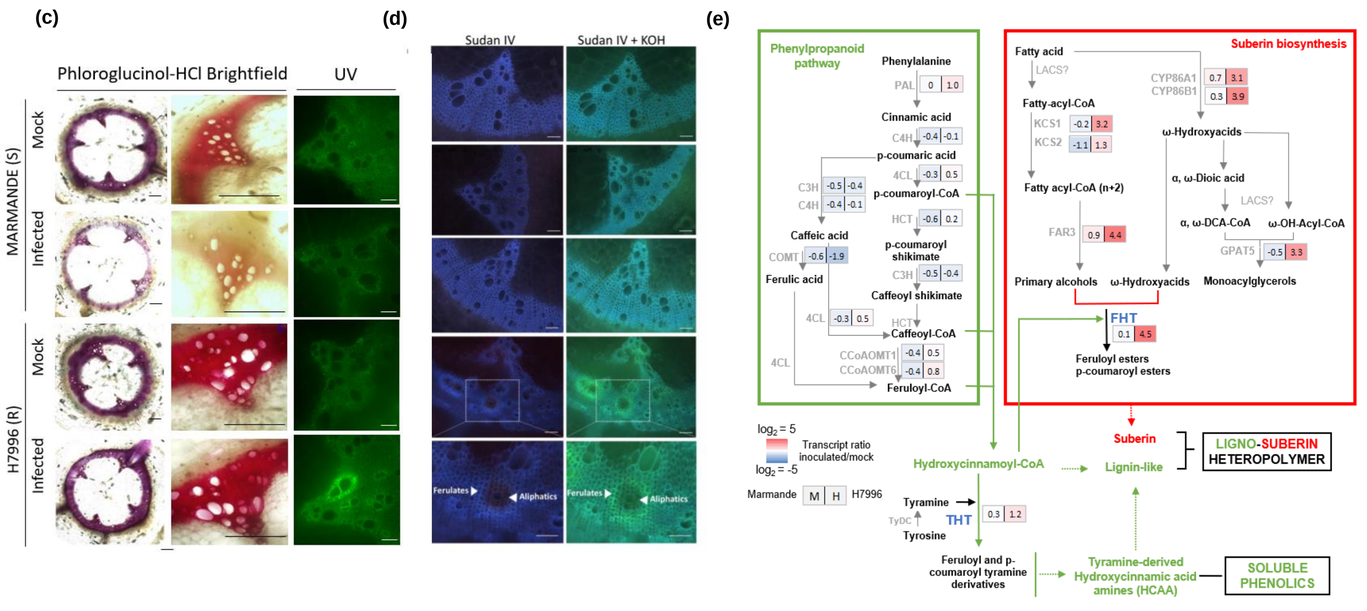
We characterized a specific feature of the highly resistant tomato cultivar Hawaii 7996 (H7996): the deposition of ligno-suberin barriers and related metabolites such as hydroxycinnamic acid amides (HCAAs) upon infection with *Ralstonia* at the root vasculature, resulting in a physical restriction of pathogen colonization throughout the plant.

To further study the contributions of these physico-chemical barriers to disease resistance, we analyzed transgenic tomato lines overexpressing genes from the ligno-suberin pathway that were significantly resistant to infection, finding structural changes in the root xylem vasculature consistent with reinforced cell walls that help blocking intruder invasion.

Resistant H7996 tomato restricts R. solanacearum colonization and induces a vascular coating response

(a) Resistance phenotype in H7996. (b) Increase in H7996 wall-bound autofluorescence when challenged with R.solanacearum infection. (c) H7996 increase in autofluorescence non-quenched by Phloroglucinol staining, normally attributed to the phenolic fraction of suberin barriers, and a lignin polymer resistant to pathogen attack, in contrast to susceptible Marmande plants.





(d) Production of suberin aliphatics in H7996 vasculature, shown as brownish areas with Sudan IV staining, and ferulate/feruloylamide deposits as a blue-to-green conversion of autofluorescence upon alkali treatment. (e) Transcriptional analysis by qRT-PCR confirmed a significant upregulation of genes coding for the ligno-suberin pathway in H7996 upon infection.

Conclusions

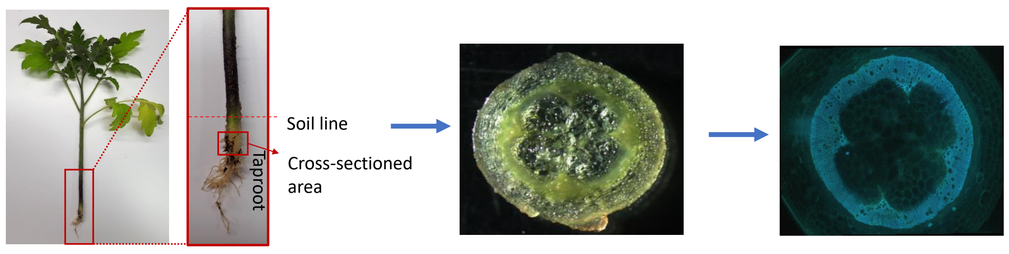
H7996 responds to R.solanacearum infection with deposition of ligno-suberin barriers and HCAAs at the root xylem vasculature

Overexpressing genes from this metabolic pathway in susceptible tomato backgrounds revealed that the production of HCAAs in SlTHT1-3 confers resistance against *R. solanacearum.*

Based on this data, we propose that these induced barriers do not only restrict pathogen colonization, but also confer antimicrobial properties to the cell walls, showing this pathway as a promising source of resistance against this devastating disease in tomato.

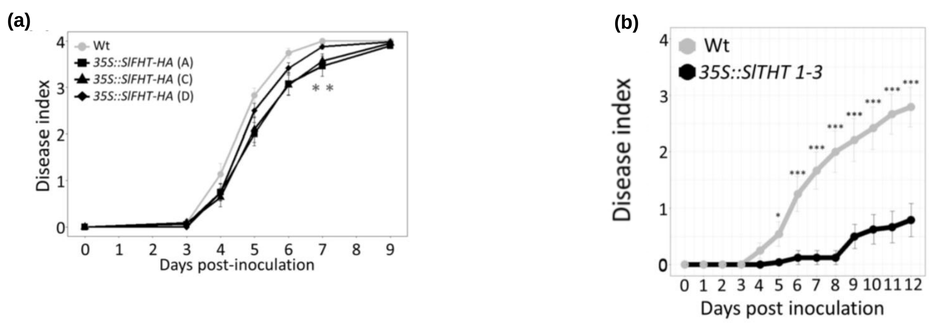
Methodology

Thin cross-sections were made out of *R.solanacearum*-infected or mock-treated plants, in the transition between the basal hypocotyl and the taproot, below the soil line. Sections were kept in 70 % ethanol at room temperature for 5-7 days to remove chlorophyll. Phloroglucinol-HCl stain was used for lignin detection under brightfield observation, and Sudan IV for detecting suberin aliphatics under UV light. An alkali treatment with 1N KOH (pH=10) was also performed to detect wall-bound ferulates as a blue-to-green autofluorescence conversion in the mounted cross-sections.

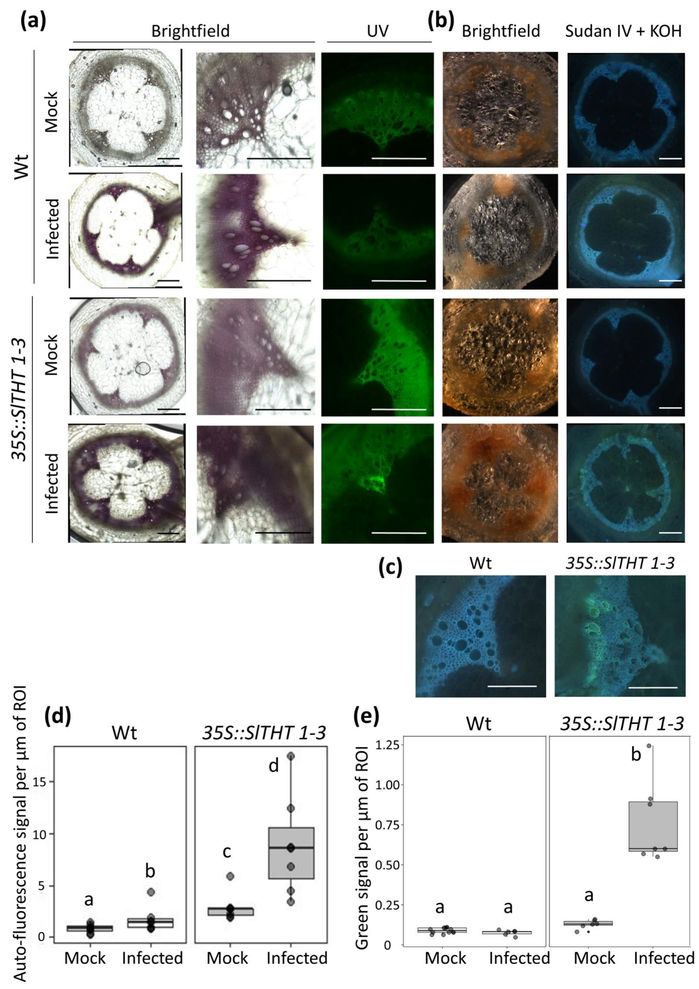


Overexpression of SlTHT1-3 in a susceptible tomato cultivar confers resistance to R. solanacearum

Genes involved in ferulate esterification into suberin aliphatics and feruloylamide synthesis, such as SlFHT and SlTHT1-3, respectively, were overexpressed in suscetible tomato, since these genes were also upregulated in H7996 upon infection. (a) SlFHT showed a slight delay in disease progression, (b) while the THT line was highly resistant to the disease.



SlTHT1-3 histological studies showing structural changes in ligno-suberin barriers: (a,d) increase in autofluorescence non quenched by phloroglucinol, presence of ferulate deposits upon KOH treatment, with no suberin aliphatic fractions (b,c,e).



References

Kashyap, Anurag, et al. "Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid amides restrict Ralstonia solanacearum colonization in resistant tomato roots." *bioRxiv* (2021).