

New Phytologist Supporting Information

Article title: Induced ligno-suberin vascular coating and tyramine-derived hydroxycinnamic acid amides restrict *Ralstonia solanacearum* colonization in resistant tomato

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Figure S11: Overexpression of *SlTHT1-3* in tomato results in restricted colonization by *R*. *solanacearum*.

Table S1: List of primers used in this study.

Table S2: Assignments of the correlation signals in the 2D HSQC spectra.



Fig. S1 Tissue used for analysis and bacterial dynamics. (a) A picture of a tomato plant is shown, highlighting in red the selected region for all cross-sections used in this work, both for histological methods and molecular analysis. The transition zone between the taproot and the hypocotyl, down the soil line, was chosen because of their implications on *R. solanacearum*-

BRIGHTFIELD

tomato interactions, as discussed in the manuscript. (**b**) Plants (4–5 weeks old) of the susceptible variety Marmande and the resistant variety H7996 were inoculated with ~1x10⁷ CFU/ml of a fluorescent *R. solanacearum* strain by soil drenching. Cross-sections of the taproot-to-hypocotyl area containing 10^5 CFU g⁻¹ of *R. solanacearum* were obtained and photographed with a fluorescence stereomicroscope under brightfield and UV light. Images from a representative experiment out of 3 with n=5 plants per cultivar. Scale bar = 500 µm.



Fig. S2 H7996 plants show mild symptoms upon challenge inoculation of *R. solanacearum*. Susceptible (Marmande) and resistant (H7996), 5-week-old tomato plants were inoculated through roots by soil-soak with ~1x10⁷ CFU/ml of *R. solanacearum* GMI1000 and incubated at 28°C. (a) Wilting progress was assayed in both cultivars by rating plants daily on a 0 to 4 disease index scale where 0 = healthy and 4 = 100% wilted. Data presented are means \pm SE of a representative experiment with n=20 plants for each cultivar, out of a total of 3 experiments. (b) The level of in planta colonization by *R. solanacearum* was calculated as colony forming units per gram of fresh taproot tissue (CFU·g⁻¹) at the indicated days post-infection (dpi). Data presented are of a

representative experiment with n=10 plants for each time point each cultivar out of a total of 3 experiments. Asterisks indicate statistically significant difference between Marmande and H7996 in a paired Student's t-test (** p-value of p < 0.01 and *** p-value of p < 0.001). Supports Figure 1 of the main manuscript.



(b)



Mock



Fig. S3 R. solanacearum-induced xylem vascular ferulic acid deposition occurs in resistant H7996, but not in susceptible Marmande. Cell wall-bound ferulic acid can be detected by emission of a blue fluorescence with UV excitation at neutral pH, which characteristically changes to stronger green emission under conditions of high pH such as in the presence of alkali. Fiveweek-old Marmande and H7996 plants were inoculated with $\sim 1 \times 10^7$ CFU/ml of R. solanacearum GMI1000 and incubated at 28°C. Taproot cross-sections were obtained at 9 dpi (a) or in plants containing a bacterial load of approximately 10^5 CFU g⁻¹ of *R. solanacearum* (**b, c**). (**a**) Autofluorescence emitted from taproot cross-section from mock-treated and infected Marmande and H7996 plants was visualized at 9 dpi under UV before and after treatment with KOH alkali (high pH above 10). In infected H7996 a green/turquoise color appears in vessel walls and surrounding xylem parenchyma cells, indicative of ferulic acid deposition in the cell walls. Scale bars = 500 μ m. (b) The same as in (a) but cross-sections were obtained from plants containing a bacterial load of approximately 10^5 CFU g⁻¹ of *R. solanacearum*. Scale bars = 500 µm. (c) Green fluorescence from ferulate deposits in the xylem and surrounding parenchyma cells was measured using ImageJ. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Plots show data from a single representative experiment (n = 6) out of a total of 3. Different letters indicate statistically significant differences (α =0.05, Fisher's least significant difference test). Supports Figure 4 of the main manuscript.



Fig. S4 Expression of suberin biosynthetic genes in xylem vasculature of taproots upon infection of *R. solanacearum*. Expression levels of tomato putative orthologs of the suberin fatty acid pathway were analyzed by qPCR in H7996 and Marmande plants infected with *R. solanacearum* or mock-treated. Xylem vascular tissue comprising of metaxylems and surrounding parenchyma cells was collected from infected plants with a *R. solanacearum* inoculum of 10⁵ CFU g^{-1} in the taproot or mock-inoculated plants of a similar age. Relative expression values were calculated using the Elongation Factor 1 alpha (eEF1 α) gene as reference. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters indicate statistically significant differences (α =0.05, Fisher's least significant difference test). Supports Figure 5 of the main manuscript.





Fig. S5 Phylogeny of Ferulovl transferase (FHT) orthologues in different plant species and expression of the putative tomato FHT ortholog in response to Ralstonia solanacearum infection. (a) Protein homologs of potato FHT gene (PGSC0003DMG400031731) were obtained from www.phytozome.jgi.doe.gov and matches with more than 80 % similarity were used for phylogenetic analysis using <u>www.phylogeny.fr</u>. (b) Gene expression of the putative tomato *FHT* ortholog (Solvc03g097500) was analyzed by qPCR. Relative expression levels were calculated using the Elongation Factor 1 alpha (*eEF1 a*, *Solyc06g005060*) as the reference gene. H7996 and Marmande plants, containing a *R. solanacearum* inoculum of 10^5 CFU g⁻¹ in the taproot were selected. Xylem vascular tissue, comprising of metaxylems and surrounding parenchyma cells was collected from taproots for RNA extraction and cDNA synthesis. Similarly, xylem tissue was collected from Marmande mock plants and H7996 mock plants. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters indicate statistically significant differences (α =0.05, Fisher's least significant difference test). Supports Figure 5 of the main manuscript.



Fig. S6 Phylogeny of tyramine hydroxycinnamoyl transferase (THT) orthologues in different plant species and expression of the tomato THT gene family members in response to R. solanacearum infection. (a) Protein homologs of tomato THT1-3 gene (Solyc08g068730) were obtained from www.phytozome.jgi.doe.gov and matches with more than 60 % similarity were used for phylogenetic analysis using the webpage www.phylogeny.fr. (b) Gene expression of the tomato THT gene family members was analyzed by qPCR. Relative expression levels were calculated using the Elongation Factor 1 alpha (eEF1 a, Solyc06g005060) as the reference gene. H7996 and Marmande plants, containing a *R. solanacearum* inoculum of 10^5 CFU g⁻¹ in the taproot were selected. Xylem vascular tissue, comprising of metaxylems and surrounding parenchyma cells was collected from taproots for RNA extraction and cDNA synthesis. Similarly, xylem tissue was collected from Marmande mock plants and H7996 mock plants. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters indicate statistically significant differences (α =0.05, Fisher's least significant difference test). Supports Figure 5 of the main manuscript.



Fig. S7 Expression of phenylpropanoid pathway genes in xylem vasculature of taproots upon invasion of *R. solanacearum*. Expression levels of tomato putative orthologs of the phenylpropanoid pathway were analyzed by qPCR in H7996 and Marmande plants infected with *R. solanacearum* or mock-treated. Xylem vascular tissue comprising of metaxylems and surrounding parenchyma cells was collected from infected plants with a *R. solanacearum* inoculum of 10^5 CFU g⁻¹ in the taproot or mock-inoculated plants of a similar age. Relative expression values were calculated using the Elongation Factor 1 alpha (*eEF1* α) gene as reference. Three biological replicates (n=3) were used, and taproots of 6 plants were used in each replicate. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters indicate statistically significant differences (α =0.05, Fisher's least significant difference test). Supports Figure 5 of the main manuscript.





Fig. S8 Immunoblots of SIFHT-HA in independent Marmande tomato lines expressing *35S::SIFHT-HA*. Immunoblots using anti-HA antibody showing SIFHT-HA protein (predicted protein size: 49kDa) levels of independent transgenic lines stably overexpressing *SlFHT-HA* on a susceptible Marmande background (*35S::SlFHT-HA*). (a) Independent lines A, B, C and D. (b) Independent lines C (siblings CI and CIII) and G. Several wild-type Marmande are included as a control (WT lanes), to ensure specificity of the observed band. Coomasie blue staining showing similar protein load in all the lanes are included.





Fig. S9 Fresh weight of 35S::SIFHT-HA plants. (a) Photographs of Wt Marmande and transgenic 35S::FHT-HA plants were taken 3 weeks after germination. (b) Fresh weight was measured at this point. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters represent statistically significant differences (α =0.05, Fisher's least significant difference test).





Fig. S10 Fresh weight of 35S::SITHT1-3 plants. (a) Photographs of Wt Marmande and transgenic 35S::THT-1-3 plants were taken 3 weeks after germination. (b) Fresh weight was measured at this point. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Different letters represent statistically significant differences (α =0.05, Fisher's least significant difference test).



(b)



Fig. S11 Overexpression of *SITHT1-3* **in tomato results in restricted colonization by** *R. solanacearum*. **(a)** Growth of *R. solanacearum* GMI1000 in planta was monitored in leaves of 35S::THT1-3 transgenic plant compared with Wt Moneymaker tomato lines over time. The bacterium was vacuum infiltrated into the leaves at a concentration of ~1x10⁵ CFU/ml and growth was recorded at 0, 3 and 6 dpi. Data are represented with box and whiskers plots: whiskers indicate variability outside the upper and lower quartiles and boxes indicate second quartile, median and third quartile. Plots show data of 6 to 8 independent plants (n=6-8) from a representative experiment out of 3. Asterisk indicates statistically significant difference between Wt and overexpression line in a paired Student's t-test (* corresponds to p-value of p < 0.05). **(b)** Representative images of tomato stem cross-sections showing colonization by the *R. solanacearum* GMI1000 GFP reporter strain at 6 dpi. *R. solanacearum* was directly injected into the xylem vasculature of the first internode through the petiole at a concentration of 10^5 CFU ml⁻¹. Colonization progress was analyzed at the point of inoculation, at higher (+0.5, +1, +2 and +3 cm) and lower -0.5, -1, -2 and -3 cm) sections. Images from a representative experiment out of 3 with *n*=5 plants each. Scale bar = 2 mm. Supports Figure 7 of the main manuscript.

Gene	Gene ID	Primers	Sequence	Usage	Origin
FHT	Solyc03g097500	part7FHTF1	GGCCCGGGATGGAGAATGGTAAACACAGT	Cloning	This paper
	, 0		GTTGC	_	
		part7FHTHAR1	GGGGATCCTTAAGCGTAGTCTGGGACGTCG		
			TATGGGTAGATCTCCATAAGTTCCTC		
FHT	Solyc03g097500	qSIFHT F1	GGTGGCTCAGGTGACAAAGT	qPCR	This paper
		qSIFHT R1	CCTCTCGCAATTTCACCCCA		
THT 1-3	Solyc08g068730	qTHT1-3F1	CCCCTTTTGACGAACCTAAA	qPCR	(Campos et al.,
		qTHT1-3R1	TTTGGATCGGAATTCCTCAA		2014)
EF	Solyc06g005060	qeEF1αF1	CCACCTCGAGATCCTAATGG	qPCR	(Campos et al.,
		qeEF1αR1	ACCCTCACGTATGCTTCCAG		2014)
PAL1	Solyc09g007920	qSIPAL1 F1	TACGTGTTTGCCTATGCTGATG	qPCR	(Rahim et al.,
		qSIPAL1 R1	CGGCCTTTAATTCGTCCTC		2019)
СОМТ	Solyc03g080180	qSICOMT F1	GGTGGTGGAACAGGGGCTACT	qPCR	(Rahim et al.,
		qSICOMT R1	TAAACAATGCTCATCGCTCCAATC		2019)
CCoAOM	Solyc02g093270	qSICCOAOMT1 F1	GAGAGCCTGAAGCCATGAAAGAGC	qPCR	(Rahim <i>et al.,</i> 2019)
T1		qSICCOAOMT1 R1	GAGCCATGGCAGTAGCAAGCAGAG		
ССоАОМ	Solyc01g107910	qSICCOAOMT6 F1	ATTTTCGAGAGGGCCCTGCTTTAC	qPCR	(Rahim <i>et al.,</i> 2019)
Т6		qSICCOAOMT6 R1	ATCCGATCACACCACCAACTTTCA		
НСТ	Solyc03g117600	qSIHCT F1	CCCTCCTCCGTGCTCGTGA	qPCR	(Rahim et al.,
		qSIHCT R1	CCCGGGTTAGTTTGAAGATTGACA		2019)
СЗН	Solyc01g096670	qSlC3H F1	CTGCAATGCGTGGCCAAGGAAGC	qPCR	(Rahim et al.,
		qSlC3H R1	TCGCGAGCAACAGCCCAGACATT		2019)
4CL	Solyc12g094520	qSL4CL F1	CGA GCA TGG AAG GGA AAA TTG	qPCR	(Rigano et al.,
		qSL4CL R1	TCA GAG TCT AGA GTG GAA GCA G		2016)
C4H	TC93956	qSIC4H F1	CTAGCTAACAACCCCGCCCA	qPCR	(Zhang et al.,
		qSIC4H R1	AACTCCTCCTGCCAACACCG		2019)
THT 7-8	Solyc08g068780	qSLTHT 7-8 F1	GGAAACTGATAAGGAGAAGGTGG	qPCR	This paper
		qSLTHT 7-8 R1	GTTTGCACGGCGTATGGAG		
THT like 4	Solyc08g068710	qSLTHT4 F1	AGTTTAGGTATGGCAAATTGCATGG	qPCR	This paper
		qSLTHT4 R1	AAGAAAACACACAGTAGCTAACAGC		
THT like 5	Solyc08g068690	qSLTHT6 F1	TCAGTCGATGGAATAGTAGCAGTT	qPCR	This paper
THT 7-8 THT like 4 THT like 5 CYP86A1		qSLTHT6 R1	TCCTCAATTTCCCCCTTGTTATG		
THT like 4 THT like 5 CYP86A1	Solyc06g076800	qSlCYP86A1 F1	GGTCTACTGGTGTATCCGCA	qPCR	This paper
		qSICYP86A1 R1	CCTTTAGGATAGTTATCGAACCTGG		
KCS1	Solyc10g009240	qSIKCS1 F1	GTCGTAGGGGTGTCACTAGC	qPCR	This paper
		qSIKCS1 R1	GTCATGAAAAACCTGAATTGCTCAG		
GPAT5	Solyc04g011600	qSIGPAT5 F2	CCCTAGGCCAATGTATGAGGTAAC	qPCR	This paper
		qSIGPAT5 R2	GTTGCTGCCAAAATCCTCTGG		
DAISY/KC	C Solyc05g009280	qDAISY F1	TCCGAGTTCATCCCAAGTCG	qPCR	This paper
S2		qDAISY R1	AACAGTATGGCTGCACCTCC		
FAR3	Solyc06g074390	qSIFAR3 F1	TGGTGCTACTGGATTTCTTGC	qPCR	This paper
		qSIFAR3 R1	TGCCACAGCCTCATTGTTGA		
THT 7-1	Solyc08g068700	qSITHT7-1 F1	GCTTGAACGCTTGGTTAGTGG	qPCR	This paper
		qSITHT7-1 R1	AGTCCTCCTTAGAGGGCTTGC	1	
CY986B1	Solyc02g014730	qSICYP86B1 F1	TCCGTTGATTTTCAAGCCAGC	qPCR	This paper
	_	qSICYP86B1 R1	тсдтсттсаасаасстстттдтд	1	

Table S1 List of primers used for cloning and qPCR analysis

Label	δ с/δн (ррm)	Assignment
Ty ₇	34.2/2.62	C ₇ /H ₇ in amides of tyramine (Ty)
Ty ₈	40.5/3.29	C ₈ /H ₈ in amides of tyramine (Ty)
$\mathbf{B}_{\boldsymbol{eta}}$	53.3/3.43	C_{β}/H_{β} in phenylcoumarans (B)
C_{β}	53.5/3.05	C_{β}/H_{β} in β - β 'resinols (C)
MeO	55.3/3.72	C/H in aromatic methoxy group
A_{γ}	59.7/3.23, 3.58	C_{γ}/H_{γ} in β – O –4' alkyl-aryl ethers (A)
I_{γ}	61.5/4.06	C_{γ}/H_{γ} in cinnamyl alcohol end-groups (I)
\mathbf{B}_{γ}	62.6/3.70	C_{γ}/H_{γ} in phenylcoumarans (B)
C_{γ}	71.1/3.80, 4.17	C_{γ}/H_{γ} in β - β resinols (B)
A_{α}	71.3/4.79	C_{α}/H_{α} in β – O –4' alkyl-aryl ethers (A)
$A_{\beta G}$	83.9/4.27	C_{β}/H_{β} in β –O–4' alkyl-aryl ethers (A) linked to a G unit
C_{α}	84.9/4.67	C_{α}/H_{α} in β - β 'resinols (C)
$A_{\beta S}$	83.6/4.28	C_{β}/H_{β} in β –O–4' alkyl-aryl ethers (A) linked to a S unit
B_{α}	86.9/5.45	C_{α}/H_{α} in phenylcoumarans (B)
S _{2,6}	104.0/6.68	C_2/H_2 and C_6/H_6 in syringyl units (S)
S' _{2,6}	106.3/7.29	C_2/H_2 and C_6/H_6 in C α -oxidized syringyl units (S')
G ₂	111.1/6.97	C ₂ /H ₂ in guaiacyl units (G)
Ty _{3,5}	114.8/6.64	C_3/H_3 and C_5/H_5 in amides of tyramine (Ty)
$G_{5/6}$	114.9/6.79	C_5/H_5 and C_6/H_6 in guaiacyl units (G)
G ₆	119.0/6.76	C ₆ /H ₆ in guaiacyl units (G)
I_{β}	128.2/6.21	C_{β}/H_{β} in cinnamyl alcohol end-groups (I)
I_{α}	128.6/6.43	C_{α}/H_{α} in cinnamyl alcohol end-groups (I)
Ty _{2,6}	129.3/6.92	C_2/H_2 and C_6/H_6 in amides of tyramine (Ty)
U_{F}	129.4/5.31	-CH=CH- in unsaturated fatty acid structures (UF)
FAm ₇	138.6/7.31	C ₇ /H ₇ in feruloyl amides (FAm)

Table S2 Assignments of the correlation signals in the 2D HSQC spectra.

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