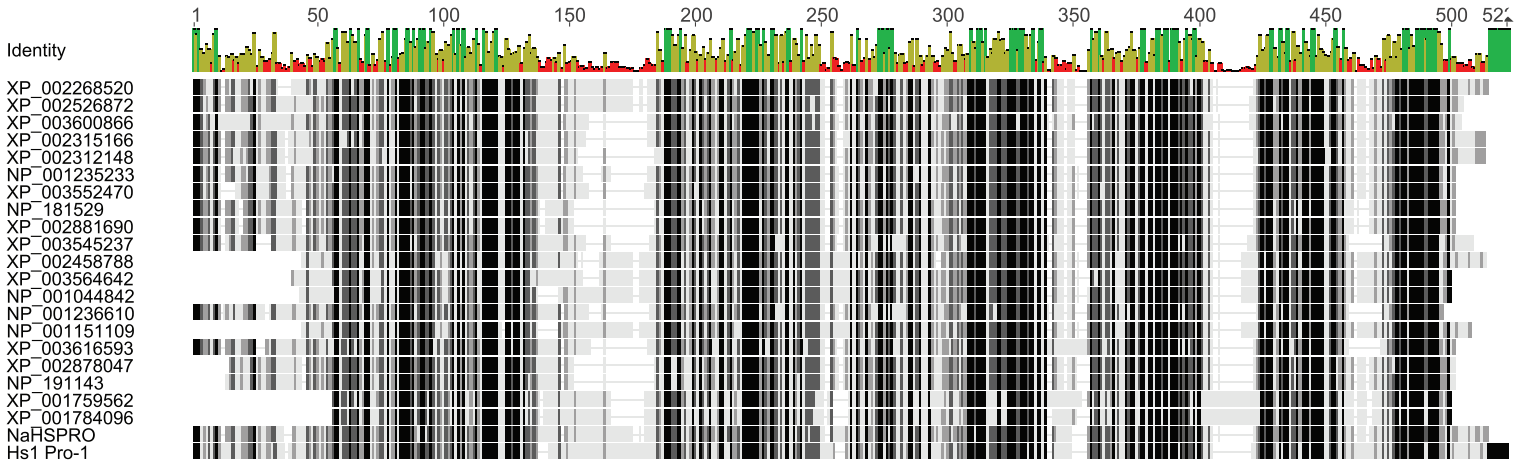
A

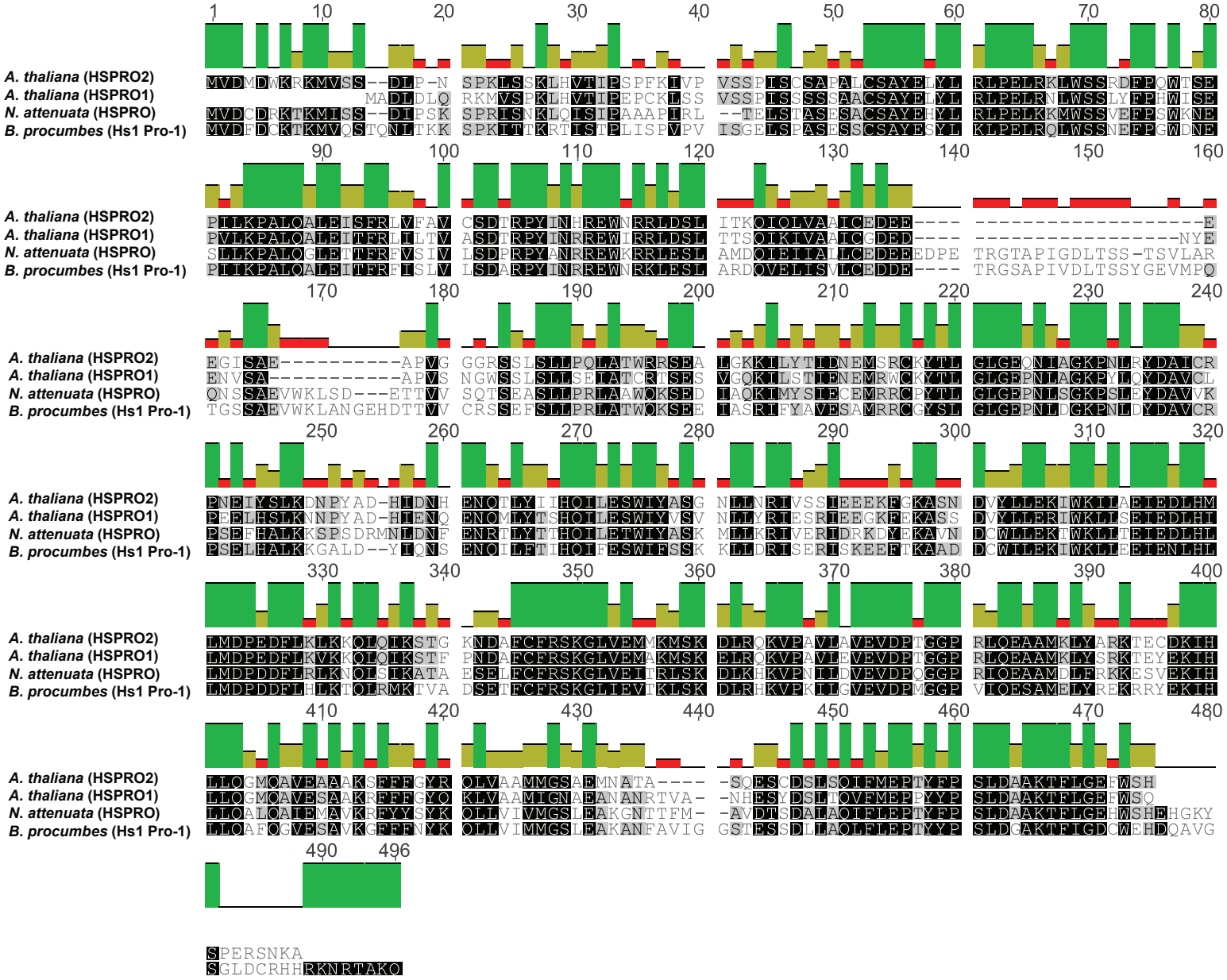


B

GenBank annotation	E-Value (blastx)	Gene ID	Organism	% Pairwise Identity
Nicotiana attenuata HSPRO	**0**	**NaHSPRO** (JQ354963)	***Nicotiana attenuata***	**100**
nematode resistance protein-like HSPRO2 isoform 1	0	XP_002268520	*Vitis vinifera*	69
conserved hypothetical protein	0	XP_002526872	*Ricinus communis*	68
predicted protein	0	XP_002315166	*Populus trichocarpa* (2)	64
Beta procumbens nematode resistance (Hs1pro-1)	0	combined AAB48305 and AAZ39087	*Beta procumbes*	63
predicted protein	0	XP_002312148	*Populus trichocarpa* (1)	61
uncharacterized protein LOC547603	0	NP_001235233	*Glycine max* (1)	58
PREDICTED: nematode resistance protein-like HSPRO2-like	0	XP_003552470	*Glycine max* (2)	58
hypothetical protein MTR_3g070230	0	XP_003600866	*Medicago truncatula*	58
PREDICTED: nematode resistance protein-like HSPRO1-like	4.14E-166	XP_003564642	*Brachypodium distachyon*	53
HS1 PRO-1 2-like protein (HSPRO2)	2.62E-175	NP_181529 (At3g55840)	*Arabidopsis thaliana* (1)	53
nematode-resistance protein	4.83E-163	NP_001151109	*Zea mays*	53
hypothetical protein ARALYDRAFT_483040	9.82E-170	XP_002881690	*Arabidopsis lyrata* (1)	53
Os01g0855600	1.01E-163	NP_001044842	*Oryza sativa*	53
hypothetical protein SORBIDRAFT_03g040300	3.51E-166	XP_002458788	*Sorghum bicolor*	53
nematode resistance HS1pro1 protein	3.66E-163	NP_001236610	*Glycine max* (4)	51
hypothetical protein ARALYDRAFT_486026	2.49E-155	XP_002878047	*Arabidopsis lyrata* (2)	51
Hs1pro-1 protein (HSPRO1)	1.69E-154	NP_191143 (At2g4000)	*Arabidopsis thaliana* (2)	50
PREDICTED: nematode resistance protein-like HSPRO2-like	7.50E-168	XP_003545237	*Glycine max* (3)	50
Nematode resistance HS1pro1 protein	1.31E-157	XP_003616593	*Medicago truncatula*	48
predicted protein	1.40E-124	XP_001759562	*Physcomitrella patens* (1)	45
predicted protein	1.28E-122	XP_001784096	*Physcomitrella patens* (2)	45

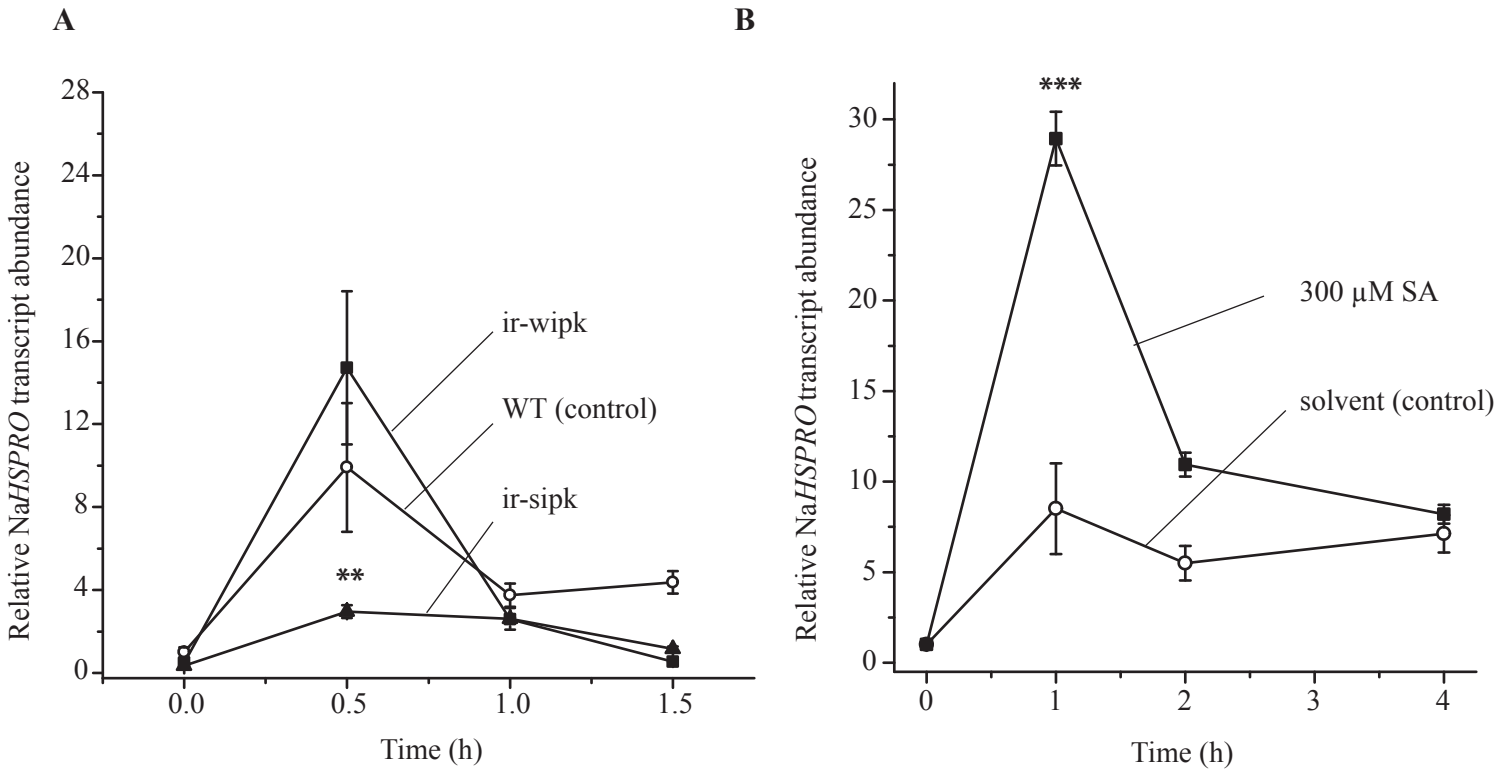
Supplemental Figure S1. Alignment of *N. attenuata* HSPRO protein sequence with close homologs in different plant species.

(a) Schematic protein sequence alignment of *N. attenuata* HSPRO (JQ354963) with the 21 closest homologs in different plant species deposited in GenBank (including *B. procumbens* Hs1^{pro-1}). The cartoon above the sequences shows the % of similarity (green bars within the overlapping regions represent identical amino acids). See materials and methods for alignment parameters. **(b)** Reference table for genes, accesion numbers and species used in (a) and Figure 1b.c



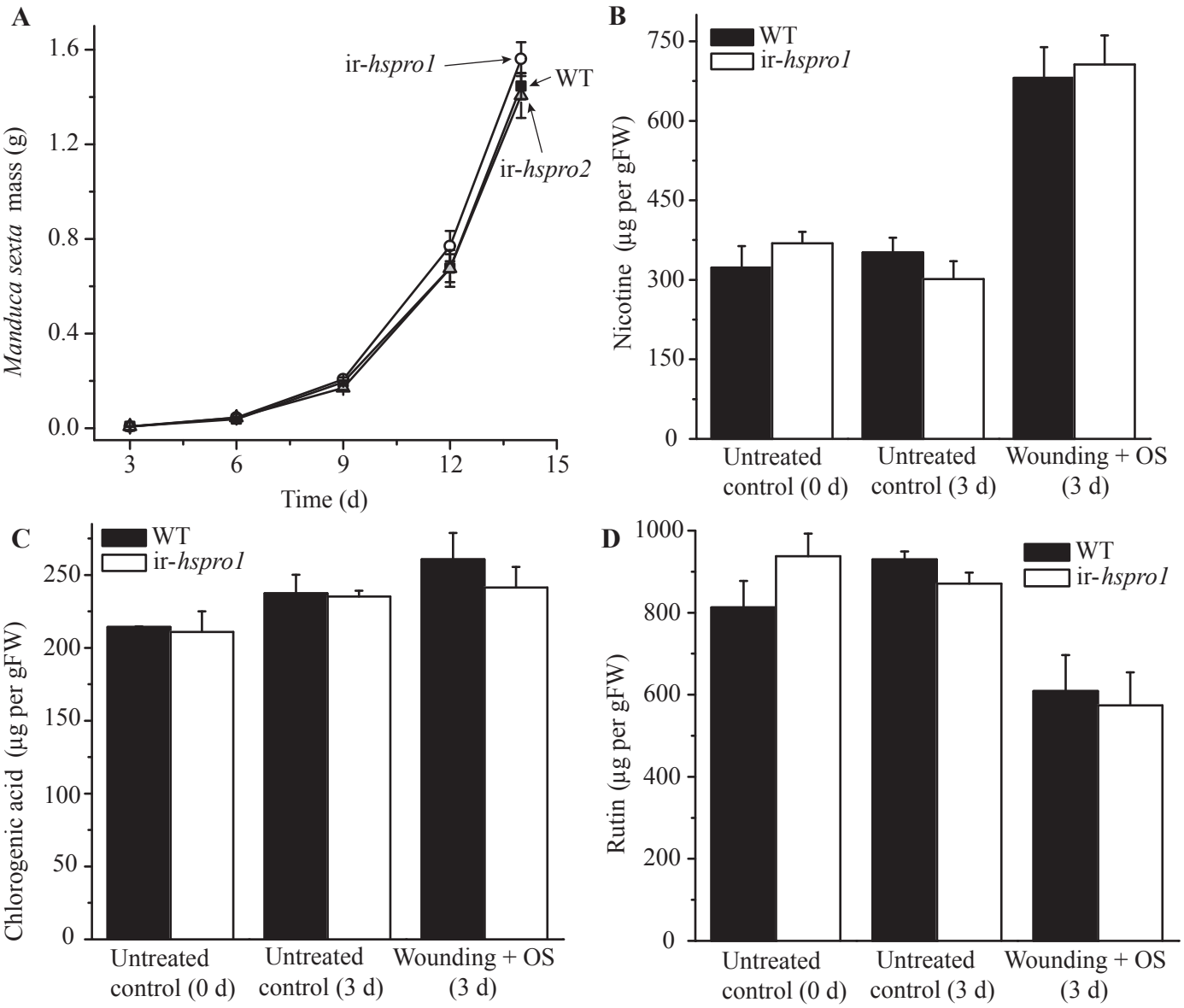
Supplemental Figure S2. Alignment of *N. attenuata* HSPRO protein sequence with homologs in *A. thaliana* and *B. procumbens*.

N. attenuata HSPRO (JQ354963); Arabidopsis HSPRO1 (NP_191143; At2g4000), Arabidopsis HSPRO2 (NP_181529; At3g55840), full lenght sugar beet (*B. procumbes)* Hs1^{pro-1} (combined AAB48305 and AAZ39087). The cartoon above the sequences represent the % of similarity (green bars within the overlapping regions shows identical amino acids in the four sequences). See materials and methods for alignment parameters.



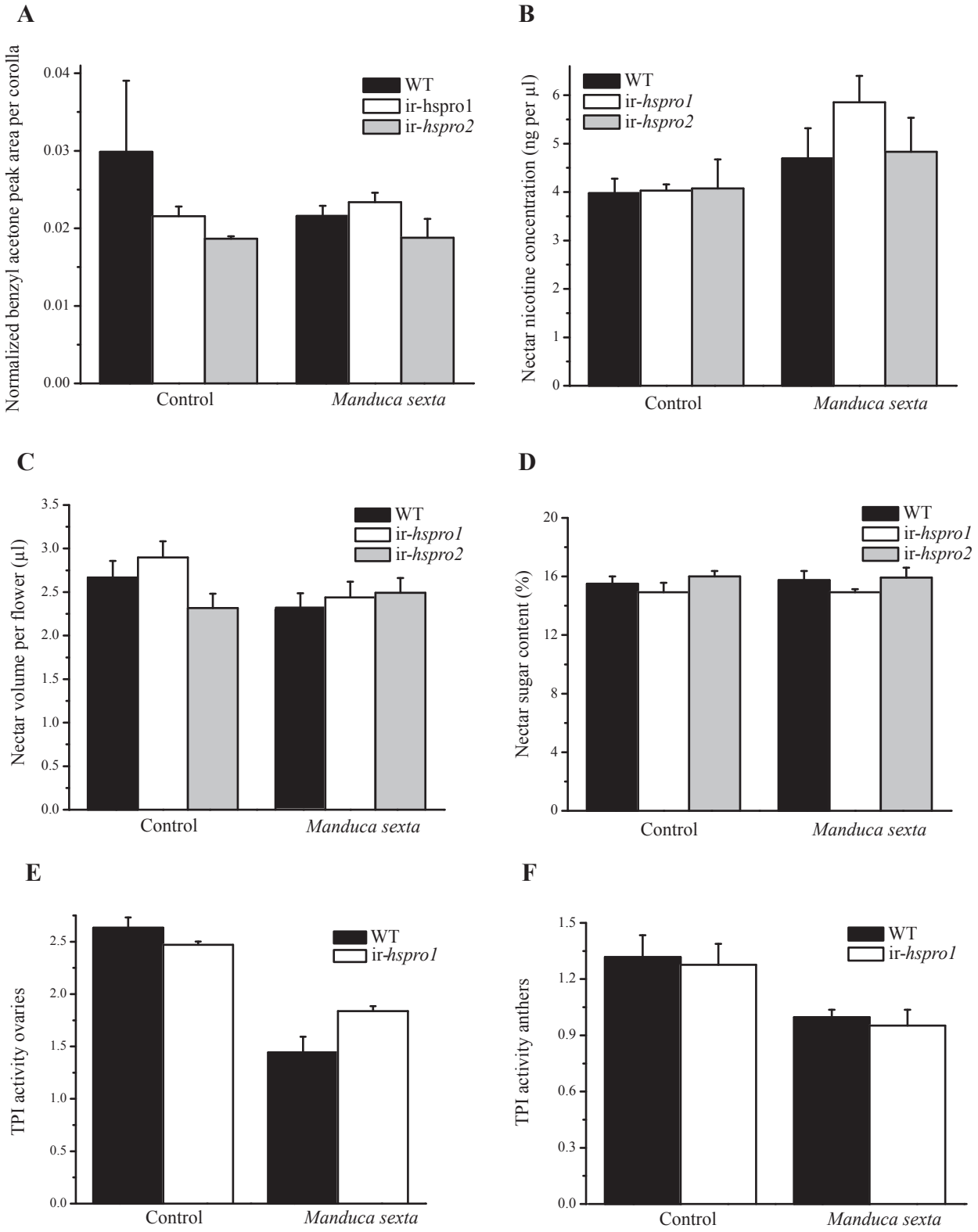
Supplemental Figure S3. Analysis of *HSPRO* expression in WT plants and in transgenic plants reduced in MAP kinase expression.

(a) Leaves of rosette-stage transgenic *N. attenuata* plants with reduced levels of SIPK (salicylic acid induced protein kinase) and WIPK (wound induced protein kinase) expression as well as control WT plants were elicited with synthetic 18:3-Glu. Total RNA was extracted from treated leaves at different times and *HSPRO* transcript levels were quantified by qPCR. *HSPRO* mRNA levels are expressed relative to the levels of the reference gene Na-*EF1A*. Quantification was performed by the ΔCt method (n=3; bars= ±S.E.). One way-ANOVA with Tukey post-hoc test (WT vs. ir-*sipk*); **: P<0.01. **(b)** Rosette stage WT plants were sprayed with 300 µM SA or control solution (see Materials and Methods for details). Leaves were harvested at different times and total RNA was extracted. *HSPRO* transcript levels were quantified as in (a) (n=3; bars= ±S.E.). Student's t-test (SA vs. control treatment); ***: P<0.001.



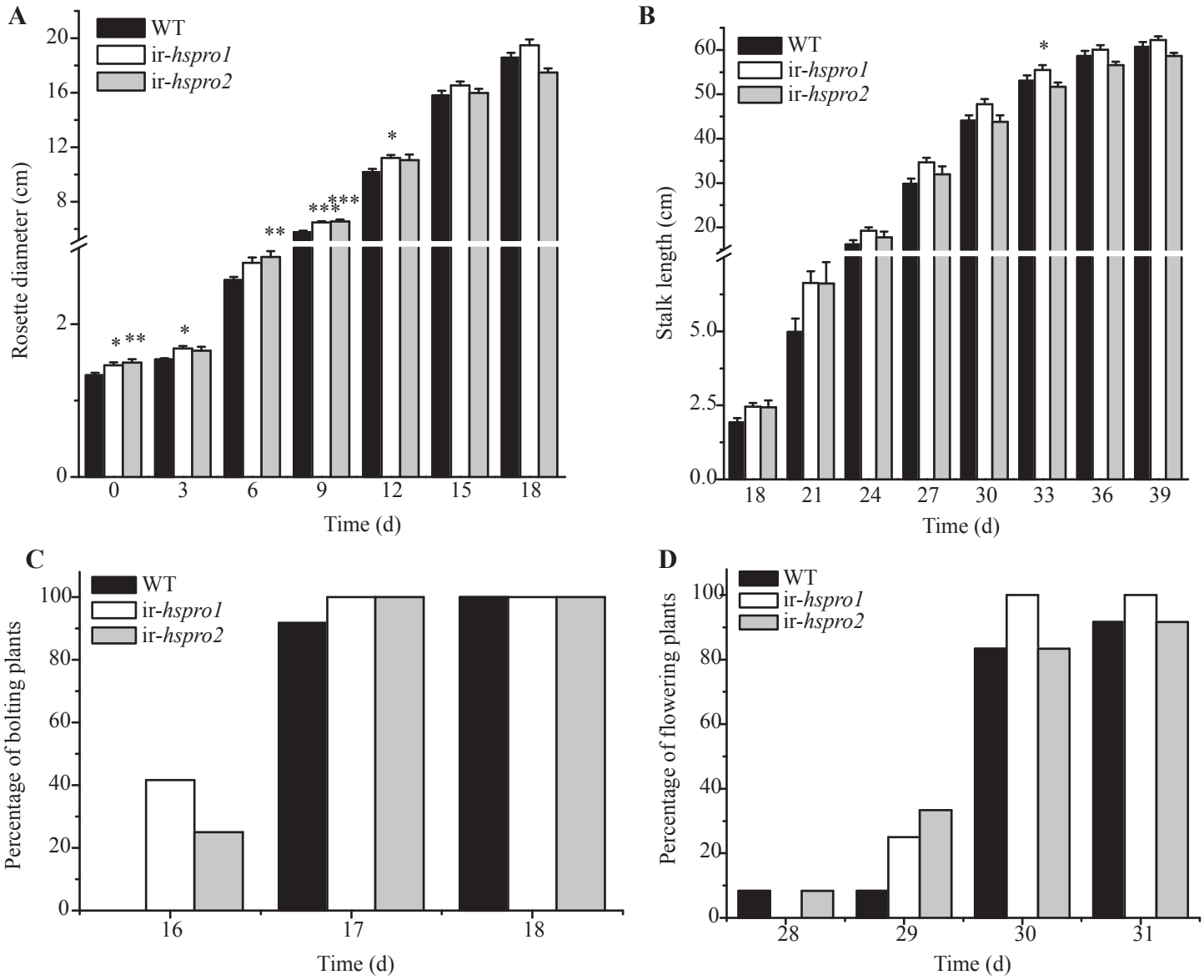
Supplemental Figure S4. Analysis of defense responses against *M. sexta* in WT and ir-*hspro* plants.

(a) Freshly hatched *M. sexta* neonates were placed on leaves of rosette stage *N. attenuata* WT and ir-*hspro* plants (one neonate per plant) and the caterpillar masses were quantified every 3 days for a period of 15 days (*n*=22 to 67; bars= ±S.E.). **(b,c,d)** Leaves from rosette stage WT and ir-*hspro1* plants were elicited with *M. sexta* OS once a day for three consecutive days. After three days (six days from the start of the treatment), leaves were harvested and used for quantification of defense metabolites by HPLC-UV. Control samples were leaves from untreated plants harvested either before the start of the treatment (0 days) or after three days (3 d) (*n*=5; bars= ±S.E.; (b) Nicotine; (c) Chlorogenic acid; (d) Rutin).

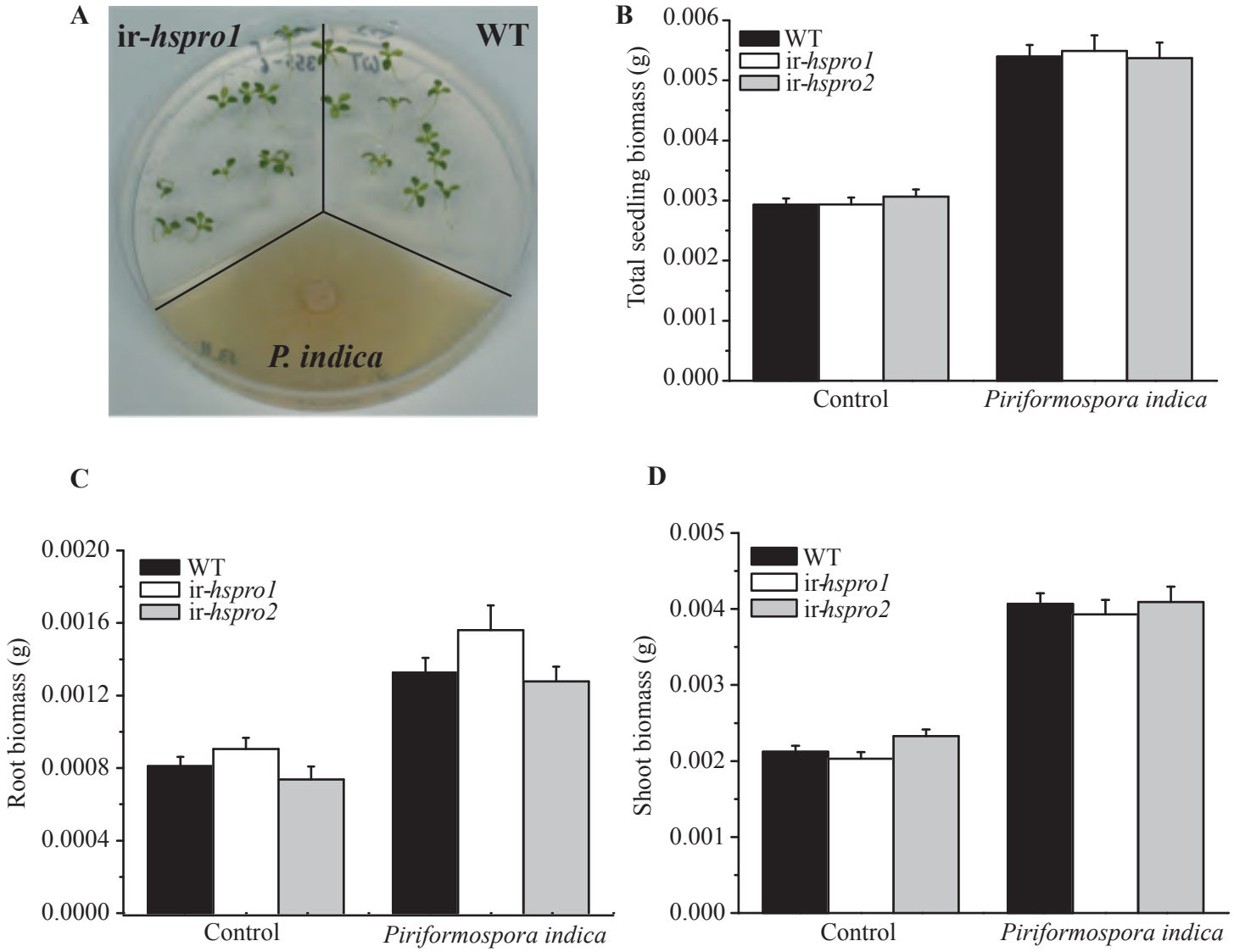


Supplemental Figure S5. Analysis of flower traits associated with the interactions of *N. attenuata* plants with insects.

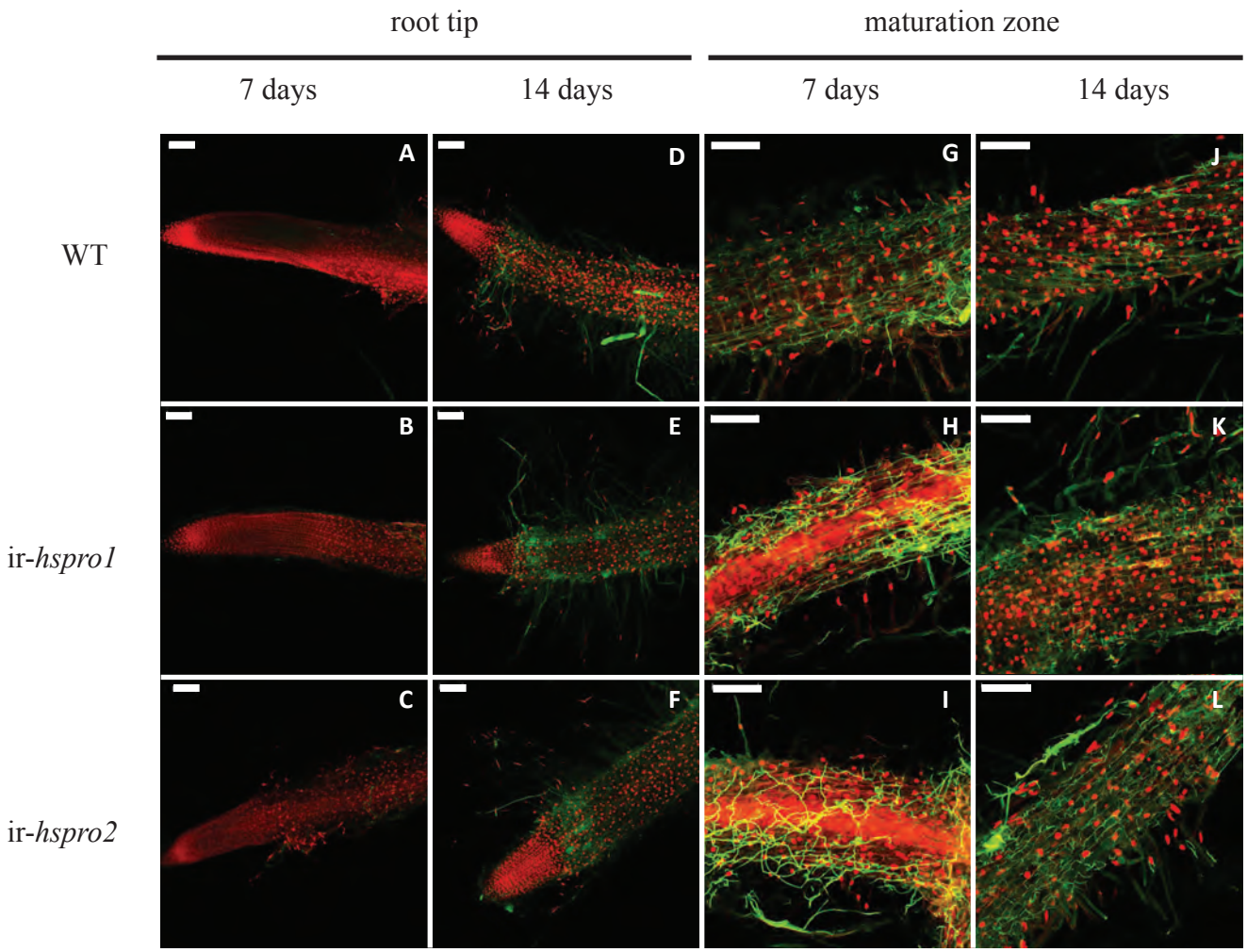
N. attenuata WT and ir-*hspro* plants were challenged with *M. sexta* larvae for 15 consecutive days. Un-attacked plants were used as controls. **(a)** After removal of the caterpillars from the plants, 30 corollas from night opening flowers per genotype and treatment were collected. Samples consisting of 10 pooled corollas (*n*=3 per genotype per treatment) were extracted with dichloromethane and benzyl acetone levels were analyzed by GC-MS. Quantification was performed with tetraline as the internal standard. Bars denote S.E. **(b)** After removal of the caterpillars from the plants, the nectar from 10 individual flowers was pooled into a 1.5 mL tube to form one sample. The nectar volume was quantified with a glass capillary (*n*=3 [3x10 corollas]; bars= ±S.E.). **(c)** Nicotine levels in nectar collected in (b) were quantified by LC-MS using $^{2}H_{3}$-nicotine as internal standard (*n*=3; bars= ±S.E.). **(d)** Nectar collected in (b) was also used to determine sugar content with a refractometer (*n*=3; bars= ±S.E.). **(e,f)** After removal of the caterpillars from the plants, ovaries (e) and anthers (f) from 10 flowers were collected into a 1.5 mL tube to form one sample. Samples were used for protein extraction and measurement of trypsin proteinase inhibitor (TPI) activity (*n*=5; bars= ±S.E.).



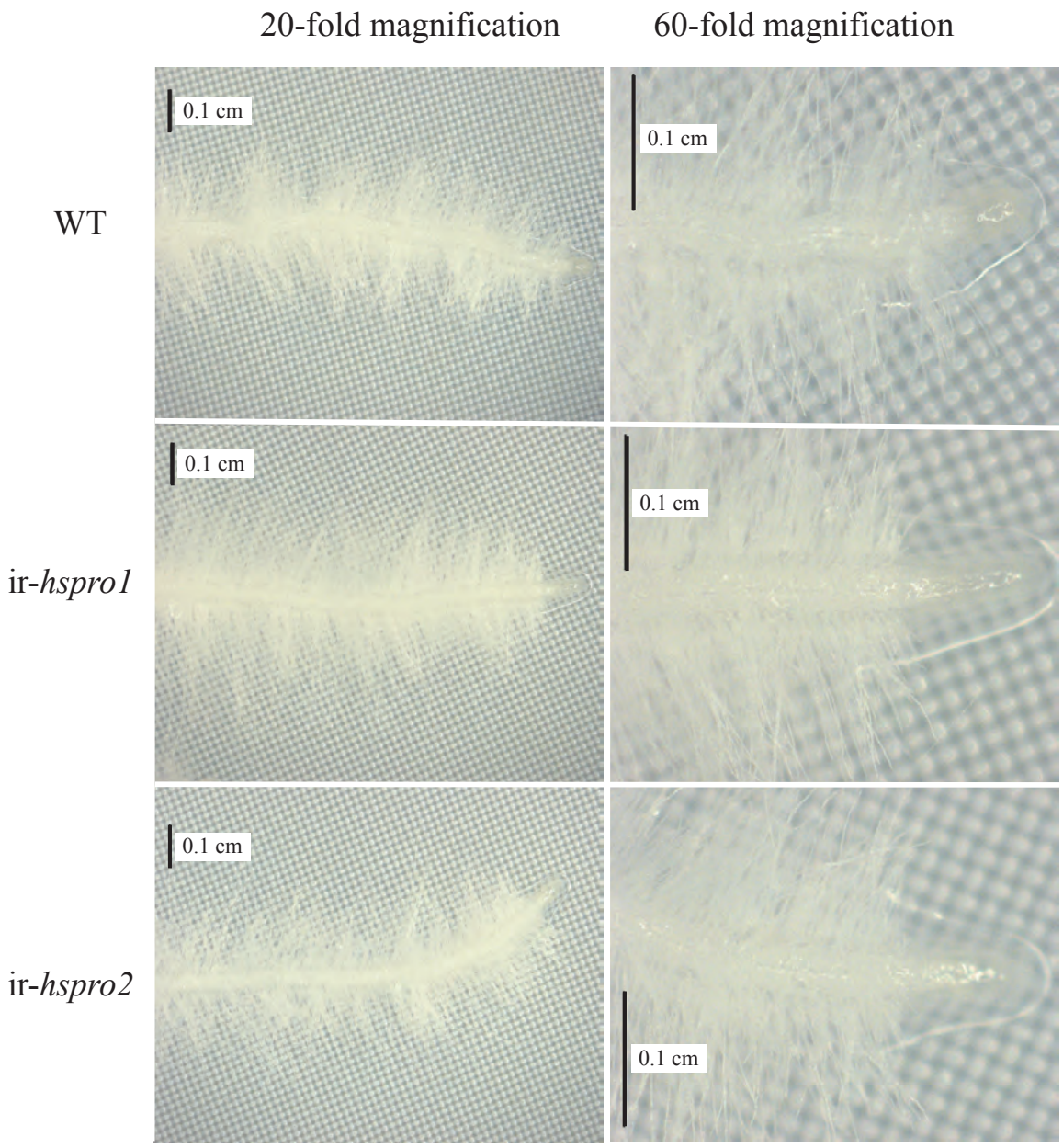
Supplemental Figure S6. Analysis of growth and developmental parameters of *P. indica*-colonized plants grown in the glasshouse.
WT and ir-*hspro* seedlings were grown on plates in the presence of *P. indica* for 14 days and the seedlings were then transferred to soil in 1L pots and grown in the glasshouse under standard growth conditions. **(a)** Rosette diameter (distance between the two longest leaves) and **(b)** stalk length were measured every three days. One way-ANOVA with Tukey post-hoc test (WT vs. ir-*hspro*); *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; $n=12$ per genotype (bars= ±S.E). **(c)** Bolting time (determined as the appearance of the reproductive meristem) and **(d)** flowering time (determined as the appearance of the first opened bud) were recorded daily ($n=12$ per genotype).



Supplemental Figure S7. Analysis of growth promotion of WT and ir-*hspro* seedlings induced by *P. indica* in a split-plate system.
(a) WT and ir-*hspro* seedlings and *P. indica* were grown in separate compartments of split Petri dishes (10 WT and 10 ir-*hspro* seedlings per plate) for 14 days. Plates without *P. indica* but with fungal media were used as controls. **(b,c,d)** Determination of fresh biomasses of total seedlings (b), shoots (c), and roots (d) was performed with a microbalance (n=26 to 29; bars= ±S.E.).



Suppl. Figure S8. Laser confocal microscopy analysis of roots from *P. indica*-colonized WT and ir-*hspro* seedlings.
(a-l) Root samples from seedlings grown in the presence of *P. indica* for 7 and 14 days were stained with WGA-AF488 (fungal structures; green) and propidium iodide (cell walls; red). Images were taken with a laser confocal microscope equipped with an argon laser. Excitation/detection was at 488/500-540 nm for WGA-AF488 and at 560/580-660 nm for propidium iodide. White bar: 100 µm.



Supplemental Figure S9. Root morphology of *P. indica*-colonized WT and ir-*hspro* seedlings.
WT and ir-*hspro* seedlings were grown in the presence and absence of *P. indica* for 14 days on a plate system. Seedling roots were visualized under a stereomicroscope at 20- and 60-fold magnifications. Bars represent 0.1 cm.

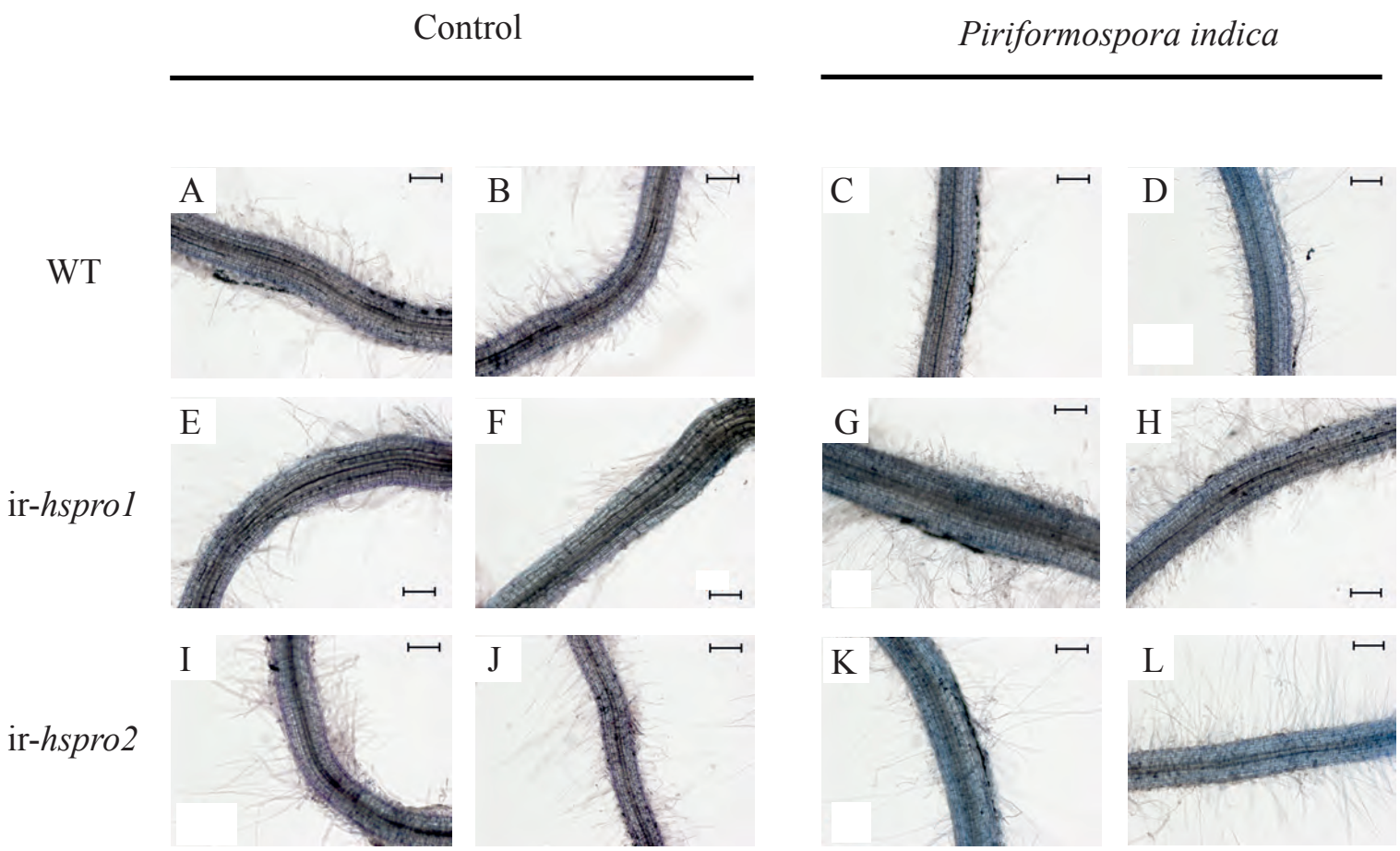
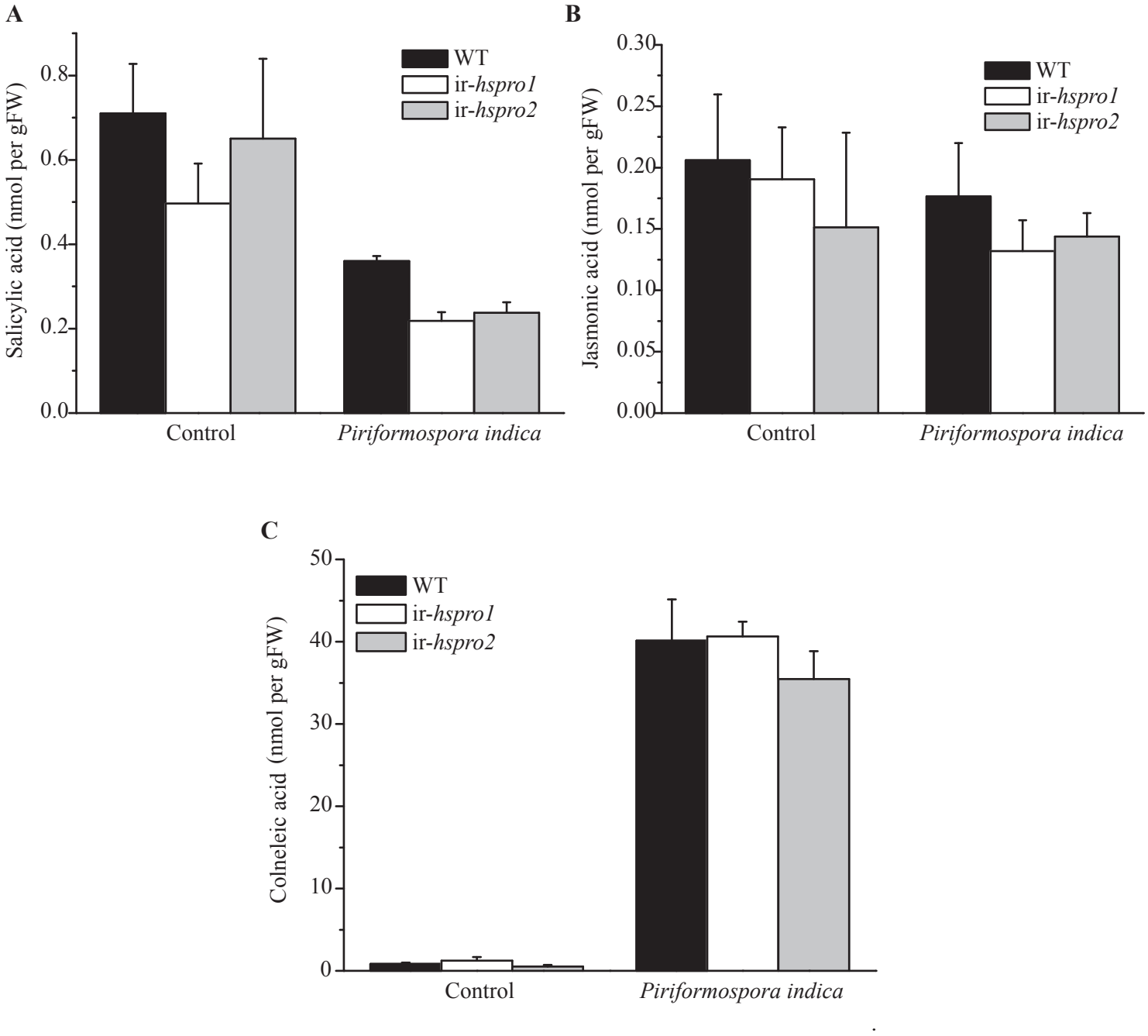


Figure S10. Analysis of root cell death in WT and ir-*hspro* seedlings.

WT and ir-*hspro* seedlings were grown in the absence (control) and presence of *P. indica* for 14 days on a plate system. Roots were excised from the seedlings and stained with trypan-blue. Images were taken with a Zeiss Imager.Z1 microscope using the AxioVision software. Bars represent 200 µm.



Supplemental Figure S11. Analysis of SA, JA and CA levels in roots of WT and ir-*hspro* seedlings. WT and ir-*hspro* seedlings were grown in the presence and absence of *P. indica* for 14 days on a plate system. Root samples were collected from the seedlings and the levels of **(a)** SA, **(b)** JA and **(c)** CA were quantified by LC-MS (n=3; bars= ±S.E.).

Supplemental Table SI. Analysis of WT and ir-*hspro* seedling's biomasses during *P. indica*-root colonization and control treatments.

Days of seedling growth	Genotype	Seedling weight (mg) ± SE [control]	*n*	Seedling weight (mg) ± SE [*P. indica*]	*n*	% growth promotion (*P.indica* vs. control)	% differential growth promotion (ir-hspro vs. WT)	*P*-value[1] (control vs. P. indica)	*P*-value[2] (WT vs. ir-hspro)
10	WT	14.2±0.9	16	30.4±1.6	20	**114**		6.33×10^{-13}	
	ir-hspro1	14.1±0.6	17	37.0±0.9	18	**162**	**42**	6.27×10^{-13}	3.62×10^{-3}
	ir-hspro2	13.8±0.4	15	41.2±0.2	18	**198**	**73**	6.27×10^{-13}	6.72×10^{-8}
	ir-hspro3	16.1±0.8	18	38.8±1.5	20	**140**	**23**	6.27×10^{-13}	2.70×10^{-5}
10	WT	20.8±2.1	20	28.6±1.8	20	**37**		0.03	
	ir-hspro1	22.8±1.7	18	35.6±1.8	18	**56**	**51**	6.13×10^{-5}	0.08
	ir-hspro2	20.9±2.0	20	32.4±1.3	20	**55**	**48**	1.62×10^{-4}	0.64
14	WT	14.5±0.8	12	28.6±1.0	17	**97**		5.77×10^{-13}	
	ir-hspro1	14.4±0.3	9	32.1±0.7	17	**123**	**26**	5.77×10^{-13}	0.02
	ir-hspro2	15.3±0.6	12	32.6±1.0	18	**113**	**17**	5.77×10^{-13}	4.08×10^{-3}
14	WT	13.2±0.9	10	39.6±1.9	24	**200**		1.97×10^{-12}	
	ir-hspro1	14.9±0.6	10	47.7±1.7	21	**220**	**10**	5.72×10^{-13}	3.51×10^{-3}
14	WT	13.5±0.7	10	38.9±1.9	11	**189**		6.27×10^{-13}	
	ir-hspro1	14.8±0.8	11	49.0±2.4	9	**231**	**22**	6.26×10^{-13}	2.05×10^{-4}
	ir-hspro2	14.7±0.4	7	47.8±1.5	6	**223**	**18**	6.26×10^{-13}	6.31×10^{-3}
14	WT	15.5±0.4	15	52.3±1.8	26	**245**		5.94×10^{-13}	
	ir-hspro1	14.4±0.6	20	60.1±1.7	22	**302**	**23**	5.94×10^{-13}	1.99×10^{-3}
	ir-hspro2	14.1±0.3	23	59.9±2.0	24	**302**	**23**	5.94×10^{-13}	1.93×10^{-3}
14	WT	11.5±0.3	9	37.2±2.5	30	**223**		1.73×10^{-6}	
	ir-hspro1	12.0±1.0	10	53.9±3.2	30	**349**	**56**	8.50×10^{-13}	5.41×10^{-4}
	ir-hspro2	13.4±0.9	10	47.9±2.6	30	**257**	**15**	1.38×10^{-10}	0.04

[1,2:] One way-ANOVA with Turkey post-hoc test (WT vs. ir-*hspro*).

Supplemental Table SIV. List of ions with differential accumulation in *P. indica*-colonized roots of ir-*hspro* compared to WT seedlings (positive mode of ionization).

Identity	log_2(FC[a])	*P*-value[b]	*m/z*	RT[s][c]	Mean peak intensity (ir-*hspro*)	S.E.	Mean peak intensity (WT)	S.E.
Unknown	-1.45	0.0348	381.789	437.273	131.929	81.947	400.507	61.602
Unknown	-1.12	0.0356	382.264	416.608	88.699	49.445	299.992	49.642
Unknown	-1.07	0.0376	450.199	165.750	77.202	43.625	272.628	50.775

[a]: FC: fold-change (ir-*hspro* vs WT)

[b]: *P*-value: Fisher´s t-test (ir-*hspro* vs WT); *n*=5

[c]: RT[s]: retention time (seconds).

Supplemental Table SV. List of primers.

Gene name	template	experiment	Forward 5'→3'	Reverse 5'→3'
Na*TEF1a*/ Na*tef1a*	cDNA/ gDNA	qRT-PCR	ACACTTCCCACATTGCTGTCA	AAACGACCCAATGGAGGGTAC
Na*HSPRO*	cDNA	qRT-PCR	CTGATCCTAGACCGTATGCGAACA	CTGACAACCGTCGTCTCATCAGA
Pi*tef1a*	gDNA	qRT-PCR	TCGTCGCTGTCAACAAGATG	GGATACAACCCCAAGACGGT
Na*HSPRO* (ORF)	cDNA	HSPRO- EGFP fusion product	ATGGTTGATTGCGATAGAAAGACAAAG ATGATATC	GGCCTTGTTACTTCTCTCTGGACTATACT TG
Na*HSPRO* (ORF + partial attB sequence)	PCR product	HSPRO- EGFP fusion product	AAAAAGCAGGCTATGGTTGATTGCGAT AG	AGAAAGCTGGGTTGGCCTTGTTACTTC
Na*HSPRO* (ORF + complete attB sequence)	PCR product	HSPRO- EGFP fusion product	GGGGACAAGTTTGTACAAAAAAGCAGG CT	GGGGACCACTTTGTACAAGAAAGCTGG GT
nptII	*nptII*- contai-ning plasmid	Probe (Southern-blot)	CCGGATCGGACGATTGCG	CGTCTGTCGAGAAGTTTCTG
At*RGA* (ORF + partial attB sequences)	cDNA	RGA-eGFP fusion product	AAAAAGCAGGCTTGAAGAGAGATCATC ACCAA	AGAAAGCTGGGTTGTACGCCGCCGTCG AGA
DCL2	gDNA	positivecontrol (diagno-stic PCR)	AAGGATGGCTCATTCCTGGTG	AGAGCTTCAACAAGCAGAGAAGG
Na*HSPRO* inverted repeat construct 5' end	gDNA	diagnostic PCR	GGAAGTTCATTTCATTTGGAG	CATACTAATTAACATCACTTAAC
Na*HSPRO* inverted repeat construct 3' end	gDNA	diagnostic PCR	GGTAACATGATAGATCATGTC	GCGAAACCCTATAGGAACCC
Na*HSPRO*	cDNA	3'-RACE	CTGATCCTAGACCGTATGCG	According to Invitrogen Kit
Na*HSPRO*	cDNA	3'-RACE	TCGAAAACCGAACGCTGTAC	According to Invitrogen Kit
Na*HSPRO*	cDNA	3'-RACE	CGACACACCAGATCCTCGAA	According to Invitrogen Kit
Na*HSPRO*	cDNA	5'-RACE	According to Invitrogen Kit	AAGCATTTTTGACGCGTAGATCC
Na*HSPRO*	cDNA	5'-RACE	According to Invitrogen Kit	GCACAACAGAGCTATG
Na*HSPRO*	cDNA	5'-RACE	According to Invitrogen Kit	TCCATCGCCAACGATTCAAGCCTC
Na*HSPRO*	cDNA	Generation of ir-hspro construct	GTCGACACGACGGTTGTCAGCCAGAC	GGATCCGTAGATCCATGTTTCGAGGATC

Supplemental Experimental Procedures S1

Full length cDNA cloning, EGFP fusion protein generation, and sequence analysis

For the cloning of the full length *HSPRO* cDNA sequence, 5 µg of total RNA were isolated from leaves of *N. attenuata* plants. The 3´RACE and 5'RACE Systems for Rapid Amplification of cDNA Ends (Invitrogen, Karlsruhe, Germany) were used following the manufacturer's instructions and the primers listed in Supplemental Table SV. The PCR products were cloned into the pGEM-T easy vector (Promega, Madison, WI) and sequenced using universal primers. Sequence alignments and phylogeny analysis were performed using BLAST (http://blast.ncbi.nlm.nih.gov) and the Geneious Pro software (version 5.4; Drummond *et al.*, 2011). Phylogenetic analysis was performed with the Jukes-Cantor genetic distance model and the Neighbor Joining tree building method with bootstrapping (602 random seed, 100 replicates and 50% support threshold).

To generate C-terminal EGFP fusion proteins, the HSPRO protein coding sequence was amplified by PCR from *N. attenuata* cDNA and the nuclear localized DELLA protein RGA (Silverstone *et al.*, 2001) was amplified from Arabidopsis Col-0 cDNA (primers listed in Supplemental Table SV). PCR products were cloned into the pJET1.2 vector (Fermentas, East Lansing, MI) and transformed into *E. coli* TOP10 strain by electroporation using standard conditions. The Gateway vector pDONR221 (Invitrogen) was used as the entry vector and p2GWF7 (Karimi *et al.*, 2007) as the destination vector for C-terminal fusions to EGFP. Leaf Arabidopsis protoplasts were isolated and transformed by the polyethylene glycol (PEG) method as previously described (Yoo *et al.*, 2007). Protoplasts were incubated for 15 h in the dark at room temperature before visualization with a Zeiss Axioplan fluorescence microscope (Carl Zeiss, Jena, Germany) using standard settings for EGFP.

Genomic DNA extraction for qPCR analysis of *P. indica* root colonization

Genomic DNA from *N. attenuata* seedlings was extracted with 2 mL of extraction buffer (2 % (w/v) CTAB, 100 mM Tris-HCl pH:8.0, 20 mM EDTA, 1.4 M NaCl, 2 % (w/v) polyvinylpyrrolidone and 0.5% (v/v) 2-mercaptoethanol) pre-heated to 65ºC. Samples were kept at 65ºC for 1 h and repeatedly mixed by inverting the tube. After centrifugation (8,000 *g* for 5 min), the supernatant was collected and transferred into 50 mL plastic tubes. This initial extraction step was repeated twice. The pooled supernatants were extracted twice with 1/3 volumes (~0.6 mL) of chloroform:isoamyl alcohol (24:1) by continuously inverting the tube for 10 min and centrifugation (8,000 *g* for 10 min). 0.1 volumes (~60 µL) of 10% (w/v)

CTAB solution were added to the sample and after mixing by inverting the tube, 1.4 volumes (~1 mL) of precipitation buffer (1% (w/v) CTAB, 50 mM Tris-HCl pH=8.0, 10 mM EDTA) were added and mixed gently. After an overnight incubation, the tubes were centrifuged at 4,500 *g* for 15 min. The supernatant was removed and the pellet dissolved in 500 µL of high-salt TE (10 mM Tris-HCl pH=8.0, 0.1 mM EDTA, 1 M NaCl) containing 0.5 µg mL^{-1} RNAse-A for 20 min at 37ºC. The genomic DNA was precipitated by mixing the sample with 1 volume (500 µL) of isopropanol and incubating the mixture for 30 min. The samples were centrifuged at 16,100 *g* for 30 min and the pellet was washed with 500 µL of 70% (v/v) ethanol and finally dissolved in 5 µL of deionized water. The genomic DNA concentration was estimated by absorbance and by comparing the signal intensities on a 0.8% (w/) agarose gel to the intensities of a DNA ladder standard of known concentration.

Quantification of defense and flower-associated traits

In the morning (6 to 8 am), the nectar from 10 flowers was collected and pooled into a 1.5 mL tube to form one sample. The nectar volume per sample was determined with a graduated glass capillary. Nectar sugar content was measured with a refractometer using a sucrose standard curve. Nectar nicotine was quantified by LC-MS (liquid chromatography-mass spectrometry; Varian 1200 Triple-Quadrupole-LC-MS system; Varian, Palo Alto, CA) (see below) from samples containing 2 µL of nectar dissolved in 400 µL of deionized water spiked with 50 pg [$^{2}H_{3}$]nicotine.

For analysis of benzyl acetone (BA) in corollas, 10 corollas from recently opened (10 to 12 pm) night flowers were pooled per sample. The samples were frozen in liquid nitrogen and homogenized in 15 mL glass vials after adding 2 mL dichloromethane containing 2 µg tetraline mL^{-1}. Corolla tissue was spun down by centrifugation at 720 *g* for 10 min at room temperature. The supernatant was transferred into new glass vials with a Pasteur pipette and washed by adding 2 mL of deionized water. An aliquot from the organic phase was transferred into a glass vial and analyzed by GC-MS with a CP-3800 GC instrument (Varian 4000) equipped with a DB-Wax column (Agilent) as previously described (Re *et al.*, 2011). For identification of the benzyl acetone peak, the retention time and mass spectra were compared to a commercial standard (Sigma). Three biological replicates were used per genotype and treatment.

TPI activity from anthers and ovaries was quantified as previously described (Van Dam *et al.*, 2001). Anthers and ovaries from 10 flowers were pooled per sample and 5 samples were analyzed per genotype and treatment. Quantification of nicotine, rutin, and

chlorogenic acid after *M. sexta* OS elicitation of leaves was performed as previously described (Keinänen *et al.*, 2001). Leaves of rosette-stage plants were elicited by wounding and *M. sexta* OS elicitation once per day for three consecutive days and leaf samples were harvested at the end of the third day (six days after the start of the treatment).

Phytohormone and divinyl ether extraction and quantification

One hundred mg of *P. indica*-colonized roots (from seedlings grown for 14 days in the plate system) were homogenized to a fine powder with a Geno/Grinder 2000 (BTC and OPS Diagnostics, Bridgewater, USA) in the presence of liquid nitrogen. Each sample (biological replicate) consisted of roots pooled from 6 to 11 seedlings and 3 biological replicates per genotype per treatment were used. One mL of ethyl acetate spiked with 200 ng [$^{2}H_2$]JA, [$^{2}H_4$]SA, and [$^{2}H_6$]ABA as IS was used for extraction. The samples were centrifuged for 15 min at 12,000 *g* (4°C) and the upper organic phase was transferred into a fresh tube. The residual leaf material/aqueous phase was re-extracted with 0.5 mL ethyl acetate without IS. The organic phases were pooled and evaporated to dryness under reduced pressure. The dry residue was reconstituted in 0.4 mL of 70/30 (v/v) methanol/water for analysis by LC-MS (Varian 1200) as previously described (Bonaventure *et al.*, 2011).

For the analysis of ET, seedlings from WT and ir-*hspro* plants were weighed and transferred into a 250 mL glass vessel. Ten seedlings were placed in each glass vessel and a total of three glass vessels (*n*=3) were used per genotype. After a 5 h incubation period (glass vessels were kept in the growth chamber under the same conditions as the agar plates), the headspace of the vessels was flushed into a laser photo-acoustic spectrometer (PAS; INVIVO, Adelzhausen, Germany) for determination of ET levels (nL $h^{-1}g^{-1}$ FW) as previously described (Körner *et al.*, 2009).

Metabolic profiling of roots

P. indica-colonized roots from ir-*hspro* and WT seedlings (grown for 14 days in the plate system) were collected for metabolic profiling. Ten roots were pooled per sample and 5 samples (biological replicates) per genotype were used. Root tissue was ground with a Geno/Grinder 2000 in the presence of liquid nitrogen and thoroughly extracted with 1 mL of 40% (v/v) methanol/50 mM aqueous sodium acetate buffer (pH: 4.8) per 100 mg of root tissue. Homogenized samples were centrifuged at 12,000 *g* for 20 min at 4°C, the supernatant was transferred into a fresh 1.5 mL microcentrifuge tube and the samples were centrifuged again using the same conditions. 100 µL of the supernatant were transferred into 2 mL glass

vials for analysis by UPLC-ToF-MS (ultra-pressure-liquid-chromatography time-of-flight mass spectrometry; Bruker Daltonik GmbH, Bremen, Germany) as previously described (Gilardoni *et al.*, 2011).

Microarray analysis

P. indica-colonized roots from ir-*hspro* and WT seedlings (grown for 14 days in the plate system) were collected for microarray analysis. Ten roots were pooled per sample and 3 samples (biological replicates) per genotype were used. Total RNA was extracted as previously described (Kistner and Matamoros, 2005) and RNA quality was checked by spectrophotometry (NanoDrop, Wilmington, DE). Genomic DNA was removed by DNAse treatment following commercial instructions (Turbo DNase; Ambion, Europe), RNA was cleaned up with RNeasy MinElute columns (Qiagen, Hilden, Germany) and the RNA quality was checked with the RNA 6000 Nano kit (Agilent, Santa Clara, CA) using an Agilent 2100 Bioanalyzer. Total RNA was used to generate labeled cRNA with the Quick Amp labeling kit (Agilent) following commercial specifications and the yield of cRNA was determined spectrophotometrically (NanoDrop). Labeled cRNA was hybridized using the Gene Expression Hybridization kit (Agilent) following commercial instructions onto a 44K custom designed 60mer *N. attenuata* Agilent microarrays as previously described (Gilardoni *et al.*, 2011; Kallenbach *et al.*, 2011). Hybridization, washing and analysis were performed as previously described (Gilardoni *et al.*, 2011; Kallenbach *et al.*, 2011). Three biological replicates were used per treatment with a total of six arrays (see Accession numbers). Data was extracted using the Agilent Feature Extraction software (version 9.5) and analyzed with the SAM (Significance Analysis of Microarrays) software (Tusher *et al.*, 2001). The *q*-values for each gene corresponded to a computed false discovery rate (FDR) of less than 4%. Changes in gene expression were considered to be significant when the Log_2 of the fold change in signal intensity (ir-*hspro* versus WT) were greater than 1 or smaller than -1.

Root and fungus staining

Root samples from seedlings grown in the presence of *P. indica* for 7 and 14 days were fixed in 0.15 % (w/v) trichloroacetic acid in 4:1 (v/v) ethanol/chloroform as previously described (Deshmukh *et al.*, 2006). Samples were stained with WGA-AF488 (fungal structures; green) and propidium iodide (cell walls; red) as previously described (Zuccaro *et al.*, 2011). Confocal laser scanning microscopy was performed using a LSM 510 Meta microscope (Carl Zeiss) equipped with an argon laser. Samples were excited at 488 nm and

light emission detected at 500-540 nm for WGA-AF488. For propidium iodide, samples were excited at 560 nm and light emission detected at 580-660 nm. Macroscopic observations of WT and ir-*hspro* seedling roots grown for 14 days on the plate system in the presence or absence of *P. indica* was carried out with a stereomicroscope (Olympus SZ51). Cell death analysis in roots was performed by trypan-blue staining as previously described (Diaz-Tielas *et al*., 2012)

M. sexta performance assays and OS collection

Larvae of the tobacco hornworm (*Manduca sexta*) were obtained from in-house colonies, generated from *M. sexta* eggs originally purchased from the Carolina Biological Supply (North Carolina, US). Eggs deposited on *N. attenuata* plants were collected and were kept in a growth chamber (Snijders Scientific) at 26°C/16 h day and 24°C/8 h night until the larvae hatched. *M. sexta* and *Spodoptera exigua* OS was collected as described by (Roda *et al.*, 2004). For *M. sexta* larval growth performance assay, freshly hatched neonates were placed carefully on leaves of rosette-stage *N. attenuata* plants (one neonate per plant). A minimum of 30 plants per genotype were used. Caterpillars were weighed every two to three days for two weeks.

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