SUPPLEMENTAL INFORMATION

Plant Hormone Quantification: IAA and SA Extraction and Quantification

Materials

Indole-3-acetic acid (IAA) and salicylic acid (SA) were purchased from Sigma-Aldrich (St. Louis, MO) and Acros Organics (Geel, Belgium), respectively. Isotope-labeled internal standards d_4-salicylic acid (d_4-SA) and indole-2,4,5,6,7-d_5-3-acetic acid (d_5-IAA) were sourced from CDN Isotopes (Pointe-Claire, Quebec). Methanol and acetonitrile (HPLC-grade) were sourced from J.T. Baker (Avantor Performance Materials) and LC-MS grade water was purchased from Honeywell Research Chemicals. Individual stock solutions of unlabeled and labeled compounds were prepared in 50% methanol and stored at -80 °C. Standard solutions were prepared fresh in 30% methanol in the linear concentration ranges of 32 pM to 10 µM. An internal standard solution was prepared in 30% methanol containing 2.5 µM d_5-IAA and d_4-SA.

Plant Hormone Extraction

Frozen plant material was extracted with 900 µL of extraction solvent (ice-cold acetonitrile/methanol; 1:1 v:v) while samples were kept cold on ice. Ten (10) microliters of the internal standard mixture and two stainless steel 5 mm beads were added to each sample tube followed by brief mixing by vortexing. Samples were placed in pre-cooled (-80 °C) Tissue Lyser II racks and homogenized for 2 min at 15 Hz. Samples were centrifuged at full speed for 5 min at 4 °C, then the supernatant was transferred to a new 2 mL tube. Samples were re-extracted with another 900 µL of extraction solvent, and then homogenized again for 2 min at 15 Hz. The

samples were then centrifuged and supernatant transferred as previously described. The extraction solvent was removed under reduced pressure with a speed-vac until completely dry. Samples were reconstituted in 30 % MeOH (200 µL) and mixed thoroughly for 30 min at 4 °C. Finally, samples were filtered through 0.8 µm PES spin-filters and 40 µL of clarified supernatant was transferred to a 96-well microplate. Two (2) µL of sample was injected onto the column.

LC-MS/MS Instrumentation

Clarified samples were analyzed on an Eksigent ekspert™ microLC 200 coupled to a Sciex 6500 QTrap® (Framingham, MA) operated with polarity-switching electrospray ionization. The LC separation was achieved using a Waters (Milford, MA) Acquity UPLC® BEH C18 1.0 × 100 mm, 1.7 µm column kept at 50 °C with a flow rate of 15 µL/min while the autosampler was set at 8 °C. The mobile phases were 0.1 % acetic acid and 3:1 acetonitrile:methanol containing 0.1 % acetic acid running a gradient of 20 % B for 4 minutes ramping to 70 % B at 7 minutes, increasing to 95 % B at 7.5 minutes, holding for 5.5 minutes, then re-equilibrate at initial conditions at 13.5 minutes for 10 minutes (total runtime is 23.5 minutes). Data analysis was completed using MultiQuant 3.0.2 (AB Sciex) by normalizing the peak areas of the unlabeled analytes relative to the peak areas of the labeled internal standards. Calibration curves were linear (*r* values = > 0.99) within the ranges provided above applying a 1/x weighting scheme.

Compound-dependent parameters

Compound	MRM Transition	Retention time (min)	DP (V)	EP (V)	CE (V)	CXP (V)
IAA	176.0 → 130.0	10.24	23	7	40	19
d_5-IAA	181.0 → 134.0	10.22	23	7	25	19
SA	137.0 → 93.0	10.20	-22	-13	-20	-10
d_4-SA	141.0 → 97.0	10.18	-41	-9	-23	-11

Source settings

Ionspray voltage: polarity switching between -4500 V in –ve to 4500 V in +ve

Curtain gas: 15

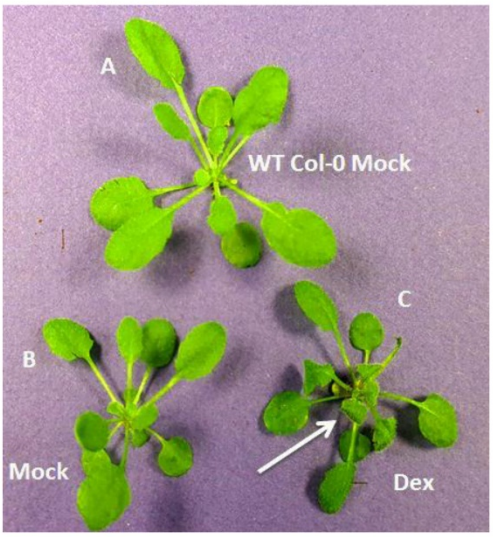
Gas 1: 35

Gas 2: 35

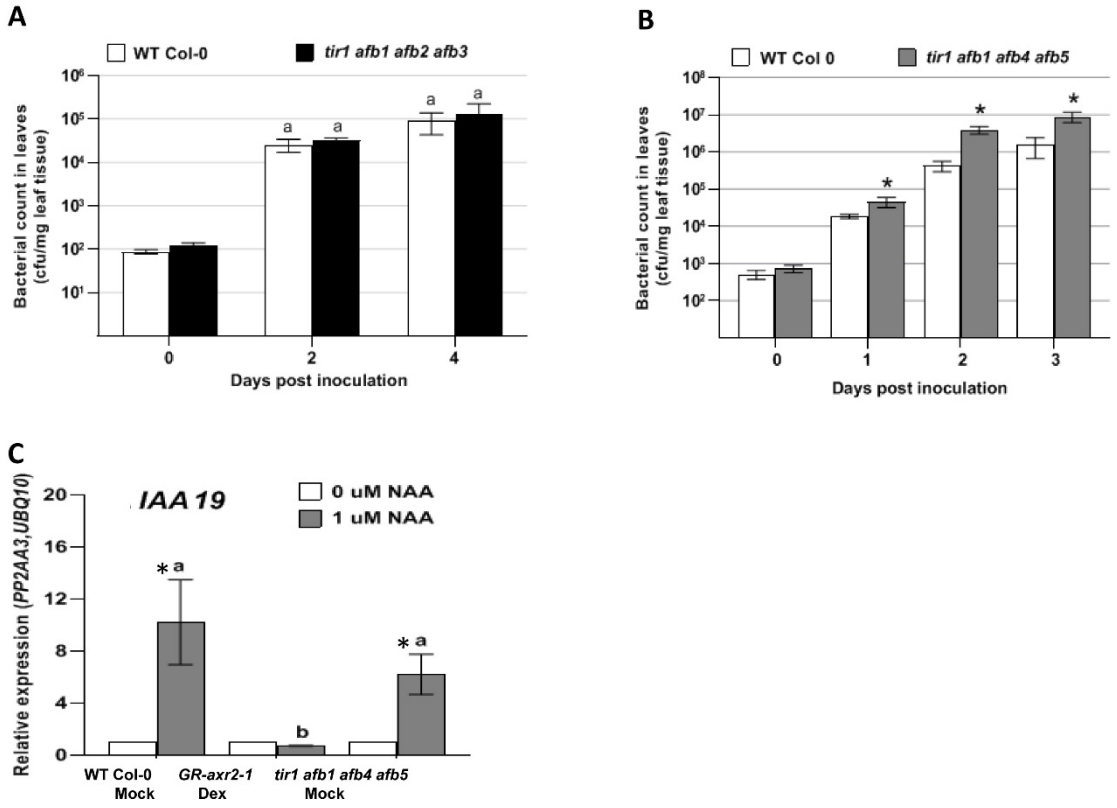
Supplemental Table 1 Primers used in this study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Reference for primers
		RT-qPCR	
GH3.3	TGTGTGAGTTTCACGCCAAT	CAAAGGAGGGACAGAGTGGA	(32)
AT2G23170			
IAA19	GAGATGTGGCAGAGAAGATG	TTCCTCAAATAAGGCACACC	(32)
AT3G15540			
PR1	GGAGCTACGCAGAACAACTAAGA	CCCACGAGGATCATAGTTGCAACTGA	(32)
AT2G14610			
PP2AA3	AACGTGGCCAAAATGATG	AACCGCTTGGTCGACTATCG	(61)
AT1G13320			
UBQ10	CGTTAAGACGTTGACTGGGAAAACT	GCTTTCACGTTATCAATGGTGTCA	(61)
At4g05320			
avrPto	ATGACGGGAGCGTCAGGAATCAAT	ATCCGTTCGGGTTCATAGTCGCAA	(30)
PSPTO_4001			
HrpL	TCAGGAAAGCTGGGAAGAC-GAAGT	ATGTTCGACGGCAGGCAATCAATG	(30)
PSPTO_1404			
mqo	GCGGCTGATGGCTCCATCGAC	CGGGACCGGATTGATGAACGAC	This work
PSPTO_1136			
cmaA	CCGTGATGTTTACCTCTGGCAC	GGACGAGTGATGTACGTAGCTGC	This work
PSPTO_4709			
hcp1	GGTCGACGCAGGCATAACGC	CTCCTTGCCGTCGTTAGTGCG	This work
PSPTO_2539			
tvrR	GGCTCGCAACGGCCCATCTG	CATGCGGTAGACGGCCAGCG	This work
PSPTO_3576			
PSPTO_5415	GCCAGGAAGGGCATGTGCTG	AATCCCTTGATGACCGGCACG	This work
16S rRNA	TAATGGCTCACCAAGGCGACG	TGGCTGGATCAGGCTTTCGC	This work
PSPTO_r01			
rpoD	GAAGTTGACGAAAGCTGGACCG	CGACGGTTGATGTCCTTGATCTC	This work
PSPTO_0537			
gyrB	CTTCAGCTGGGACATTCTGGC	AACCGCCTTCGTACTTGAACAG	This work
PSPTO_0004			
recA	TAGAACTTCAGCGCGTTACC	GCCAACTGCCTGGTTATCT	(36)
PSPTO_4033			
		Genotyping (PCR) and Cloning	
sid2-2 sm108F	TTCTTCATGCAGGGGAGGAG	AAGCAAAATGTTTGAGTCAGCA	(32)
WT- Sm30F	CAACCACCTGGTGCACCAGC		
GR-axr2-1	GR: GCCATCGTCAAAAGGGAAGG		This work
axr2		TGACTCTAACTCGGTAAGGTTCAT	This work
TopoGW_GR__F1	CACCATGATTCAGCAAGCCACTGC		This work
GR_AXR2_R1		TGAGGTTCATAAGTTGGCCGAT	This work
		CATTTTTTGATGAAACAGAAGCT	
GR_AXR2_F1	AGCTTCTGTTTCATCAAAAAATG		This work
	ATCGGCCAACTTATGAACCTCA		
AXR2_V1_R1		TCAAGATCTGTTCTTGCAGTACT	This work

Supplemental Figures

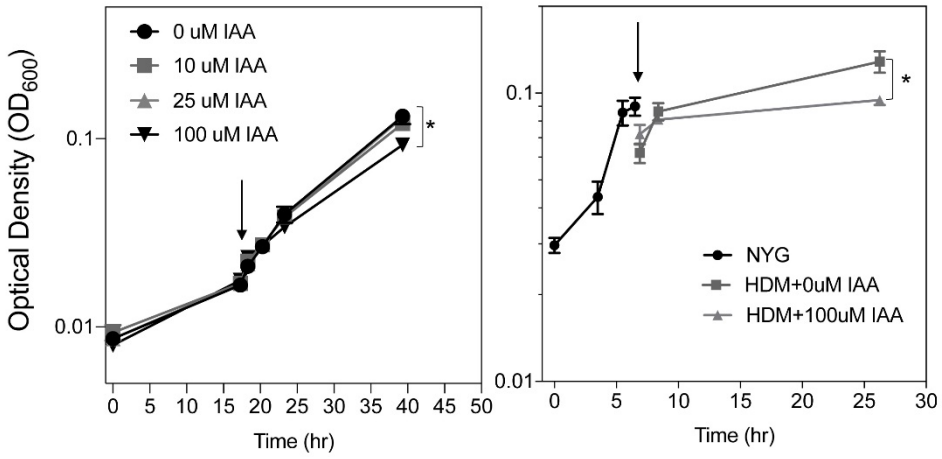


Supplemental Fig. 1. Dex treatment of *GR-axr2*-1 transgenic plants results in abnormal leaf morphology. **(A)** WT Col-0; **(B)** and **(C)** *GR-axr2*-1 transgenic plants, 1 day after spraying with 0.1 % Ethanol (Mock) or 10 mM Dexamethasone in 0.1 % Ethanol (Dex). Arrow indicates curled leaves that did not expand normally.



Supplemental Fig. 2. Growth of *Pto*DC3000 and expression of auxin-responsive genes *IAA19* in auxin receptor mutants.

(A) Growth of *Pto*DC3000 in the *tir1 afb1 afb2 afb3* mutant. Similar results were observed in 3 additional independent experiments. **(B)** Growth of *Pto*DC3000 in WT Col-0 and *tir1 afb1 afb4 afb5* plants. Values are an average ± SEM for data from 4 biological replicates (leaves) for day 0 and 8 replicates for days 1, 2, and 3. Results were analyzed using ANOVA, followed by a Tukey's post test, and asterisks (*) indicates significant differences between WT Col-0 and *tir1 afb1 afb4 afb5* (4X) mutant plants with $P < 0.05$ at each time point. Plants were inoculated with a bacterial suspension of 10^6 cfu ml^{-1}. Samples from this experiment were used for gene expression experiments shown in Fig. 5. **(C)** Expression of the auxin-responsive gene *IAA19* after NAA treatment. Values are an average ± SEM for 3 biological replicates for Col-0 WT Mock, *GR-axr2-1* and *tir1 afb1 afb4 afb5*. Samples indicated by different lower-case letters are significantly different ($p<0.05$). Similar results were obtained in a second independent experiment. * indicates significant difference between treatment (0 and 1 µM NAA) with $p<0.05$. Results were analyzed using ANOVA, followed by a Tukey's post test. Samples indicated by different letters are significantly different at $p<0.05$.



Supplemental Figure 3. Impact of IAA on growth of *Pto*DC3000 in culture

(A) Growth of *Pto*DC3000 in Hrp De-repressing Medium (HDM) containing the indicated concentrations of IAA. IAA was added to the cultures at mid-log (arrow), and growth was monitored by quantifying cell density (OD_{600}) over ~ 24 hours. **(B**) *Pto*DC3000 was grown in NYG to an OD of ~0.1, cells were collected via centrifugation, resuspended in NYG or HDM (arrow) containing the indicated concentrations of IAA and grown for ~24 hours. Growth was monitored by quantifying cell density (OD_{600}). Cells were collected at 1.5 hours after transfer to HDM for RNA isolation. Cell cultures were grown in triplicate for both experiments. Data points are the average of 3 biological replicates per treatment and error bars represent S.E.M. A student's t-test was performed to compare the effect of the IAA treatments on bacterial growth to Mock treatment with DMSO. * $p<0.05$. The data shown in panel B are from cultures used to monitor the effect of IAA on PtoDC3000 gene expression shown in figure 4.