SUPPORTING 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

SUPPLEMENTARY TABLE

Supplementary Table S1. Primers used in this study

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Reference for primers
		RT-qPCR	
GH3.3 *AT2G23170*	TGTGTGAGTTTCACGCCAAT	CAAAGGAGGGACAGAGTGGA	(Mutka 2013)
IAA19 *AT3G15540*	GAGATGTGGCAGAGAAGATG	TTCCTCAAATAAGGCACACC	(Mutka 2013)
PR1 *AT2G14610*	GGAGCTACGCAGAACAACTAAGA	CCCACGAGGATCATAGTTGCAACTGA	(Mutka 2013)
PP2AA3 *AT1G13320*	AACGTGGCCAAAATGATG	AACCGCTTGGTCGACTATCG	(Czechowski et al. 2005)
UBQ10 At4g05320	CGTTAAGACGTTGACTGGGAAAACT	GCTTTCACGTTATCAATGGTGTCA	(Czechowski et al. 2005)
avrPto *PSPTO_4001*	ATGACGGGAGCGTCAGGAATCAAT	ATCCGTTCGGGTTCATAGTCGCAA	(Anderson et al. 2014)
HrpL *PSPTO_1404*	TCAGGAAAGCTGGGAAGAC-GAAGT	ATGTTCGACGGCAGGCAATCAATG	(Anderson et al. 2014)
mqo *PSPTO_1136*	GCGGCTGATGGCTCCATCGAC	CGGGACCGGATTGATGAACGAC	This work
cmaA *PSPTO_4709*	CCGTGATGTTTACCTCTGGCAC	GGACGAGTGATGTACGTAGCTGC	This work
hcp1 *PSPTO_2539*	GGTCGACGCAGGCATAACGC	CTCCTTGCCGTCGTTAGTGCG	This work
tvrR *PSPTO_3576*	GGCTCGCAACGGCCCATCTG	CATGCGGTAGACGGCCAGCG	This work
PSPTO_5415	GCCAGGAAGGGCATGTGCTG	AATCCCTTGATGACCGGCACG	This work
16S rRNA *PSPTO_r01*	TAATGGCTCACCAAGGCGACG	TGGCTGGATCAGGCTTTCGC	This work
rpoD *PSPTO_0537*	GAAGTTGACGAAAGCTGGACCG	CGACGGTTGATGTCCTTGATCTC	This work

gyrB	CTTCAGCTGGGACATTCTGGC	AACCGCCTTCGTACTTGAACAG	This work
PSPTO_0004			
recA	TAGAACTTCAGCGCGTTACC	GCCAACTGCCTGGTTATCT	(Smith et al.2018)
PSPTO_4033			
Genotyping (PCR) and Cloning			
sid2-2 sm108F	TTCTTCATGCAGGGGAGGAG	AAGCAAAATGTTTGAGTCAGCA	(Mutka 2013)
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