

New Phytologist Supporting Information

Article title: Aldoximes are precursors of auxins in Arabidopsis and maize Authors: Veronica C. Perez¹, Ru Dai¹, Bing Bai¹, Breanna Tomiczek², Bryce C. Askey¹, Yi Zhang⁴, Garret M. Rubin⁴, Yousong Ding⁴, Alexander Grenning², Anna K. Block³, and Jeongim Kim^{1,5} Article acceptance date: 28 April 2021

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Α

Data from A high resolution map of the Arabidopsis thaliana developmental transcriptance based on RNA-seg profiling: Klepikova et al., 2016, Plant J. BBIJ059-1070. Total RNA was extracted with RNeasy Plank Kin and Illumina CAMA Bibraries were generated using the respective maniforturer's protocols. CDAR was then sequenced using Illumina HIS+R2000 with a 50bp read length. The read data are publicly available in HCB1's Sequence Read Archive under the BioProject ID 314076 (accession) PDIAR214000, Reads were signed to the reference TARISO genome (Lamesch et al., 2012), using Tophat (Tragning Hist al., 2006), Delaxit Tophist attings and Jib pressure. 2015). Reads were filtered so that only uninterrupted reads corresponding to a region within exactly one gene were used for RPKM calculation. If a gene's expression level is not displayed, this indicates the reads for this gene did not pass the filtering criteria. RPKM values were compiled using an in-house R script. Powered by CARISON DATA State State



Fig. S1. Expression profile of *CYP79A2* in *Arabidopsis thaliana*. A) Whole-plant expression profile. B) Seed-specific expression profile. Images from TAIR.



Fig. S2. LC-MS confirmation of the identity of an unknown peak as benzyl glucosinolate. A) MS trace of benzyl glucosinolate standard. B-C) TIC of WT (B) and the *CYP79A2* overexpression line (C) showing an unknown peak in *CYP79A2* overexpression plants that is absent in wild-type plants. D-E) MS spectrum (D) and MS/MS fragmentation pattern (E) of benzyl glucosinolate in the *CYP79A2* overexpression line. LC/MS analysis suggests that the peak is benzyl glucosinolate.

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Fig. S3. Mass spectra of D₅-PAA and D₅-PAOx fed *sur1* samples. A) Precursor ion spectrum and product spectrum of D₅-PAA analyzed with direct infusion MS. B-C) Mass fragmentation pattern of *sur1* fed with D₅-PAA (B) or D₅-PAA standard (C). Parent ion \rightarrow product ion (m/z 140.077 \rightarrow 96) was monitored for D₅-PAA.



Fig. S4. Overexpression of *ZmCYP79A61* in *Arabidopsis* increases free IAA and PAA content. A) Relative expression of *ZmCYP79A61* in wild type, and *ZmCYP79A61* overexpression lines (*ox-11/WT*, *ox-12/WT*). (N=3). B) Benzyl glucosinolate contents in 2-week-old wild type, and *ZmCYP79A61* overexpression line (*ox-11/WT*, *ox-12/WT*). C) Representative 3-week-old wild type, and the strong *ZmcYP79A61* overexpression line (*ox-11/WT*). D and E) Free IAA (D) and free PAA (E) content of 2-week-old wild type, and *ZmCYP79A61* overexpression line (*ox-11/WT*). D and E) Free IAA (D) and free PAA (E) content of 2-week-old wild type, and *ZmCYP79A61* overexpression line (*ox-11/WT*) (N=3). (F) Hypocotyl length of 2-week-old wild type and *ZmCYP79A61* overexpression line (*ox-11/WT*) (N=6). Data represent mean \pm SD. The means were compared by one-way ANOVA, and statistically significant differences (P < 0.05) were identified by Tukey's test and indicated by letters to represent difference among groups.



Fig. S5. Morphology and auxin content of wild type and *yuc6-1D*. Related to Figure 1. A) Representative 3-week-old wild type and *yuc6-1D* plants. B, C) Free IAA and PAA content of 2-week-old wild type and *yuc6-1D* plants (N=3). Data represent mean \pm SD. The means were compared by one-way ANOVA, and statistically significant differences (P < 0.05) were identified by Tukey's test and indicated by letters to represent difference between groups.



Fig. S6 . Mass spectra of D5-PAA and D5-PAOx fed maize samples. A) Precursor ion spectrum and product spectrum of D₅-PAA analyzed with direct infusion MS. B-C) Mass fragmentation pattern of maize fed with D₅-PAA (B) or D₅-PAA standard (C). Parent ion \rightarrow product ion (m/z 140.077 \rightarrow 96) was monitored for D₅-PAA.

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Fig. S7. Mass spectra of D₅-IAA and D₅-IAOx fed maize samples. A) Precursor ion spectrum and product spectrum of D₅-IAA analyzed with direct infusion MS. B-C) Mass fragmentation pattern of maize fed with D₅-IAA (B) or D₅-IAA standard (C). Parent ion \rightarrow product ion (m/z 181.102 \rightarrow 134.083) was monitored for D₅-IAA.



Fig. S8. The expression of the genes functioning in glucosinolates biosynthesis in *ox-2* compared to wild type. (A) A volcano plot showing p-values and $\log_2(\text{fold change})$ of genes identified from RNAseq analysis. The blue or red colored points represent genes that showed a p-value < 0.05. (B) A heatmap showing expression levels of glucosinolate biosynthesis genes in *CYP79A2* overexpression line *ox-2* compared to wild type.



Fig. S9. Indole glucosinolate content in *CYP79A2* overexpression lines. 2-week-old whole aerial parts were extracted to measure relative amount of benzyl glucosinolate. Data represent mean \pm SD (N=4). The means were compared by one-way ANOVA, and statistically significant differences (P < 0.05) were identified by Tukey's test and indicated by letters to represent difference among groups.



Fig. S10. Expression profile of CYP79A61 (GRMZM2G138248) homologs in *Zea mays.* The expression data were extracted from Maize eFP Browser at bar.utoronto.ca. Tissue-specific expression patterns of CYP79A61 (A), GRMZM2G105185 (B), GRMZM2G178351 (C), and GRMZM2G011156 (D) are shown.



Fig. S11. Root phenotype of *CYP79A2* overexpression lines. Related to Figure 1. A,B) Representative 8-day-old *CYP79A2* overexpression lines compared with their controls. C) Main or primary root length of 10-day-old wild type and *CYP79A2* overexpression lines *ox-1* and *ox-2* (N=20). Data represent mean \pm SD. The means were compared by one-way ANOVA, and statistically significant differences (P < 0.05) were identified by Tukey's test and indicated by letters to represent difference among groups. Scale bar indicates 1 cm.

 Table S1 Primers used in this study.

Primer name	Sequences (5' to 3')
CYP79A2-Forward	GGGAGAAATCCTAGTGTGTGG
CYP79A2-Reverse	GTAACATGTACGTCATGGCTGAC
ZmCYP79A61-	CACTACTCCCCCAATCTCCT
Forward	CACTACTOCOGOAATOTCOT
ZmCYP79A61-	
Reverse	AGTAGAGAAGGCCGAGGGAG
Tubulin 3-Forward	TGGTGGAGCCTTACAACGCTACTT
Tubulin 3-Reverse	TTCACAGCAAGCTTACGGAGGTCA
KFB1-Forward	TGGTGTATGACGTGGCAGAAGACA
KFB1-Reverse	ACCGATGACATGGAATTTGCCAGC
KFB20-Forward	AGCTTCCTCCGATTCCTGGTCAAA
KFB20-Reverse	CACGCGCCATTTGGAAGTGAGAAA
KFB39-Forward	AGACGAATGTCAAGGATTCGCCGT
KFB39-Reverse	ACCATGAATCTGTCGCTGGATCGT
KFB50-Forward	GCGTTTATAACGCAACGCTGGACA
KFB50-Reverse	TCTCCGGCGAAGAAATCCAGAACA
P367	CGAATTTCATCGTAATCGG
P368	TTCGATACCTTCAAGATCAAT

Table S2 GO term analysis of the genes that are up-regulated in CYP79A2
overexpression line ox-2 compared to wild type.

GO Term	Description	Count ^a	Fold Enrichment ^{b,c}	FDR
GO:0009631	cold acclimation	14	5.705794228	1.34E-04
GO:0007623	circadian rhythm	17	3.64280088	0.00267338
GO:0009414	response to water deprivation	38	2.830985462	2.00E-05
GO:0042254	ribosome biogenesis	19	2.781144168	0.01843619
GO:0009409	response to cold	39	2.711138251	2.05E-05
GO:0009873	ethylene-activated signaling pathway	22	2.554629339	0.01843619
GO:0009734	9734 auxin-activated signaling pathway		2.516126552	0.01843619
GO:0009737	response to abscisic acid	45	2.373966235	5.50E-05
GO:0016567	protein ubiquitination	41	1.868862113	0.01902625

a. 'Count' represents the number of genes that were used as queries and related to the specific GO term.
b. 'Fold Enrichment' was normalized to the percentage of background genes (all expressed genes) involved in the corresponding GO term.
c. GO terms organized from largest to smallest fold enrichment.

Table S3 GO term analysis of the genes that are down-regulated in CYP79A2overexpression line ox-2 compared to wild type.

GO Term	Description	Count	Fold Enrichment	FDR
GO:0051131	chaperone-mediated protein complex assembly	7	15.05382469	6.72E-04
GO:0030490	maturation of SSU-rRNA	6	9.462404092	3.29E-02
GO:0000462	maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	14	8.07766203	6.80E-06
GO:0000162	tryptophan biosynthetic process	8	7.278772379	1.02E-02
GO:0009695	jasmonic acid biosynthetic process	7	6.623682864	4.65E-02
GO:0010584	pollen exine formation	7	5.914002558	8.07E-02
GO:0042335	cuticle development	7	5.710071435	0.08850562
GO:0008652	cellular amino acid biosynthetic process	14	4.799770192	1.25E-03
GO:0006364	rRNA processing	14	4.301092769	3.48E-03
GO:0009553	embryo sac development	10	3.240549347	1.91E-01
GO:0009611	response to wounding	27	3.209609428	1.48E-04
GO:0009617	response to bacterium	13	3.014981696	8.85E-02
GO:0009408	response to heat	20	2.957001279	7.32E-03
GO:0006633	fatty acid biosynthetic process	16	2.957001279	3.45E-02
GO:0080167	response to karrikin	14	2.587376119	1.75E-01

Table S4 A full list of genes that are up-regulated in CYP79A2 overexpression line ox-2,ref2-1, and ref5-1 (p-value < 0.05 for all genotypes).</td>

AGI Number Name Short Description				
AT3G49580	LSU1	response to low sulfur 1		
AT1G09080	BIP3	heat shock protein 70 (Hsp 70) family protein		
AT2G04050	AT2G04050	MATE efflux family protein		
AT4G12470	AZI1	azelaic acid induced 1		
AT4G14690	ELIP2	chlorophyll A-B binding family protein		
AT4G05070	WIP2	wound-responsive family protein		
AT2G21660	GRP7	cold, circadian rhythm, and RNA binding 2		
AT2G27830	AT2G27830	hypothetical protein		
AT5G24470	PRR5	two-component response regulator-like protein		
AT3G59940	KFB50	galactose oxidase/kelch repeat superfamily protein		
AT1G80440	KFB20	galactose oxidase/kelch repeat superfamily protein		
AT1G05680	UGT74E2	uridine diphosphate glycosyltransferase 74E2		
ATMG00660	ORF149	hypothetical protein		
AT5G10140	FLC	K-box region and MADS-box transcription factor family		
AT3C5/000	SM7	integrase-type DNA-binding superfamily protein		
AT5G54490	DRD1	ninoid-binding protein 1		
A13034430				
AT3G55580	30 TCF1 regulator of chromosome condensation (RCCT) T			
AT3G49570	LSU3	response to low sulfur 3		
AT3G62960	ROXY8	thioredoxin superfamily protein		
AT2G24550	AT2G24550	major centromere autoantigen B-like protein		
AT1G21520	AT1G21520	hypothetical protein		
AT1G76530	PILS4	auxin efflux carrier family protein		
AT2G05380	GRP3S	glycine-rich protein 3 short isoform		
AT1G13670	AT1G13670	hypothetical protein		
AT2G18690	AT2G18690	transmembrane protein		
AT3G14210	ESM1	GDSL-like lipase/acylhydrolase superfamily protein		
AT4G03510	RMA1	RING membrane-anchor 1		
AT5G43260	AT5G43260	chaperone protein dnaJ-like protein		
AT4G27657	AT4G27657	hypothetical protein		
AT3G03990	D14	alpha/beta-Hydrolases superfamily protein		
AT5G23020	IMS2	2-isopropylmalate synthase 2		
AT3G62550	AT3G62550	adenine nucleotide alpha hydrolases-like superfamily		
AT4G36040	J11	chaperone DnaJ-domain superfamily protein		
AT1G76930	EXT4	extensin 4		
AT3G15760	AT3G15760	cytochrome P450 family protein		
AT4G37370	CYP81D8	cytochrome P450, family 81, subfamily D, polypeptide 8		
AT5G46710	AT5G46710) PLATZ transcription factor family protein		
AT4G01670	AT4G01670	hypothetical protein		
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AT2G28900	OEP16-1	outer plastid envelope protein 16-1		
AT1G78000	SULTR1;2	sulfate transporter 1		
AT5G66010	AT5G66010	RNA-binding (RRM/RBD/RNP motifs) family protein		
AT4G34138	UGT73B1	UDP-glucosyl transferase 73B1		
AT2G40520	AT2G40520	nucleotidyltransferase family protein		
AT4G34750	SAUR49	SAUR-like auxin-responsive protein family		
AT4G17940	AT4G17940	tetratricopeptide repeat (TPR)-like superfamily protein		
AT1G32450	NRT1.5	nitrate transporter 1.5		
AT3G22120	CWLP	cell wall-plasma membrane linker protein		
AT3G23870	DUF803	protein of unknown function		
AT2G30230	AT2G30230	6,7-dimethyl-8-ribityllumazine synthase		
AT2G23170	GH3.3	auxin-responsive GH3 family protein		
AT3G13310	DJC66	chaperone DnaJ-domain superfamily protein		
AT4G29190	OZF2	zinc finger C-x8-C-x5-C-x3-H type family protein		
AT4G04330	RbcX1	chaperonin-like RbcX protein		
AT2G36800	DOGT1	don-glucosyltransferase 1		
AT4G27740	AT4G27740	Yippee family putative zinc-binding protein		
AT2G43540	AT2G43540	transmembrane protein		
AT1G17860	AtKTI5	Kunitz family trypsin and protease inhibitor protein		
AT2G44130	KFB39	galactose oxidase/kelch repeat superfamily protein		
AT1G73500	MKK9	MAP kinase kinase 9		
AT1G07420	SMO2-1	sterol 4-alpha-methyl-oxidase 2-1		
AT1G56300	AT1G56300	chaperone DnaJ-domain superfamily protein		
AT1G78380	GSTU19	glutathione S-transferase TAU 19		
AT2G47000	ABCB4	ATP binding cassette subfamily B4		
AT2052000		Adenine nucleotide alpha hydrolases-like superfamily		
A13G53990	AIUSP	protein		
AT2G02410	AT2G02410	yacP-like NYN domain protein		
AT5G40720	AT5G40720	C3H4 type zinc finger protein		
AT1G70420	DUF1645	DNA ligase-like protein, putative		
AT1G49650	AT1G49650	alpha/beta-Hydrolases superfamily protein		
AT4G17900	AT4G17900	PLATZ transcription factor family protein		
AT2G05520	GRP-3	glycine-rich protein 3		
AT5G26340	MSS1	major facilitator superfamily protein		
AT2C14000		Class I glutamine amidotransferase-like superfamily		
A13G14990	DJTA	protein		
AT1G71030	MYBL2	MYB-like 2		
AT3G53000	PP2-A15	phloem protein 2-A15		
AT4G40065	AT4G40065	other RNA		
AT4G01026	PYL7	PYR1-like 7		
AT2G46600	AT2G46600	calcium-binding EF-hand family protein		
AT5G39050	PMAT1	HXXXD-type acyl-transferase family protein		
AT1G12200	FMO	flavin-binding monooxygenase family protein		
AT2C/7900	AT2C47900	B-box type zinc finger protein with CCT domain-		
A1204/080	71204/030	containing protein		

AT5G63790	NAC102	NAC domain containing protein 102		
AT3G44160	P39	outer membrane OMP85 family protein		
AT2G33550	ASR3	homeodomain-like superfamily protein		
AT3G59210	AT3G59210	F-box/RNI-like superfamily protein		
AT4G11300	DUF793	protein of unknown function		
AT5G62350	AT5G62350	plant invertase/pectin methylesterase inhibitor superfamily protein		
AT4G02050	STP7	sugar transporter protein 7		
AT5G45380	DUR3	urea-proton symporter DEGRADATION OF UREA 3		
AT1G02300	AtcathB1	cysteine proteinases superfamily protein		
AT1G76955	AT1G76955	expressed protein		
AT5G20960	AO1	aldehyde oxidase 1		
AT5G14940	AT5G14940	major facilitator superfamily protein		
AT1G69850	NRT1:2	nitrate transporter 1:2		
AT2G16600	ROC3	rotamase CYP 3		
AT5G28830	AT5G28830	calcium-binding EF hand family protein		
AT3G26740	CCL	CCR-like protein		
AT5G23240	DJC76	DNAJ heat shock N-terminal domain-containing protein		
AT5G24210	AT5G24210	alpha/beta-Hydrolases superfamily protein		
AT2G45560	CYP76C1	cytochrome P450, family 76, subfamily C, polypeptide 1		
AT5G65310	HB5	homeobox protein 5		
AT2G18170	MPK7	MAP kinase 7		
AT5G26040	HDA2	histone deacetylase 2		
AT1G80920	J8	chaperone DnaJ-domain superfamily protein		
AT5G59780	MYB59	myb domain protein 59		
AT4G25640	DTX35	detoxifying efflux carrier 35		
AT2G32800	AP4.3A	protein kinase family protein		
AT1G73650	DUF1295	protein of unknown function		
AT4G14716	ARD1	acireductone dioxygenase 1		
AT5G11580	AT5G11580	regulator of chromosome condensation (RCC1) family protein		
AT5G58200	AT5G58200	calcineurin-like metallo-phosphoesterase superfamily protein		

AGI Number	Name	Short Description		
AT1G01610	GPAT4	glycerol-3-phosphate acyltransferase 4		
AT1G02690	IMPA-6	importin alpha isoform 6		
AT1G03440	AT1G03440	leucine-rich repeat (LRR) family protein		
AT1G11670	AT1G11670	MATE efflux family protein		
AT1G24260	SEP3	K-box region and MADS-box transcription factor family protein		
AT1G25450	KCS5	3-ketoacyl-CoA synthase 5		
AT1G33811	AT1G33811	GDSL-like lipase/acylhydrolase superfamily protein		
AT1G48480	RKL1	receptor-like kinase 1		
AT1G53700	WAG1	PsPK3-type kinase		
AT1G66250	AT1G66250	O-Glycosyl hydrolases family 17 protein		
AT1G69390	MINE1	homologue of bacterial MinE 1		
AT1G69770	CMT3	chromomethylase 3		
AT1070070	ити	glucose-methanol-choline (GMC) oxidoreductase family		
ATIGIZATO	піп	protein		
AT1G77270	AT1G77270	hypothetical protein		
AT2G19910	AT2G19910	RNA-dependent RNA polymerase family protein		
AT2G35190	NPSN11	putative plant snare 11		
AT2G38110	GPAT6	glycerol-3-phosphate acyltransferase 6		
AT2G47440	AT2G47440	tetratricopeptide repeat (TPR)-like superfamily protein		
AT3G01345	AT3G01345	Expressed protein		
AT3G02380	COL2	CONSTANS-like 2		
AT3G04290	LTL1	Li-tolerant lipase 1		
AT3G11670	DGD1	UDP-Glycosyltransferase superfamily protein		
AT3G16150	ASPGB1	N-terminal nucleophile aminohydrolases (Ntn hydrolases) superfamily protein		
AT3G23670	KINESIN- 12B	phragmoplast-associated kinesin-related protein		
AT3G23730	XTH16	xyloglucan endotransglucosylase/hydrolase 16		
AT3G24500	MBF1C	multiprotein bridging factor 1C		
AT3G49900	AT3G49900	phototropic-responsive NPH3 family protein		
AT4G13260	YUC2	flavin-binding monooxygenase family protein		
AT4G17860	DUF239	protein of unknown function		
AT4G18570	AT4G18570	tetratricopeptide repeat (TPR)-like superfamily protein		
AT4G18960	AG	K-box region and MADS-box transcription factor family		
AT4G18970	AT4G18970	GDSL-like lipase/acylhydrolase superfamily protein		
AT4G21760	BGLU47	beta-glucosidase 47		
AT4G21770	AT4G21770) pseudouridine synthase family protein		
AT4G28230	AT4G28230) hypothetical protein		
AT4G28680	TYRDC	L-tyrosine decarboxylase		

Table S5 A full list of genes that are down-regulated in CYP79A2 overexpression line ox-2, ref2-1, and ref5-1 (p-value < 0.05 for all genotypes).</td>

AT4G28780	AT4G28780	GDSL-like lipase/acylhydrolase superfamily protein		
AT4G31840	ENODL15	early nodulin-like protein 15		
AT4G32830	AUR1	ataurora1		
AT5G02540	AT5G02540	NAD(P)-binding Rossmann-fold superfamily protein		
AT5G07180	ERL2	ERECTA-like 2		
AT5G11160	APT5	adenine phosphoribosyltransferase 5		
AT5G12050	AT5G12050	rho GTPase-activating protein		
AT5G13240	MAF1	transcription regulator		
AT5G18460	DUF239	protein of unknown function		
AT5G20270	HHP1	heptahelical transmembrane protein 1		
AT5G25090	ENODL13	early nodulin-like protein 13		
AT5G37300	WSD1	O-acyltransferase (WSD1-like) family protein		
ATEC20440	RBCS3B	ribulose bisphosphate carboxylase (small chain) family		
A15G50410		protein		
AT5G40460	SMR6	cyclin-dependent kinase inhibitor SMR3-like protein		
AT5G41080	GDPD2	PLC-like phosphodiesterases superfamily protein		
AT5G43500	ARP9	actin-related protein 9		
AT5C44635	MCM6	minichromosome maintenance (MCM2/3/5) family		
A13044033		protein		
AT5G45670	AT5G45670	GDSL-like Lipase/Acylhydrolase superfamily protein		
AT5G47380	DUF547	protein of unknown function		
AT5G51750	SBT1.3	subtilase 1.3		
AT5G53660	GRF7	growth-regulating factor 7		
AT5G59150	RABA2D	RAB GTPase homolog A2D		
AT5G64310	AGP1	arabinogalactan protein 1		
AT5G67460	AT5G67460 AT5G67460 O-Glycosyl hydrolases family 17 protein			

Table S6 GO term analysis of the genes that are up-regulated in CYP79A2overexpression line ox-2, ref2, and ref5 compared to wild type.

GO Term	Description	Count	Fold Enrichment	FDR
GO:2000762	regulation of phenylpropanoid metabolic process	3	100.3562387	0.07125823