

# Supplementary Material to ‘Thaxtomin A affects CESA-complex density, expression of cell wall genes, cell wall composition, and causes ectopic lignification in *Arabidopsis thaliana* seedlings’

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**Table S1.** Quantitative real-time RT-PCR results and primer sequences.

AGI	Gene Name	Annotation	$\Delta C_T^*$ Control	$\Delta C_T^*$ Thax. A	$\Delta\Delta C_T^{**}$ Thax. A	$\Delta C_T^*$ Isoxaben	$\Delta\Delta C_T^{**}$ Isoxaben	Forward Primer	Reverse Primer
At4g32410	CESA1	cellulose synthase	2.90	4.16	-1.26	3.64	-0.74	GAGCTGAGATGGAGCGGTGT	CTGCTCGTTCCTCCACCAAT
At4g39350	CESA2	cellulose synthase	5.16	6.65	-1.49	6.01	-0.85	AAGCAGATTCATGCGCTAGAGA	CAACATTTGACTGGGACGA
At5g05170	CESA3	cellulose synthase	3.03	4.35	-1.32	3.81	-0.78	GGAGTTGAAGGTGCTGGTTTTG	CCAGGCTCATTGCGACATT
At5g09870	CESA5	cellulose synthase	4.35	6.68	-2.33	5.53	-1.18	CATCGTCCCTGAGATTAGCAACT	AGCAATCGACCCGAAAAGTG
At5g64740	CESA6	cellulose synthase	2.72	4.37	-1.65	3.65	-0.93	GGAAAATTCATCGTCCCGAG	AGAAGAGCGCCATGAAGAGG
At5g49720	KOR1	membrane-bound endo-1,4-beta-D-glucanase	2.85	3.85	-1.00	3.25	-0.40	ATGGAGGTTAATAGAGTTGAATCATG	AATATTGCAGCGGCTGTGG
At3g08550	KOB1	plasma membrane protein	5.86	7.14	-1.28	6.67	-0.81	AATTGCGTCAACAGCAACTGA	TGTTACGGTACCAGCTTAGCA
At1g67490	KNF	alpha-glucosidase I	6.02	6.96	-0.94	6.90	-0.88	AGCAATTTGATAAGGAACGTGGTT	GATGTACCCCGTCTCGTAATAGTTT
At5g17420	CESA7	xylem-specific cellulose synthase	7.42	9.86	-2.44	11.09	-3.67	GAGCTGGGTTGGATCTATGGC	AATGCATCTTGAATCCCGTCA
At4g18780	CESA8	xylem-specific cellulose synthase isomer	7.35	9.99	-2.64	11.31	-3.96	AGCTATTTGCCCTTACCAGGC	CATGCTTGCTAGGTTTGATAGCG
At5g15630	COBL4	member of the COBRA family	9.52	12.20	-2.68	13.40	-3.88	CGAGAACAAAAAGAGCTGCG	TTCTTGTTAGAATCTTAGAATCAGCCT
At5g54690	IRX8	putative galacturonosyltransferase	8.92	11.38	-2.46	12.62	-3.70	TCATTGGCTTGACGAGAACTTAA	CCCAGCTGCCACAACTCAG
At2g37090	IRX9	putative family 43 glycosyl transferase	7.45	10.20	-2.75	11.52	-4.07	AAGGCACCAACAGGATTCG	CCTCCAAACTACTTGCTTCACAT
At1g02730	CslD5	cellulose synthase-like gene	5.12	7.88	-2.76	7.89	-2.77	CGAATGCACAACAGAGGATGG	CACGGAAAGCGTCTCTTTTTTG
At2g24630	CslC8	cellulose synthase-like gene	8.27	10.72	-2.45	10.51	-2.24	TAACCGTGCCACATTTCAAGG	AACGCCACCTAGCCTGAAC
At3g61130	GAUT1	putative galacturonosyltransferase	6.46	7.58	-1.12	7.38	-0.92	CCGAGTAGACCCCTCCTTT	CATCAGGAGTTGCTGGATTCTC
At2g46480	GAUT2	putative galacturonosyltransferase	13.86	17.12	-3.26	17.79	-3.93	AACCTGAACGAAAACCGGAC	CCCTGGTGGCAATGTTCTAG
At4g38270	GAUT3	putative galacturonosyltransferase	8.19	8.85	-0.66	8.61	-0.42	GATTTTGCAAGCCAGCAAA	CCTTTCTGACGTAAAATCTGCC
At5g47780	GAUT4	putative galacturonosyltransferase	6.06	6.93	-0.87	6.39	-0.33	CTGCCAAAAGACTGCTATAGAAAAGC	GGCCAGTGTGTTGCTCCATTG
At2g30575	GAUT5	putative galacturonosyltransferase	7.69	9.39	-1.70	8.66	-0.97	CTTCGACCTGGGAGTAAAAGGA	ACTGGCAATCCCTGCTT
At3g25140	GAUT8	Quasimodo1	4.10	5.21	-1.11	4.53	-0.43	TGGCAAAATCTGAACGAGAACA	TCAGTCCCGGTGGTAACGTC
At2g20810	GAUT10	putative galacturonosyltransferase	6.85	8.11	-1.26	7.76	-0.91	TCATTCTCTCAAAGCTGGTCAT	CGGTAACGTCGCTTAGGAATAGA
At1g19300	GATL1	putative galacturonosyltransferase	5.97	7.94	-1.97	7.01	-1.04	GATGGCGATGCAAAAGAGAATGA	CAATAAAAACGGTGGTAAACGAAC
At3g62660	GATL7	putative galacturonosyltransferase	7.95	6.76	1.19	6.76	1.19	GCCGTTCTGTTGGTGTTCG	CACCGATGCTCTATCGGAGC
At1g24170	GATL8	putative galacturonosyltransferase	5.55	6.97	-1.42	5.92	-0.37	TTTGGTGTGCGGGTAATG	TCTAAACCATGTTGGTTCCATC
At3g53190	PecL	pectatelyase family protein	4.34	7.77	-3.43	7.25	-2.91	AATCCGCCTCATTATCACC	CTGCCACCAACGCCAAG
At5g53370	PME	pectinesterase family protein	4.23	5.82	-1.59	3.79	0.44	TGGGAAAAGTATCGCCGATG	ACCAGTCGACGAAAAGTG
At2g47550	PME	pectinesterase family protein	8.03	5.57	2.46	5.72	2.31	TTTTCTCAAAGGTTGGTGC	GCTTGTGGTGGCAATCG
At4g38420	PME	pectinesterase family protein, SKS9	6.11	4.13	1.98	5.72	0.39	CGCTCCACCACTCAGGTATATCC	CATTGTCCAGTGCCACATATACG

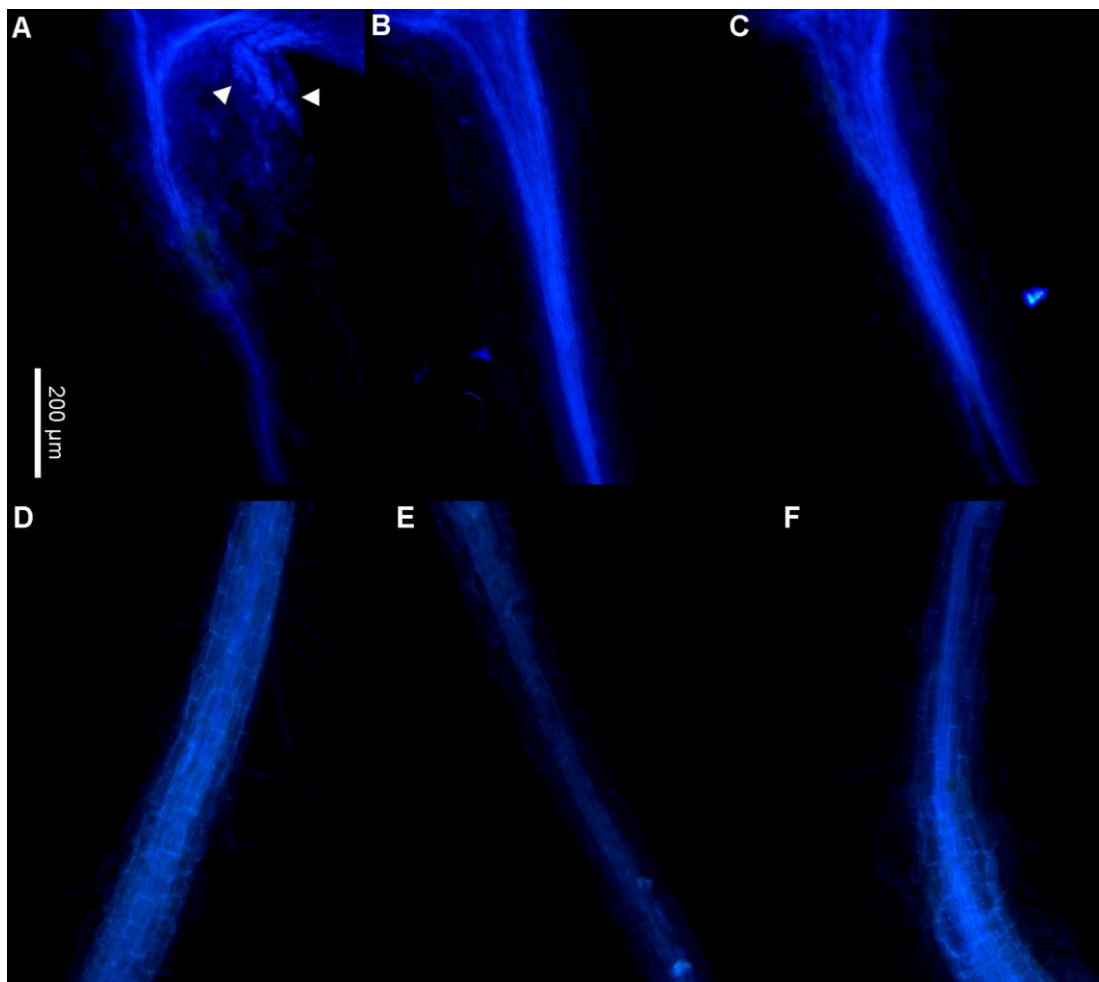
<i>At5g48450</i>	<i>PME</i>	pectinmethylesterase	6.54	7.65	-1.11	7.34	-0.80	GCACATACAACAAGGGTGATGC	CATGCACCAGGAAACACCTG
<i>At5g45280</i>	<i>PAE</i>	Pectinacetylerase, putative	11.15	10.12	1.03	9.67	1.48	GCTTCAAACCGTTCAAGAGACC	TCGTGCGGATCGAACA
<i>At4g19410</i>	<i>PAE</i>	pectinacetylerase, putative	2.24	5.25	-3.01	4.17	-1.93	GCCTCTTGGTCCGGTGACAA	CGACTGCTTTCGCAATTCTCG
<i>At2g06850</i>	<i>XTH4</i>	endoxyloglucan transferase (EXGT-A1) gene	1.87	7.02	-5.15	3.91	-2.04	CCCAACTTGGGCTTTTGACC	GTGCCAGTGTATTTGTGCGAGGA
<i>At4g03210</i>	<i>XTH9</i>	xyloglucan endotransglucosylase/hydrolases	2.87	6.85	-3.98	5.46	-2.59	TCATTGTGTCAACGAAGGCG	TTGATTCAAACCCAGCTCCAG
<i>At4g30290</i>	<i>XTH19</i>	putat. xyloglucan endotransglycosylase/hydrolase	5.24	5.85	-0.61	4.93	0.31	TTTTTACCGTTGATGGGATTCCA	ATGGGACTCCACGTGACTCG
<i>At4g25810</i>	<i>XTH23</i>	xyloglucan endotransglycosylase-related protein	4.05	1.54	2.51	0.87	3.18	COGCTTACTATTTGAAATCCCCT	AAGGATCACCCTTAGGTTTCCC
<i>At5g53250</i>	<i>AGP22</i>	arabinogalactan protein 22	5.03	9.72	-4.69	7.73	-2.70	CATCTCCGTGATTCTTTTGCC	ATCGATGTTCCATCGCTGG
<i>At1g35230</i>	<i>AGP5</i>	encodes arabinogalactan-protein	6.93	5.05	1.88	4.23	2.70	CATCTCCGTGATTCTTTTGCC	ATCGATGTTCCATCGCTGG
<i>At5g07570</i>	<i>HRGP</i>	glycine/proline-rich protein	12.96	13.53	-0.57	10.72	2.24	CGTCTACTTCCCCATCCGGA	GCAGGTCCAGCTTCTCCAGA
<i>At1g76930</i>	<i>HRGP</i>	encodes an Arabidopsis extensin gene	-1.01	-2.46	1.45	-2.92	1.91	TCGATGTATCCCCCTTGGTAA	CCTGGACTGATACCGGATGAATA
<i>At3g50570</i>	<i>HRGP</i>	hydroxyproline-rich glycoprotein family protein;	6.48	7.4	-0.92	4.61	1.87	TGGAGTCGACGACGATACACA	TTCTCCCGTCAACGATCTTGT
<i>At1g05570</i>	<i>CALS1</i>	encodes a callose synthase 1 catalytic subunit	5.57	6.55	-0.98	6.47	-0.90	TCCCGGCTCGTCCCTAATA	GTTGGGAAACGGGATGTTTG
<i>At4g04970</i>	<i>GSL1</i>	encodes a gene similar to callose synthase	8.4	9.22	-0.82	9.54	-1.14	TTCTGGCTTCGATTGGCTCA	CCATAGCCATGCAATGAAATCA
<i>At2g30490</i>	<i>C4H</i>	encodes a cinnamate-4-hydroxylase	2.18	0.79	1.39	0.94	1.24	TCGCCGCGATTGAGACAAC	GCTCTGCAATTCCCCACTCG
<i>At1g51680</i>	<i>4CL1</i>	encodes an isoform of 4-coumarate:CoA ligase	3.39	1.77	1.62	2.10	1.29	TACTGATGTTGCTGTTGTGCGCAAT	GGAACCTCACCAGCTGCTTCTTCT
<i>At3g21240</i>	<i>4CL2</i>	encodes an isoform of 4-coumarate:CoA ligase	5.18	3.36	1.82	3.64	1.54	TGCTAAGTTTCTAACGCCAAGC	GCTTCTGTATCCCATAGCCCT
<i>At1g65060</i>	<i>4CL3</i>	encodes an isoform of 4-coumarate:CoA ligase	7.67	10.08	-2.41	10.38	-2.71	CAAGATAGTCTTCGTCGCCGT	CCGTATACCATATCCCTGGC
<i>At5g04330</i>	<i>F5H</i>	ferulate-5-hydroxylase, putative	8.05	8.54	-0.49	9.08	-1.03	CATGGATGTGATGTTCCGGAGG	GGTTAGCACCCATTGCGATTGC
<i>At5g48930</i>	<i>HCT</i>	hydroxycinnamoyltransferase	3.62	2.87	0.75	3.10	0.52	TTCCCGAAGTTGATCACTCCG	GTCACCTGCAAAACGAGAAGC
<i>At1g15950</i>	<i>CCR1</i>	cinnamoyl CoA reductase isoform	3.66	3.63	0.03	3.81	-0.15	GACCCTGAGGCTGTGCTTGA	GCCGTAACAATACCAATTCTTGTT
<i>At1g80820</i>	<i>CCR2</i>	cinnamoyl CoA reductase isoform	11.52	7.79	3.73	9.11	2.41	GAGGTTGTTGAGATTCTGGCCA	TTCTCGTCCGAACACTTGGTG
<i>At5g54160</i>	<i>COMT1</i>	caffeic acid/5-hydroxyferulic acid O-methyltransferase	2.10	1.82	0.28	1.70	0.40	ATCTCCACATGTCATCGAAGA	TCTCCTCAACATGCTCAATACC
<i>At3g19450</i>	<i>CAD4</i>	cinnamyl alcohol dehydrogenase isoform	4.55	5.64	-1.09	5.28	-0.73	ATCCTATGGTTCCTGGGCACGA	TCTCCTACGGTGAACCTGCTCACA
<i>At4g39330</i>	<i>CAD9</i>	dehydrogenase of unknown function	2.74	8.55	-5.81	6.85	-4.11	TGTTCTCGGAAGGAAAATGGTTG	CTCTTGTGTCTCCTTCATCCCTCC
<i>At5g24780</i>	<i>VSP1</i>	acid phosphatase	7.37	5.57	1.80	6.60	0.77	CACTGTGCGAATCTCAAGGCTG	CGTTTGGCTTGAGTATGAGATGC
<i>At5g24770</i>	<i>VSP2</i>	acid phosphatase	9.44	5.71	3.73	7.87	1.57	ACGCAAAATATGGATACGGAACAG	CGGTCCCTAACCAACACAGTA
<i>At3g12500</i>	<i>CHI-B</i>	basic chitinase	6.77	5.58	1.19	5.82	0.95	ACGAGGAAGAAGGAGGTCGC	CTGTAGCCCATCCACCTGTGATTT
<i>At5g44420</i>	<i>PDF1.2</i>	plant defensin	8.27	9.24	-0.97	3.92	4.35	TTTGCTGCTTTCGACGCAC	CACTTGGCTTCTCGCAACT
<i>At2g14610</i>	<i>PR1</i>	Pathogenesis-related gene 1	9.05	5.81	3.24	8.83	0.22	CGTGCAATGGAGTTTGTGGTC	TTTGGCACATCCGAGTCTCAC
<i>At1g75040</i>	<i>PR5</i>	Thaumatococcus-like protein	8.3	6.54	1.76	8.04	0.26	ACAGGCTGCAACTTTGACGC	TTAGACCGCCACAGTCTCCG
<i>At2g43570</i>	<i>CHI</i>	chitinase, putative	9.71	7.63	2.08	9.32	0.39	TTCATGGCCGAGCTAATTC	CGATTTGCGGTTTGGAGATG

\* $\Delta C_T = C_T$  (gene of interest) -  $C_T$  (*UBQ10* reference gene).  $C_T$  = threshold cycle number.

\*\* $\Delta\Delta C_T = \Delta C_T$  (Control) -  $\Delta C_T$  (Thax A).

**Figure S1.** Aniline blue staining of *Arabidopsis* seedlings treated with thaxtomin A or isoxaben.

Deposition of ectopic callose was analyzed in the elongation zones of hypocotyls (A-C) or roots (D-F) of 6-days-old, liquid culture grown, etiolated *Arabidopsis* seedlings treated with 200 nM thaxtomin A during two days (A, D), methanol-treated control seedlings (B, E) or seedlings treated with 5 nM isoxaben (C, F). Typical staining patterns are shown. Arrow heads in panel A point towards ectopic callose deposition in thaxtomin A-treated hypocotyls. Conductive tissue in all conditions including untreated (not shown) or methanol-treated (B, E) seedlings showed autofluorescence.



**Figure S2.** Effect of thaxtomin A on GFP-TUA6-labeled cortical microtubules.

Microtubules were analyzed in etiolated *A. thaliana* hypocotyl cells as shown in Figure 4A.

Representative snap-shots (four-frame-averaged) of untreated (A-C) and 200 nM Thaxtomin

A treated (D-F) wild-type seedlings are presented.

