

Note

## Gene expression profile of *Arabidopsis* plants that overexpress *ZEITLUPE/LOV KELCH PROTEIN1*: up-regulation of auxin-inducible genes in hypocotyls

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**Abstract** ZEITLUPE (ZTL) is a blue-light photoreceptor with an F-box motif in *Arabidopsis thaliana*. The molecular mechanisms underlying the ZTL-dependent control of the circadian rhythm and repression of photoperiodic flowering are relatively well characterized. ZTL also positively regulates hypocotyl elongation under light, but the molecular mechanisms remain unknown. Using microarray analysis, we showed that 194 genes, including 17 SMALL AUXIN UP RNA (SAUR) genes (SAUR9, 15, 16, 19, 20, 22–24, 28, 29, 61–67) and two AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) genes (IAA7, 29), were up-regulated and 283 genes were down-regulated in ZTL-overexpressing *Arabidopsis* seedlings. The results were confirmed for SAUR22, 23, and IAA29 by real-time quantitative reverse-transcription PCR. Application of the polar auxin transport inhibitor N-1-naphthalphthalamic acid (NPA), the auxin antagonist  $\alpha$ -(phenyl ethyl-2-one)-indole-3-acetic acid (PEO-IAA), or the auxin biosynthesis inhibitor 5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (YUCASIN) inhibited hypocotyl elongation enhanced in ZTL-overexpressing seedlings. Our data suggest the involvement of auxin and auxin-inducible genes, including SAURs and IAA, in hypocotyl elongation in ZTL-overexpressing seedlings.

**Key words:** *Arabidopsis thaliana*, auxin, hypocotyl elongation, SAUR, ZEITLUPE.

*Arabidopsis* ZEITLUPE (ZTL) or LOV KELCH PROTEIN1 is a blue-light photoreceptor F-box protein (Kiyosue and Wada 2000; Somers et al. 2000). ZTL determines the period of circadian oscillation by regulating polyubiquitination and subsequent degradation of two circadian clock proteins, TIMING OF CAB EXPRESSION1 (TOC1) (Más et al. 2003) and PSEUDO RESPONSE REGULATOR (PRR)5 (Kiba et al. 2007). ZTL represses photoperiodic flowering by inhibiting the function of the GIGANTEA (GI)-FLAVIN BINDING, KELCH REPEAT, F-BOX1 (FKF1) complex (Imaiizumi et al. 2005; Kim et al. 2013; Sawa et al. 2007; Song et al. 2012; Takase et al. 2011). Another function of ZTL is to promote hypocotyl elongation in light-grown plants. ZTL-overexpressing plants have elongated hypocotyls under blue, red, or white light, whereas *ztl* mutants have shorter hypocotyls under red or white light (Kevei et al. 2006; Kiyosue and Wada 2000; Nelson et al. 2000; Somers et al. 2000). In the dark, hypocotyl length of both ZTL-overexpressing and knock-out seedlings is the same as that of control plants (Kevei et al. 2006;

Kiyosue and Wada 2000; Nelson et al. 2000; Somers et al. 2000). Although the molecular mechanisms of ZTL function in circadian rhythm determination and photoperiodic flowering are relatively well characterized, the effect of ZTL on hypocotyl elongation still remains underexplored. To identify the genes that may enhance hypocotyl elongation caused by ZTL-overexpression under white light, we performed microarray and real-time PCR analyses.

First, seeds of two independent ZTL-overexpressing lines, *ZTL*<sub>ox1</sub> and *ZTL*<sub>ox2</sub> (Kiyosue and Wada 2000), and wild-type Columbia accession (Col) were surface-sterilized with 0.1% (v/v) sodium hypochlorite, sown on 1.5% (w/v) agar containing 1/2-basal-salt Murashige and Skoog medium and incubated at 4°C for 7 days in the dark and then at 22°C for 8 days under continuous white light (80  $\mu$ mol·m<sup>-2</sup>·s<sup>-1</sup>). Scion Image software (<http://www.mediafire.com/download/2tmpv0gibwcwwv1/Scion+Image.zip>) was used to measure the hypocotyl length. Under white light, the lengths of hypocotyls of the ZTL-

Abbreviations: AUX/IAA, auxin/indole-3-acetic acid; EIR1, ETHYLENE INSENSITIVE ROOT1; ERF1, ETHYLENE RESPONSE FACTOR1; FKF1, FLAVIN-BINDING KELCH REPEAT F-BOX 1; PRR, PSEUDO RESPONSE REGULATOR; SAUR, SMALL AUXIN UP RNA; TOC1, TIMING OF CAB EXPRESSION1; ZTL, ZEITLUPE.

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overexpressing plants were significantly greater than those of the wild type (Figure S1), in line with previous reports (Kiyosue and Wada 2000; Nelson et al. 2000).

Next, we grew *ZTLox1*, *ZTLox2*, and wild-type Col seedlings (two biological replicates: Col1 and Col2) axenically under continuous white light ( $80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ ) for 8 days and isolated total RNA with an RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The RNA quality was checked using an Agilent 2100 Bioanalyzer platform (Agilent Technologies, Böblingen, Germany). Single-color hybridization of *Arabidopsis* RNA to Agilent Whole *Arabidopsis* Genome Oligo Microarrays  $4 \times 44$  K V4 (G2519F-021169) (Agilent Technologies) was performed for each sample by Miltenyi Biotec KK (Tokyo, Japan) as previously described (Miyazaki et al. 2015). Briefly, hybridization was performed according to the Agilent 60-mer oligo microarray processing protocol using an Agilent Gene Expression Hybridization Kit (Agilent Technologies). To produce Cy3-labeled cRNA, the RNA samples were amplified and labeled using an Agilent Low Input Quick Amp Labeling Kit (Agilent Technologies) following the manufacturer's protocol. The Cy3-labeled fragmented cRNA (1.65  $\mu\text{g}$ ) in hybridization buffer was hybridized overnight (17 h, 65°C) to Agilent Whole *Arabidopsis* Genome Oligo Microarrays  $4 \times 44$  K V4 in a hybridization chamber and oven as recommended by Agilent. Fluorescence signals were detected using an Agilent Microarray Scanner System (Agilent Technologies). The Agilent Feature Extraction Software (FES, 10.7.3.1) was used to read out and process the microarray image files. For determination of differential gene expression FES derived output data files were further analyzed using the Rosetta Resolver gene expression data analysis system (Rosetta Biosoftware, Rosetta Inpharmatics, Seattle, WA, USA).

A total of 194 genes were up-regulated (fold change  $>2$ ;  $p < 0.01$ ) and 283 genes were down-regulated (fold change  $<0.5$ ;  $p < 0.01$ ) in both *ZTL*-overexpressing lines (Figure 1). These up-regulated genes are listed in Table S1 and down-regulated genes in Table S2. Up-regulation of *ZTL* in *ZTL*-overexpressing lines (AT5G57360; 30-fold in *ZTLox1* and 40-fold in *ZTLox2*) validated this microarray analysis. The up-regulation of clock genes *PRR3* (AT5G60100; 6.4-fold in *ZTLox1*, 6.5-fold in *ZTLox2*), *PRR5* (AT5G24470; 2.5-fold in *ZTLox1*, 2.1-fold in *ZTLox2*), *PRR7* (AT5G02810; two probes: 5.4- and 4.9-fold in *ZTLox1*; 3.0- and 3.4-fold in *ZTLox2*), *PRR9* (AT2G46790; 6.5-fold in *ZTLox1*, 3.5-fold in *ZTLox2*), and *GI* (AT1G22770; two probes: 4.4- and 2.5-fold in *ZTLox1*; 2.8- and 3.5-fold in *ZTLox2*) in *ZTL*-overexpressing plants was consistent with the involvement of *ZTL* in circadian clock regulation. The down-regulation of flowering time genes *CONSTANS* (AT5G15840; -5.5-fold in *ZTLox1*, -2.1-fold in

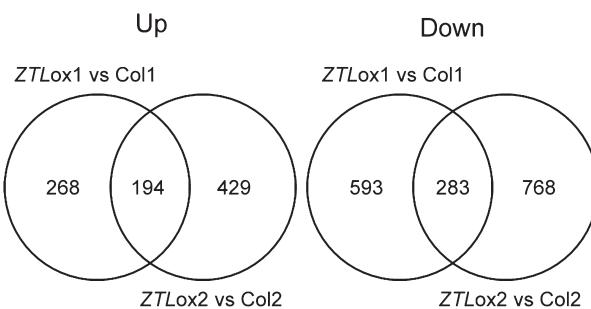


Figure 1. Venn diagrams showing the number of genes differentially expressed in *ZTL*-overexpressing seedlings (*ZTLox1* and *ZTLox2*) in comparison with wild-type Columbia seedlings (Col1 and Col2). A total of 194 genes were up-regulated (fold change  $>2$ ;  $p < 0.01$ ) in both *ZTL*-overexpressing lines in comparison with Col. A total of 283 genes were down-regulated (fold change  $<0.5$ ;  $p < 0.01$ ) in both *ZTL*-overexpressing lines in comparison with Col.

*ZTLox2*) and *FLOWERING LOCUS T* (AT1G65480; -9.2-fold in *ZTLox1*, -8.7-fold in *ZTLox2*) was consistent with the involvement of *ZTL* in flowering time regulation. Pathway analysis of up-regulated and down-regulated genes with the Gene Functional Classification Tool in DAVID Bioinformatics Resources 6.7 (<http://david.abcc.ncifcrf.gov/home.jsp>) classified them into several groups (Tables S3, S4). Among the down-regulated genes, those involved in flavonoid biosynthesis (11 genes) were detected as the group with the smallest  $p$ -value (1.21E-10; Table S3). Among the up-regulated genes, those involved in the response to auxin (19 genes) were detected as the group with the smallest  $p$ -value (4.03E-11; Table S4). The latter group included 17 *SMALL AUXIN UP RNA* (*SAUR*) genes (*SAUR9, 15, 16, 19, 20, 22–24, 28, 29*, and *61–67*) and 2 *AUXIN/INDOLE-3-ACETIC ACID* (*AUX/IAA*) genes (*IAA7* and *29*) (Table 1).

We performed real-time quantitative reverse-transcription PCR (qRT-PCR) to confirm the up-regulation of *SAUR22*, *SAUR23*, and *IAA29* in hypocotyls of 8-day-old *ZTL*-overexpressing plants in comparison with wild-type plants grown as for microarray analysis. RNA was isolated from hypocotyls with RNAiso Plus (Takara, Otsu, Japan). The reverse transcription reaction was performed with an oligo dT primer and a PrimeScript II 1st Strand cDNA Synthesis Kit (Takara). PCR was performed with SYBR Premix Ex Taq II (Takara) and a StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA). The PCR primers are listed in Table S5. The expression level of *Actin2* (*ACT2*) was used to normalize the expression levels of the target genes. The expression of *SAUR22*, *SAUR23*, and *IAA29* was significantly higher ( $>5$  fold) in both *ZTL*-overexpressing lines in comparison with wild-type plants, which confirms that these genes are up-regulated in the hypocotyls of *ZTL*-overexpressing seedlings (Figure 2).

To evaluate the role of auxin in enhanced hypocotyl

Table 1. Genes up-regulated in *ZTL*-overexpressing seedlings in comparison with control seedlings identified with the Gene Functional Classification Tool within DAVID Bioinformatics Resources 6.7.

Array Element	Gene locus	Gene name	<i>ZTLox1</i> vs Col1		<i>ZTLox2</i> vs Col2	
			Fold Change	p-value	Fold Change	p-value
A_84_P196504	AT5G18010	SAUR19	10.30	4.25.E-19	8.82	1.98.E-18
A_84_P141269	AT5G18050	SAUR22	6.57	6.53.E-17	10.64	2.37.E-19
A_84_P94979	AT5G18060	SAUR23	6.10	2.64.E-16	7.23	2.49.E-17
A_84_P147028	AT5G18020	SAUR20	5.02	7.33.E-15	6.68	1.05.E-16
A_84_P272980	AT5G18080	SAUR24	4.36	1.14.E-13	4.37	1.16.E-13
A_84_P15930	AT1G29460	SAUR65	4.04	4.03.E-13	4.07	5.71.E-13
A_84_P537376	AT1G29420	SAUR61	3.92	1.21.E-06	3.73	7.00.E-03
A_84_P16734	AT4G38850	SAUR15	3.89	1.47.E-10	4.49	4.67.E-09
A_84_P12944	AT4G36110	SAUR9	3.54	2.60.E-03	2.21	1.96.E-03
A_84_P19713	AT1G29450	SAUR64	2.98	3.56.E-10	4.53	4.19.E-14
A_84_P20189	AT3G03830	SAUR28	2.76	1.82.E-08	2.10	4.00.E-05
A_84_P55550	AT4G32280	IAA29	2.69	2.72.E-07	4.86	8.81.E-14
A_84_P11207	AT1G29500	SAUR66	2.63	7.87.E-09	2.85	1.15.E-09
A_84_P17680	AT4G38860	SAUR16	2.49	3.18.E-08	3.33	3.03.E-11
A_84_P21135	AT3G03820	SAUR29	2.31	4.74.E-07	8.00	5.41.E-16
A_84_P279980	AT1G29440	SAUR63	2.29	2.64.E-07	2.98	3.62.E-10
A_84_P22555	AT1G29510	SAUR67	2.23	5.26.E-07	3.12	1.54.E-10
A_84_P760471	AT3G23050	IAA7	2.21	1.65.E-06	2.06	9.12.E-06
A_84_P10257	AT1G29430	SAUR62	2.01	9.40.E-04	3.78	5.00.E-05

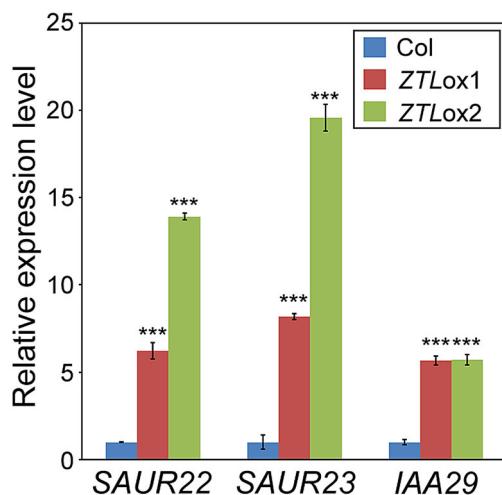


Figure 2. Expression of *SAUR22*, *SAUR23*, and *IAA29* in hypocotyls of wild-type Columbia (Col) and two *ZTL*-overexpressing seedlings (*ZTLox1* and *ZTLox2*) quantified by real-time quantitative reverse-transcription PCR. The expression levels were normalized by using the levels of actin transcript (*ACT2*). The values for *ZTLox1* and *ZTLox2* are shown relative to wild-type Col. Error bars represent standard deviation ( $n=3$ ; technical repeats); \*\*\*  $p<0.001$  (Student's *t*-test) in comparison with wild-type Col.

elongation in *ZTL*-overexpressing seedlings, we used three auxin inhibitors. Col, *ZTLox1*, and *ZTLox2* seedlings were grown as above in the absence or presence of the auxin transport inhibitor *N*-1-naphthalphthalamic acid (NPA), the auxin antagonist  $\alpha$ -(phenyl ethyl-2-one)-indole-3-acetic acid (PEO-IAA), or the auxin biosynthesis inhibitor 5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (YUCASIN). In the presence of 1  $\mu$ M NPA, 5  $\mu$ M PEO-IAA, or 25  $\mu$ M YUCASIN, the

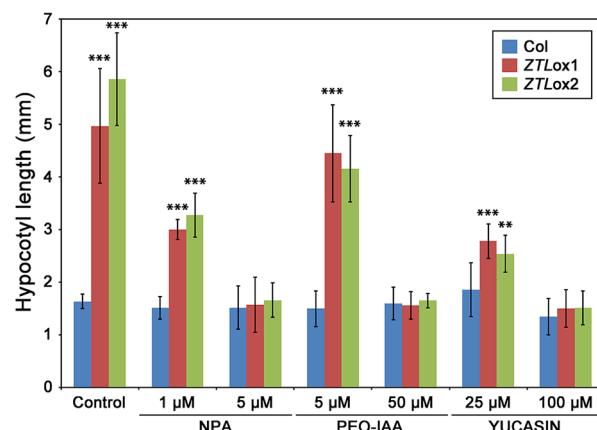


Figure 3. Inhibition of hypocotyl elongation by auxin inhibitors. Seedlings were grown under continuous white light for 8 days on 1.5% (w/v) agar containing 1/2-basal-salt Murashige and Skoog medium with 1% (v/v) dimethyl-sulfoxide (DMSO) in the absence or presence of indicated concentrations of *N*-1-naphthalphthalamic acid (NPA),  $\alpha$ -(phenyl ethyl-2-one)-indole-3-acetic acid (PEO-IAA), or 5-(4-chlorophenyl)-4*H*-1,2,4-triazole-3-thiol (YUCASIN). Error bars represent standard deviation ( $n=5$ –10); \*\*  $p<0.01$ , \*\*\*  $p<0.001$  (Student's *t*-test) in comparison with wild-type Col.

hypocotyl length of *ZTL*-overexpressing seedlings was reduced, although their hypocotyls were still significantly longer than those of Col (Figure 3). Enhanced hypocotyl elongation in *ZTL*-overexpressing seedlings was completely inhibited by 5  $\mu$ M NPA, 50  $\mu$ M PEO-IAA, or 100  $\mu$ M YUCASIN. These results strongly suggest the involvement of auxin in the enhancement of hypocotyl elongation in *ZTL*-overexpressing seedlings.

Auxin regulates the expression of a large number

of genes involved in growth, development, and differentiation (Abel and Theologis 1996), including the *AUX/IAA* and *SAUR* families of the early (primary) auxin-inducible genes (Hagen and Guilfoyle 2002; Woodward and Bartel 2005). *AUX/IAA* genes encode transcriptional repressors and function in negative-feedback pathways to attenuate auxin responses (Tiwari et al. 2001). The expression of *SAUR* genes is usually associated with tissue elongation (Franklin et al. 2011; Roig-Villanova et al. 2007). The sequences of *SAUR*19–24 are highly identical to each other, and these six genes are located in a tandem array on chromosome 5, which suggests their functional redundancy (Hagen and Guilfoyle 2002). Plants overexpressing *SAUR*19 have longer hypocotyls than wild-type plants under continuous light (Franklin et al. 2011), and plants overexpressing a *GFP-SAUR*19, 21, 23, or 24 fusion gene also have elongated hypocotyls under long-day conditions (Spartz et al. 2012). *SAUR*19 stimulates plasma membrane H<sup>+</sup>-ATPase by inhibiting the activity of type 2C protein phosphatases, which leads to cell expansion via an acid-growth mechanism (Spartz et al. 2014). It is noteworthy that the expression of *SAUR*22 and *SAUR*23 in *ZTLox*2 was higher than that in *ZTLox*1 and the hypocotyls of *ZTLox*2 were longer than those of *ZTLox*1 (Figures 2, S1).

Auxin transcriptionally activates the expression of genes for expansins, which are involved in cell wall loosening necessary for growth (Cosgrove 2000). The up-regulation of the expression of genes for expansins A8 (AT2G40610; two probes: 2.9- and 2.7-fold in *ZTLox*1; 4.5- and 3.9-fold in *ZTLox*2; Table S1) and A11 (AT1G20190; 2.4-fold in *ZTLox*1, 2.5-fold in *ZTLox*2; Table S1) might contribute to hypocotyl elongation in *ZTL*-overexpressing seedlings grown under light.

We also observed up-regulation of the expression of genes for gibberellin 20-oxidase (GA5) (AT4G25420; 2.2-fold in *ZTLox*1, 5.0-fold in *ZTLox*2; Table S1) and a gibberellin-regulated protein (AT5G14920; two probes: 2.3-fold for both probes in *ZTLox*1; 5.6- and 5.0-fold in *ZTLox*2; Table S1). Two genes involved in ethylene signaling were down-regulated (Table S2): *ETHYLENE RESPONSE FACTOR*1 (*ERF*1) (AT3G23240; –2.3-fold in *ZTLox*1, –2.5-fold in *ZTLox*2) and *ETHYLENE INSENSITIVE ROOT*1 (*EIR*1) (AT5G57090; –2.8-fold in both *ZTLox*1 and *ZTLox*2). These data suggest *ZTL*-dependent changes in gibberellin and ethylene signaling. Gibberellins stimulate cell elongation in *Arabidopsis* hypocotyls, whereas ethylene predominantly inhibits cell elongation, although it can also stimulate elongation under certain conditions (Cowling and Harberd 2007; Vandenbussche et al. 2007). The up-regulation of gibberellin-related genes and the down-regulation of ethylene-related genes in *ZTL*-overexpressing seedlings indicate that these hormones may also contribute to the

effect of *ZTL* on hypocotyl elongation.

We found that expression of the gene for phytochrome-interacting factor (PIF) 4 was up-regulated in *ZTL*-overexpressing seedlings (AT2G43010; 4.2-fold in *ZTLox*1, 3.1-fold in *ZTLox*2; Table S1). PIF4 is a basic helix-loop-helix transcription factor that promotes hypocotyl elongation by activating the expression of genes involved in auxin biosynthesis, auxin signaling components, cell wall loosening, and cell expansion (Hornitschek et al. 2012; Leivar and Quail 2011; de Lucas and Prat 2014; Sun et al. 2013). Therefore, the up-regulation of PIF4 might lead to elevation of auxin biosynthesis and up-regulation of *SAUR*s in *ZTL*-overexpressing seedlings. The expression of PIF4 is diurnally regulated by the evening complex (EC), which consists of EARLY FLOWERING (ELF)3, ELF4, and LUX ARRHYTHMO (LUX)/PHYTOCLOCK1 (PCL1) (Nusinow et al. 2011). The expression of EC genes is under circadian control; EC binds to the PIF4 promoter to repress PIF4 (Nusinow et al. 2011). Plants overexpressing *ZTL* show very short circadian rhythm or arrhythmicity under continuous light (Somers et al. 2004), which may reduce the EC amount, activity, or both. If so, this might be a reason for the up-regulation of PIF4 and enhancement of hypocotyl elongation in *ZTL*-overexpressing seedlings grown under light. Further study is necessary to evaluate the role of PIF4 and EC in *ZTL*-mediated hypocotyl elongation.

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A_84_P10332	AT5G66110	metal ion binding	0.377	2.381	1.4.E-07	0.506	3.208	2.8.E-10
A_84_P761538	AT3G22142	structural constituent of cell wall	0.376	2.377	2.0.E-25	0.791	6.185	0.0.E+00
A_84_P14450	AT2G28900	OEP16 (OUTER ENVELOPE PROTEIN 16); P-P-bond-hydrolysis-driven protein transmembrane transporter	0.375	2.373	9.7.E-08	0.560	3.628	2.9.E-12
A_84_P21014	AT2G05530	glycine-rich protein	0.375	2.373	5.9.E-06	0.412	2.580	5.0.E-07
A_84_P806732	AT1G20620	CAT3 (CATALASE 3); catalase	0.375	2.369	1.0.E-07	0.760	5.751	4.0.E-16
A_84_P549629	AT5G58140	PHOT2 (NON PHOTOTROPIC HYPOCOTYL 1-LIKE); kinase	0.374	2.367	6.5.E-06	0.488	3.078	1.0.E-05
A_84_P23180	AT3G53800	armadillo/beta-catenin repeat family protein	0.374	2.365	1.1.E-07	0.639	4.351	6.4.E-14
A_84_P806759	AT1G20620	CAT3 (CATALASE 3); catalase	0.373	2.360	1.1.E-07	0.784	6.084	1.6.E-16
A_84_P20058	AT2G30420	myb family transcription factor	0.372	2.354	6.7.E-07	0.490	3.093	2.7.E-08
A_84_P69244	AT2G45560	CYP76C1 (cytochrome P450, family 76, subfamily C, polypeptide 1); heme binding / iron ion binding / monooxygenase	0.372	2.353	1.8.E-07	0.582	3.818	2.5.E-12
A_84_P22976	AT2G36270	AB15 (ABA INSENSITIVE 5); DNA binding / transcription activator/ transcription factor	0.371	2.351	1.8.E-07	0.398	2.502	4.3.E-08
A_84_P809730	AT5G14920	gibberellin-regulated family protein	0.371	2.348	1.3.E-07	0.747	5.587	8.0.E-16
A_84_P842922	AT3G13980	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G54200.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN69469.1)	0.364	2.315	3.1.E-07	0.333	2.154	1.9.E-06
A_84_P790043	AT3G07390	no match	0.364	2.312	2.3.E-03	0.613	4.107	1.0.E-05
A_84_P21135	AT3G03820	auxin-responsive protein, putative	0.364	2.311	4.7.E-07	0.903	8.002	5.4.E-16
A_84_P131456	AT5G14920	gibberellin-regulated family protein	0.362	2.302	2.1.E-07	0.703	5.042	3.7.E-15
A_84_P16857	AT5G41890	GDSL-motif lipase/hydrolase family protein	0.362	2.301	6.8.E-03	0.359	2.287	1.5.E-04
A_84_P279980	AT1G29440	auxin-responsive family protein	0.360	2.291	2.6.E-07	0.474	2.979	3.6.E-10
A_84_P21864	AT1G73805	calmodulin binding	0.359	2.287	3.0.E-07	0.332	2.149	1.6.E-06
A_84_P811729	AT2G33830	dormancy/auxin associated family protein	0.357	2.275	2.9.E-07	0.469	2.944	4.0.E-10
A_84_P255590	AT4G23870	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G11020.1)	0.355	2.266	3.2.E-07	0.325	2.114	1.9.E-06
A_84_P597910	AT1G68870	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G26210.1)	0.355	2.265	1.7.E-06	0.438	2.743	2.3.E-08
A_84_P750656	AT1G53160	SPL4 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 4); DNA binding	0.354	2.261	1.0.E-05	0.395	2.482	1.0.E-05
A_84_P789621	AT1G24148	other RNA	0.353	2.255	2.3.E-06	0.417	2.613	2.0.E-07
A_84_P768857	AT5G44572	unknown protein	0.353	2.252	3.8.E-07	0.443	2.773	1.8.E-09
A_84_P719823	AT1G1362	enzyme inhibitor/ pectinesterase	0.352	2.251	1.8.E-03	0.595	3.938	2.3.C-04
A_84_P839042	AT1G1925	Encodes a Stigma-specific Stig1 family protein	0.350	2.241	1.0.E-05	0.522	3.330	4.4.E-10
A_84_P22555	AT1G29510	SAUR68 (SMALL AUXIN UPREGULATED 68)	0.349	2.233	5.3.E-07	0.495	3.125	1.5.E-10
A_84_P825225	AT5G65080	AGL68/MAF5 (MADS AFFECTING FLOWERING 5); transcription factor	0.349	2.233	9.1.E-04	0.507	3.211	6.0.E-05
A_84_P16678	AT4G25420	GA5 (GA REQUIRING 5); gibberellin 20-oxidase/ gibberellin 3-beta-dioxygenase	0.348	2.229	3.5.E-06	0.703	5.044	1.1.E-13
A_84_P814887	AT1G75900	family II extracellular lipase 3 (EXL3)	0.347	2.224	5.2.E-07	0.624	4.204	1.2.E-13
A_84_P811724	AT2G33830	dormancy/auxin associated family protein	0.347	2.223	5.2.E-07	0.420	2.628	7.0.E-09
A_84_P834622	AT1G02300	cathepsin B-like cysteine protease, putative	0.346	2.217	5.9.E-07	0.348	2.229	5.3.E-07
[AT4G38825, similar to auxin-responsive protein, putative [Arabidopsis thaliana] (TAIR:AT5G18030.1); similar to auxin-induced protein-like [Brassica rapa] (GB:ABL97983.1); contains InterPro domain Auxin responsive SAUR protein (InterPro:IPR003676);[AT4G38820, unknown protein]								
A_84_P765839	AT38825;AT4G3		0.346	2.216	7.3.E-07	1.021	10.498	3.3.E-16
A_84_P299810	AT1G11925	Encodes a Stigma-specific Stig1 family protein	0.345	2.215	1.4.E-06	0.466	2.925	9.8.E-10
A_84_P760471	AT3G23050	IAA7 (AUXIN RESISTANT 2)	0.345	2.212	1.7.E-06	0.313	2.055	9.1.E-06
A_84_P15583	AT3G46970	ATPHS2/PHS2 (ALPHA-GLUCAN PHOSPHORYLASE 2); phosphorylase/ transferase, transferring glycosyl groups	0.344	2.207	6.3.E-07	0.407	2.551	1.5.E-08
A_84_P844205	AT4G23160	protein kinase family protein	0.344	2.206	9.5.E-03	0.424	2.656	5.7.E-03
A_84_P856901	AT5G16030	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G02500.1); similar to hypothetical protein [Cleome spinosa] (GB:ABD96956.1)	0.340	2.190	8.0.E-07	0.474	2.978	3.3.E-10
A_84_P838988	AT5G26570	PWD (PHOSPHOGLUCAN WATER DIKINASE); catalytic	0.337	2.173	1.7.E-06	0.402	2.525	5.4.E-08
A_84_P519104	AT3G25716	DVL6/RTFL16 (ROTUNDIFOLIA LIKE 16)	0.336	2.168	1.0.E-06	0.378	2.390	8.2.E-08
A_84_P841512	AT3G28370	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G28350.1); similar to hypothetical protein OsI_021564 [Oryza sativa (indica cultivar-group)] (GB:EAZ00332.1)	0.335	2.165	1.2.E-06	0.503	3.183	9.6.E-11
A_84_P148558	AT5G64490	binding	0.334	2.156	4.6.E-06	0.362	2.302	2.2.E-06
A_84_P235073	AT1G73870	zinc finger (B-box type) family protein	0.333	2.152	1.2.E-06	0.482	3.031	2.1.E-10
A_84_P17108	AT1G71000	DNAJ heat shock N-terminal domain-containing protein	0.333	2.151	8.9.E-03	0.693	4.930	1.4.E-14
A_84_P14446	AT2G19190	FRK1 (FLG22-INDUCED RECEPTOR-LIKE KINASE 1); kinase	0.330	2.136	1.8.E-06	0.359	2.285	3.3.E-07
similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G27980.1); similar to unnamed protein product [Vitis vinifera] (GB:CAO71037.1); contains InterPro domain Molecular chaperone, heat shock protein, Hsp40, DnaJ (InterPro:IPR015609)								
A_84_P80689	AT5G62720	integral membrane HPP family protein	0.329	2.134	1.5.E-06	0.422	2.640	6.3.E-09
A_84_P577078	AT5G35480	unknown protein	0.327	2.123	1.7.E-06	0.419	2.623	7.7.E-09
A_84_P13568	AT2G33830	dormancy/auxin associated family protein	0.324	2.110	2.0.E-06	0.369	2.336	1.5.E-07
A_84_P115312	AT3G47860	apolipoprotein D-related	0.322	2.099	2.3.E-06	0.509	3.230	4.3.E-11
A_84_P105486	AT3G28370	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT3G28350.1); similar to hypothetical protein OsI_021564 [Oryza sativa (indica cultivar-group)] (GB:EAZ00332.1)	0.322	2.097	2.4.E-06	0.441	2.758	2.1.E-09
A_84_P23021	AT2G29090	CYP707A2 (cytochrome P450, family 707, subfamily A, polypeptide 2); oxygen binding	0.321	2.096	6.7.E-06	0.652	4.484	6.1.E-13
A_84_P289354	AT2G15890	MEE14 (maternal effect embryo arrest 14)	0.320	2.087	2.6.E-06	0.347	2.223	5.2.E-07
A_84_P55946	AT1G18265	contains InterPro domain Protein of unknown function DUF593 (InterPro:IPR007656)	0.319	2.083	3.0.E-05	0.350	2.240	1.0.E-05
A_84_P813465	AT2G15890	MEE14 (maternal effect embryo arrest 14)	0.318	2.079	2.9.E-06	0.323	2.104	2.2.E-06
A_84_P16577	AT1G21520	unknown protein	0.318	2.077	3.3.E-06	0.386	2.433	5.7.E-08
A_84_P754400	AT1G24148	other RNA	0.315	2.067	6.9.E-06	0.365	2.317	5.9.E-07
A_84_P17209	AT1G31690	copper ion binding	0.315	2.063	6.0.E-06	0.370	2.342	4.0.E-07
A_84_P812392	AT4G33720	pathogenesis-related protein, putative	0.313	2.058	3.2.E-03	0.541	3.472	1.1.E-03
A_84_P79415	AT2G18300	basic helix-loop-helix (BHLH) family protein	0.313	2.057	3.9.E-06	0.436	2.731	2.8.E-09
A_84_P20612	AT5G26570	PWD (PHOSPHOGLUCAN WATER DIKINASE); catalytic	0.312	2.052	4.0.E-06	0.536	3.439	9.8.E-12
A_84_P55630	AT1G02300	cathepsin B-like cysteine protease, putative	0.312	2.050	4.2.E-06	0.352	2.248	4.0.E-07
A_84_P521683	AT2G46970	PIL1 (PHYTOCHROME INTERACTING FACTOR 3-LIKE 1); transcription factor	0.312	2.049	5.3.E-04	0.744	5.545	4.7.E-12
A_84_P847330	AT3G46970	ATPHS2/PHS2 (ALPHA-GLUCAN PHOSPHORYLASE 2); phosphorlyase/ transferase, transferring glycosyl groups	0.310	2.041	6.8.E-06	0.529	3.384	4.1.E-11
A_84_P761148	AT3G22886	MIR167A; miRNA	0.310	2.040	3.0.E-03	0.344	2.208	6.5.E-03
A_84_P802615	AT5G62360	invertase/pectin methylesterase inhibitor family protein	0.308	2.034	5.2.E-06	0.337	2.174	9.8.E-07
A_84_P847119	AT5G62360	invertase/pectin methylesterase inhibitor family protein	0.307	2.028	5.5.E-06	0.348	2.226	5.0.E-07
similar to unknown protein [Arabidopsis thaliana] (TAIR:AT4G33980.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN75496.1)								
A_84_P164883	AT5G42900		0.306	2.025	5.7.E-06	0.679	4.773	1.1.E-14
A_84_P13577	AT1G36940	unknown protein	0.304	2.014	6.6.E-06	0.350	2.238	4.4.E-07
A_84_P242433	AT3G25020	disease resistance family protein	0.304	2.013	2.0.E-05	0.398	2.503	3.1.E-07
A_84_P17881	AT5G62360	invertase/pectin methylesterase inhibitor family protein	0.304	2.012	6.7.E-06	0.312	2.050	4.1.E-06
A_84_P10257	AT1G29430	auxin-responsive family protein	0.303	2.008	9.4.E-04	0.578	3.783	5.0.E-05
A_84_P103276	AT5G02160	unknown protein	0.301	2.001	7.7.E-06	0.430	2.693	3.8.E-09







A_84_P19565	AT4G37430	CYP91A2 (CYTOCHROME P450 MONOOXYGENASE 91A2); oxygen binding	-0.33311	-2.15335	1.23E-06	-0.37235	-2.35695	1.18E-07
A_84_P545036	AT4G39795	senescence-associated protein-related	-0.33179	-2.14678	1.99E-06	-0.43183	-2.70289	3.93E-09
A_84_P10354	AT1G27940	PGP13 (P-GLYCOPROTEIN 13); ATPase, coupled to transmembrane movement of substances	-0.33021	-2.13899	0.00023	-0.53443	-3.42315	1.17E-10
A_84_P17762	AT5G23030	TET12 (TETRASPARIN12)	-0.33013	-2.13862	3.65E-06	-0.36087	-2.29547	3.40E-07
A_84_P802975	AT4G17340	DELTA-TIP2/TIP2.2 (tonoplast intrinsic protein 2.2); water channel	-0.33011	-2.13852	1.42E-06	-0.39129	-2.46201	3.76E-08
A_84_P10804	AT1G26770	ATEXPA10 (ARABIDOPSIS THALIANA EXPANSIN A10)	-0.3295	-2.13549	1.49E-06	-0.31769	-2.07822	2.94E-06
A_84_P558829	AT2G29350	SAG13 (Senescence-associated gene 13); oxidoreductase	-0.32828	-2.12949	0.0072	-0.94742	-8.85967	1.53E-18
A_84_P831307	AT1G32450	proton-pump oligopeptide transport (POT) family protein	-0.32821	-2.12896	3.25E-06	-0.31826	-2.08084	8.74E-06
A_84_P14717	AT4G01630	ATEXPA17 (ARABIDOPSIS THALIANA EXPANSIN A17)	-0.32781	-2.1272	1.75E-06	-0.35124	-2.24513	5.02E-07
A_84_P79425	AT5G20820	auxin-responsive protein-related	-0.32726	-2.1245	1.73E-06	-0.44297	-2.7731	1.84E-09
A_84_P19141	AT2G47460	ATMYB12/MYB12 (MYB DOMAIN PROTEIN 12); DNA binding / transcription activator/transcription factor	-0.3269	-2.12273	1.89E-06	-0.61935	-4.16241	1.77E-13
A_84_P149248	AT1G21120	O-methyltransferase, putative	-0.32643	-2.12045	1.79E-06	-0.33306	-2.15307	1.20E-06
[AT3G46250, pseudogene, similar to leaf senescence-associated receptor-like protein kinase, serine/threonine-specific receptor protein kinase, Arabidopsis thaliana, PIR:S71277; blastp match of 31% identity and 2.8e-15 P-value to GP 9837280 gb JAG00510.1  AF285172 leaf senescence-associated receptor-like protein kinase [Phaseolus vulgaris];[AT3G46270, receptor protein kinase-related]								
A_84_P22048	AT2G18550	ATHB21/HB-2 (homeobox-2); DNA binding / transcription factor	-0.32349	-2.10613	0.00001	-0.45251	-2.83471	3.49E-09
A_84_P762771	AT3G52561	unknown protein	-0.32342	-2.10583	0.00001	-0.401	-2.51769	7.95E-08
A_84_P150278	AT2G34610	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G30190.1); similar to unnamed protein product [Tetraodon nigroviridis] (GB:CAG12037.1)	-0.32324	-2.10573	3.48E-06	-0.53171	-3.4018	1.58E-11
A_84_P799508	AT1G02220	ANAC003 (Arabidopsis NAC domain containing protein 3); transcription factor	-0.32227	-2.10245	2.52E-06	-0.5255	-3.35352	1.90E-11
A_84_P857567	AT4G30170	peroxidase, putative	-0.32267	-2.10218	2.29E-06	-0.36815	-2.33427	1.67E-07
A_84_P182054	AT5G37690	GDSL-motif lipase/hydrolase family protein	-0.32181	-2.09804	2.34E-06	-0.33252	-2.15039	1.24E-06
A_84_P288500	AT3G47710	bHLH family protein	-0.32176	-2.09779	0.00015	-0.73142	-5.38786	7.14E-11
A_84_P21046	AT2G38600	acid phosphatase class B family protein	-0.32088	-2.09352	3.69E-06	-0.46383	-2.90958	6.05E-10
A_84_P22181	AT3G28210	PMZ; zinc ion binding	-0.32065	-2.09242	2.52E-06	-0.36445	-2.31446	1.86E-07
A_84_P230859	AT3G22550	senescence-associated protein-related	-0.32034	-2.09094	2.66E-06	-0.37308	-2.36091	1.16E-07
A_84_P869736	AT5G62165	AGL42 (AGAMOUS LIKE 42); transcription factor	-0.31861	-2.0826	0.00006	-0.51699	-3.28843	2.81E-10
A_84_P63350	AT1G29720	protein kinase family protein	-0.31584	-2.06938	4.91E-06	-0.4262	-2.66807	7.66E-09
A_84_P833808	AT1G17420	LOX3 (Lipoxygenase 3); iron ion binding / lipoxygenase/ metal ion binding / oxidoreductase, acting on single donors with incorporation of molecular oxygen, incorporation of two atoms of similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G04860.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN68625.1); contains InterPro domain C2 calcium-dependent membrane targeting (InterPro:IPR000008)	-0.31582	-2.06926	3.42E-06	-0.58941	-3.88513	6.43E-13
A_84_P245475	AT2G25460	UNE11 (unfertilized embryo sac 11); pectinesterase inhibitor	-0.31546	-2.06757	5.87E-06	-0.35003	-2.23887	4.68E-07
A_84_P22381	AT4G31320	auxin-responsive protein, putative / small auxin up RNA (SAUR_C)	-0.31531	-2.06686	3.43E-06	-0.4378	-2.74029	2.48E-09
A_84_P823058	AT3G04630	WDL1 (WD2-LIKE 1)	-0.31527	-2.06665	4.47E-06	-0.36064	-2.29423	3.83E-07
A_84_P12677	AT3G23630	AT1P77 (Arabidopsis thaliana isopentenyltransferase 7); transferase, transferring alkyl or aryl (other than methyl) groups	-0.31453	-2.06314	5.73E-06	-0.31428	-2.06196	4.66E-06
A_84_P10500	AT1G15640	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G15620.1); similar to hypothetical protein [Vitis vinifera] (GB:CAN70531.1)	-0.31347	-2.05812	0.00029	-0.35999	-2.29084	0.00001
A_84_P16270	AT1G33610	leucine-rich repeat family protein	-0.31286	-2.05525	0.00002	-0.46593	-2.92367	1.34E-09
A_84_P835471	AT2G34080	cysteine proteinase, putative	-0.30896	-2.03684	0.00003	-0.42798	-2.67906	3.11E-06
A_84_P543604	AT1G47400	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G47395.1)	-0.30866	-2.03545	7.55E-06	-0.52284	-3.33304	3.79E-11
A_84_P23409	AT5G10130	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G47395.1)	-0.30764	-2.03068	6.17E-06	-0.7919	-6.19301	1.51E-16
A_84_P22377	AT4G30430	TET9 (TETRASPARIN9)	-0.30652	-2.02543	9.23E-06	-0.48864	-3.08063	8.20E-10
A_84_P24008	AT3G04320	endopeptidase inhibitor	-0.30613	-2.02362	0.00089	-0.3647	-2.31581	6.68E-07
A_84_P557480	AT5G15725	unknown protein	-0.30505	-2.02055	6.36E-06	-0.67486	-4.72997	1.54E-14
A_84_P573814	AT1G59171	similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G58936.1); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G58643.1); similar to unknown protein [Arabidopsis thaliana] (TAIR:AT1G59312.1); contains InterPro domain Protein of unknown function DUF941 (InterPro:IPR009286)	-0.30505	-2.01858	0.00059	-0.61729	-4.1428	0.00001
A_84_P17313	AT2G37770	aldo/keto reductase family protein	-0.30431	-2.01518	0.00093	-0.33124	-2.14409	0.00008
A_84_P20772	AT5G04370	NAMT1; S-adenosylmethionine-dependent methyltransferase	-0.30411	-2.01423	6.59E-06	-0.61219	-4.09439	2.11E-13
A_84_P577990	AT5G44570	unknown protein	-0.3036	-2.01185	0.00001	-0.3925	-2.46887	4.90E-08
A_84_P11789	AT3G43250	cell cycle control protein-related	-0.30304	-2.00927	0.00176	-0.60065	-3.987	7.29E-13
A_84_P11200	AT5G45920	carboxylesterase	-0.30265	-2.00749	0.00325	-0.42642	-2.66945	7.45E-09
A_84_P567338	AT5G55570	heat shock protein binding / unfolded protein binding	-0.30242	-2.00639	8.65E-06	-0.34905	-2.23385	5.45E-07
			-0.30104	-2.00005	0.00001	-0.42111	-2.63703	1.51E-08

**Table S3. Pathway analysis of genes down-regulated in *ZTL*-overexpressors performed with the Gene Functional Classification Tool in DAVID Bioinformatics Resources 6.7.**

Annotation Cluster 1: Enrichment Score: 7.660176315895672

Term	Count	%	PValue
flavonoid biosynthetic process	11	4.489796	1.21E-10
flavonoid metabolic process	11	4.489796	2.58E-10
phenylpropanoid metabolic process	15	6.122449	1.48E-09
secondary metabolic process	20	8.163265	5.99E-08
phenylpropanoid biosynthetic process	12	4.897959	7.54E-08
cellular amino acid derivative metabolic process	16	6.530612	1.74E-07
cellular amino acid derivative biosynthetic process	13	5.306122	6.96E-07
aromatic compound biosynthetic process	13	5.306122	2.06E-06

Annotation Cluster 2: Enrichment Score: 4.8640217871452025

Term	Count	%	PValue
flavonoid biosynthetic process	11	4.489796	1.21E-10
flavonoid metabolic process	11	4.489796	2.58E-10
anthocyanin biosynthetic process	4	1.632653	1.19E-04
anthocyanin metabolic process	4	1.632653	3.50E-04
pigment biosynthetic process	4	1.632653	0.059527
pigment metabolic process	4	1.632653	0.084623

Annotation Cluster 3: Enrichment Score: 3.577382072650882

Term	Count	%	PValue
response to endogenous stimulus	28	11.42857	1.70E-06
response to organic substance	31	12.65306	2.22E-06
response to ethylene stimulus	13	5.306122	1.23E-05
transcription	30	12.2449	5.08E-05
response to hormone stimulus	23	9.387755	1.33E-04
ethylene mediated signaling pathway	9	3.673469	3.55E-04
two-component signal transduction system (phosphorelay)	10	4.081633	4.01E-04
response to carbohydrate stimulus	9	3.673469	0.001003
cellular response to hormone stimulus	13	5.306122	0.001588
hormone-mediated signaling	13	5.306122	0.001588
regulation of transcription	35	14.28571	0.001967
intracellular signaling cascade	18	7.346939	0.002879
regulation of transcription, DNA-dependent	22	8.979592	0.003476
regulation of RNA metabolic process	22	8.979592	0.003706

Annotation Cluster 4: Enrichment Score: 3.2353612172106825

Term	Count	%	PValue
response to UV	7	2.857143	9.19E-05
response to light stimulus	15	6.122449	2.97E-04
response to radiation	15	6.122449	4.18E-04
response to abiotic stimulus	22	8.979592	0.010034

Annotation Cluster 5: Enrichment Score: 1.9675766001400188

Term	Count	%	PValue
regulation of anthocyanin biosynthetic process	3	1.22449	0.00442
regulation of flavonoid biosynthetic process	3	1.22449	0.008702
regulation of anthocyanin metabolic process	3	1.22449	0.009974
regulation of secondary metabolic process	3	1.22449	0.035145

Annotation Cluster 6: Enrichment Score: 1.7606299652332218

Term	Count	%	PValue
cell wall modification	7	2.857143	0.002615
external encapsulating structure organization	8	3.265306	0.030836
cell wall organization	7	2.857143	0.06481

---

**Annotation Cluster 7: Enrichment Score: 1.1809446630620486**

Term	Count	%	PValue
response to far red light	4	1.632653	0.010184
response to red light	3	1.22449	0.111796
response to red or far red light	4	1.632653	0.251675

---

**Annotation Cluster 8: Enrichment Score: 0.7446376091400814**

Term	Count	%	PValue
response to oxidative stress	8	3.265306	0.027992
response to reactive oxygen species	5	2.040816	0.055332
response to hydrogen peroxide	4	1.632653	0.117843
cellular response to reactive oxygen species	3	1.22449	0.253278
cellular response to oxidative stress	3	1.22449	0.257067
oxygen and reactive oxygen species metabolic process	3	1.22449	0.306275
response to inorganic substance	7	2.857143	0.476071
cellular response to stress	5	2.040816	0.637024

---

**Annotation Cluster 9: Enrichment Score: 0.5391032334165797**

Term	Count	%	PValue
organic acid biosynthetic process	8	3.265306	0.124756
carboxylic acid biosynthetic process	8	3.265306	0.124756
fatty acid biosynthetic process	4	1.632653	0.221805
cellular amino acid biosynthetic process	4	1.632653	0.290393
amine biosynthetic process	4	1.632653	0.346308
fatty acid metabolic process	4	1.632653	0.374193
lipid biosynthetic process	6	2.44898	0.418754
nitrogen compound biosynthetic process	4	1.632653	0.894487

---

**Annotation Cluster 10: Enrichment Score: 0.37690502440290247**

Term	Count	%	PValue
transmembrane receptor protein tyrosine kinase signaling pathway	3	1.22449	0.384197
enzyme linked receptor protein signaling pathway	3	1.22449	0.384197
cell surface receptor linked signal transduction	3	1.22449	0.501393

---

**Annotation Cluster 11: Enrichment Score: 0.21246079467630907**

Term	Count	%	PValue
growth	4	1.632653	0.461355
cell growth	3	1.22449	0.645776
regulation of cell size	3	1.22449	0.675135
regulation of cellular component size	3	1.22449	0.702504

---

**Annotation Cluster 12: Enrichment Score: 0.07072851963326712**

Term	Count	%	PValue
flower development	3	1.22449	0.689065
reproductive structure development	6	2.44898	0.873657
reproductive developmental process	6	2.44898	0.920814
post-embryonic development	6	2.44898	0.9404

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**Annotation Cluster 13: Enrichment Score: 0.045019450030184975**

Term	Count	%	PValue
protein amino acid phosphorylation	9	3.673469	0.860406
phosphate metabolic process	10	4.081633	0.91216
phosphorus metabolic process	10	4.081633	0.912609
phosphorylation	9	3.673469	0.92228

**Table S4. Pathway analysis of genes up-regulated in *ZTL*-overexpressors performed with the Gene Functional Classification Tool in DAVID Bioinformatics Resources 6.7.**

Annotation Cluster 1: Enrichment Score: 8.026434844463541

Term	Count	%	PValue
response to auxin stimulus	19	11.80124	4.03E-11
response to endogenous stimulus	26	16.14907	2.59E-08
response to hormone stimulus	25	15.52795	3.01E-08
response to organic substance	27	16.77019	2.49E-07

Annotation Cluster 2: Enrichment Score: 7.700409336795261

Term	Count	%	PValue
response to abiotic stimulus	31	19.25466	1.17E-09
response to temperature stimulus	17	10.55901	6.08E-09
response to cold	12	7.453416	1.11E-06

Annotation Cluster 3: Enrichment Score: 4.057457021955261

Term	Count	%	PValue
response to red or far red light	10	6.21118	4.39E-06
response to light stimulus	15	9.31677	7.73E-06
response to radiation	15	9.31677	1.13E-05
red or far red light signaling pathway	6	3.726708	1.06E-04
intracellular signaling cascade	10	6.21118	0.127068

Annotation Cluster 4: Enrichment Score: 3.005851716823043

Term	Count	%	PValue
response to red or far red light	10	6.21118	4.39E-06
response to red light	5	3.10559	7.57E-04
post-embryonic development	9	5.590062	0.288941

Annotation Cluster 5: Enrichment Score: 2.1461282184755133

Term	Count	%	PValue
circadian rhythm	6	3.726708	9.23E-06
rhythmic process	6	3.726708	2.88E-05
regulation of circadian rhythm	3	1.863354	0.006785
regulation of transcription	25	15.52795	0.010854
transcription	16	9.937888	0.056232
two-component signal transduction system (phosphorelay)	5	3.10559	0.077141
regulation of transcription, DNA-dependent	11	6.832298	0.279358
regulation of RNA metabolic process	11	6.832298	0.285047

Annotation Cluster 6: Enrichment Score: 1.4573214047782048

Term	Count	%	PValue
response to fungus	10	6.21118	0.004958
defense response	16	9.937888	0.008209
defense response to fungus	9	5.590062	0.009175
cell killing	4	2.484472	0.372049
killing of cells of another organism	4	2.484472	0.372049

Annotation Cluster 7: Enrichment Score: 1.2193856300758492

Term	Count	%	PValue
starch metabolic process	4	2.484472	0.001962
starch catabolic process	3	1.863354	0.003967
cellular polysaccharide catabolic process	3	1.863354	0.005289
glucan catabolic process	3	1.863354	0.0333
cellular glucan metabolic process	4	2.484472	0.063432
glucan metabolic process	4	2.484472	0.100167
polysaccharide catabolic process	3	1.863354	0.103065
cellular polysaccharide metabolic process	4	2.484472	0.104684
polysaccharide metabolic process	4	2.484472	0.218409

cellular carbohydrate catabolic process	3	1.863354	0.289049
carbohydrate catabolic process	3	1.863354	0.444369
macromolecule catabolic process	4	2.484472	0.883978

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Annotation Cluster 8: Enrichment Score: 1.0067600602344602

Term	Count	%	PValue
systemic acquired resistance	3	1.863354	0.023041
defense response, incompatible interaction	4	2.484472	0.033789
innate immune response	4	2.484472	0.329742
immune response	4	2.484472	0.366024

---

Annotation Cluster 9: Enrichment Score: 0.3579734088771785

Term	Count	%	PValue
developmental growth involved in morphogenesis	3	1.863354	0.318941
unidimensional cell growth	3	1.863354	0.318941
developmental growth	3	1.863354	0.382969
cell morphogenesis	3	1.863354	0.454283
cell growth	3	1.863354	0.468965
regulation of cell size	3	1.863354	0.497622
cellular component morphogenesis	3	1.863354	0.509274
regulation of cellular component size	3	1.863354	0.525292
growth	3	1.863354	0.543169

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Annotation Cluster 10: Enrichment Score: 0.35013923663701424

Term	Count	%	PValue
flower development	4	2.484472	0.24369
regulation of post-embryonic development	3	1.863354	0.286323
post-embryonic development	9	5.590062	0.288941
reproductive structure development	7	4.347826	0.452165
reproductive developmental process	7	4.347826	0.549398
seed development	3	1.863354	0.831547
fruit development	3	1.863354	0.850066

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Annotation Cluster 11: Enrichment Score: 0.1271781828743214

Term	Count	%	PValue
response to cadmium ion	3	1.863354	0.697609
response to inorganic substance	4	2.484472	0.764793
response to metal ion	3	1.863354	0.778591

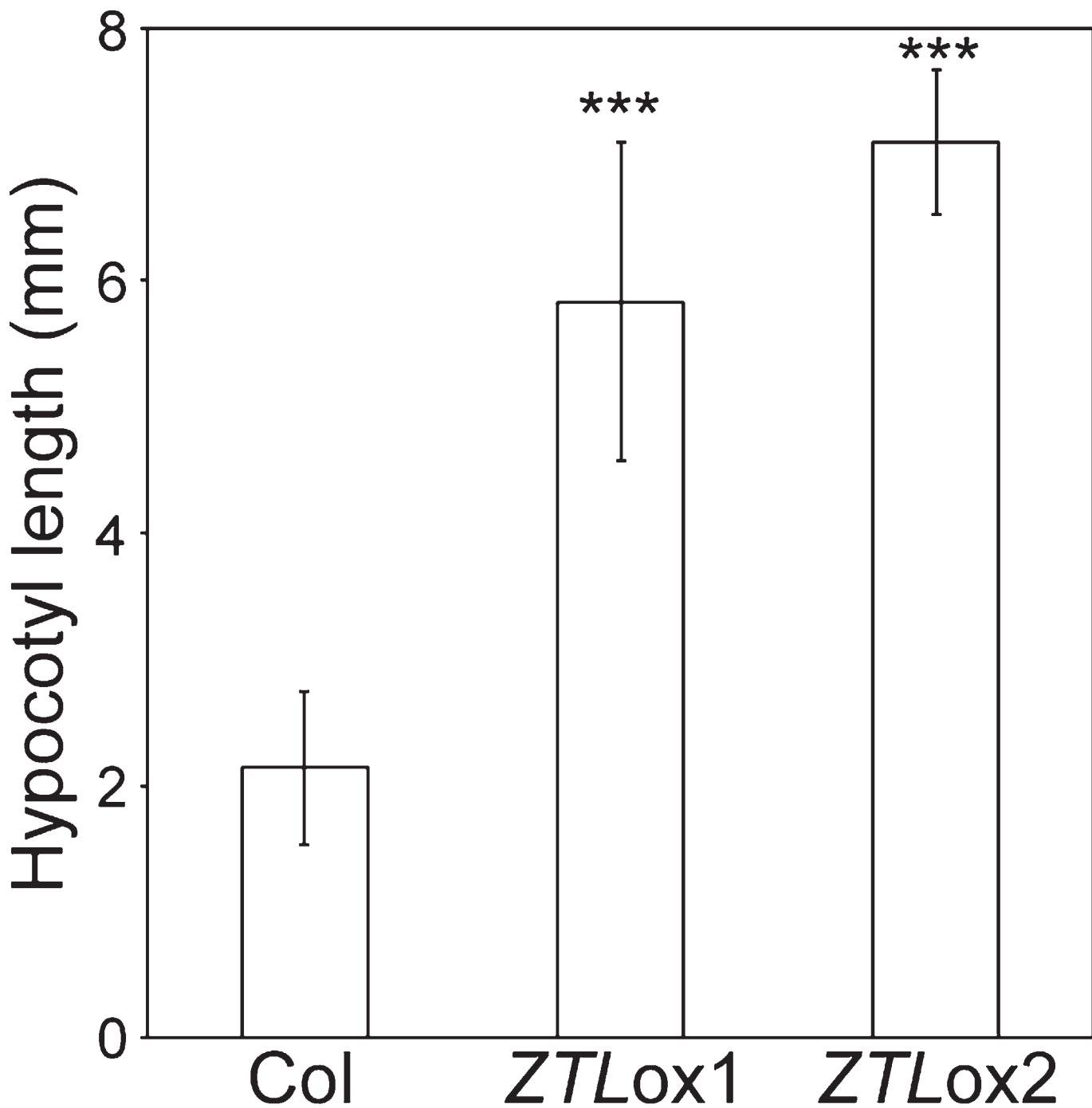
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Annotation Cluster 12: Enrichment Score: 0.06411649297992181

Term	Count	%	PValue
phosphate metabolic process	8	4.968944	0.838337
phosphorus metabolic process	8	4.968944	0.838929
phosphorylation	7	4.347826	0.874631
protein amino acid phosphorylation	6	3.726708	0.90067

Table S5. PCR primers used for expression analysis.

Genes	Forward sequence	Reverse sequence
ACT2	5'-GGTAACATTGTGCTCAGTGGTGG-3'	5'-AACGACCTTAATCTTCATGCTGC-3'
SAUR22	5'-CCTCTTTCAAGCTCTGCTCAGT-3'	5'-TTGGAGCCGAGAAGTCACATT-3'
SAUR23	5'-AGTGTACGTAGGAGAGAGCCAGAA-3'	5'-ATCGGATGATCGAACCCAAA-3'
IAA29	5'-CTTCCAAGGGAAAGAGGGTGAC-3'	5'-TTCCGCAAAGATCTTCATGTAAC-3'



**Supplemental Figure S1.** Hypocotyl length of *ZTL*-overexpressing (*ZTLox1* and *ZTLox2*) and wild-type Columbia (Col) *Arabidopsis thaliana* seedlings. Seeds were incubated for 7 days in the dark on 1.5% (w/v) agar containing 1/2-basal-salt Murashige and Skoog medium at 4°C, and then exposed to continuous white light (80  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) for 8 days at 22°C. Error bars represent standard deviation ( $n = 15\text{--}16$ ); \*\*\* $P < 0.001$  (Student's *t*-test) in comparison with wild-type Col.