

Supplemental Material

Supplementary Figure S1 shows cytological characterization of *Phytophthora brassicae* and *Botrytis cinerea* infection.

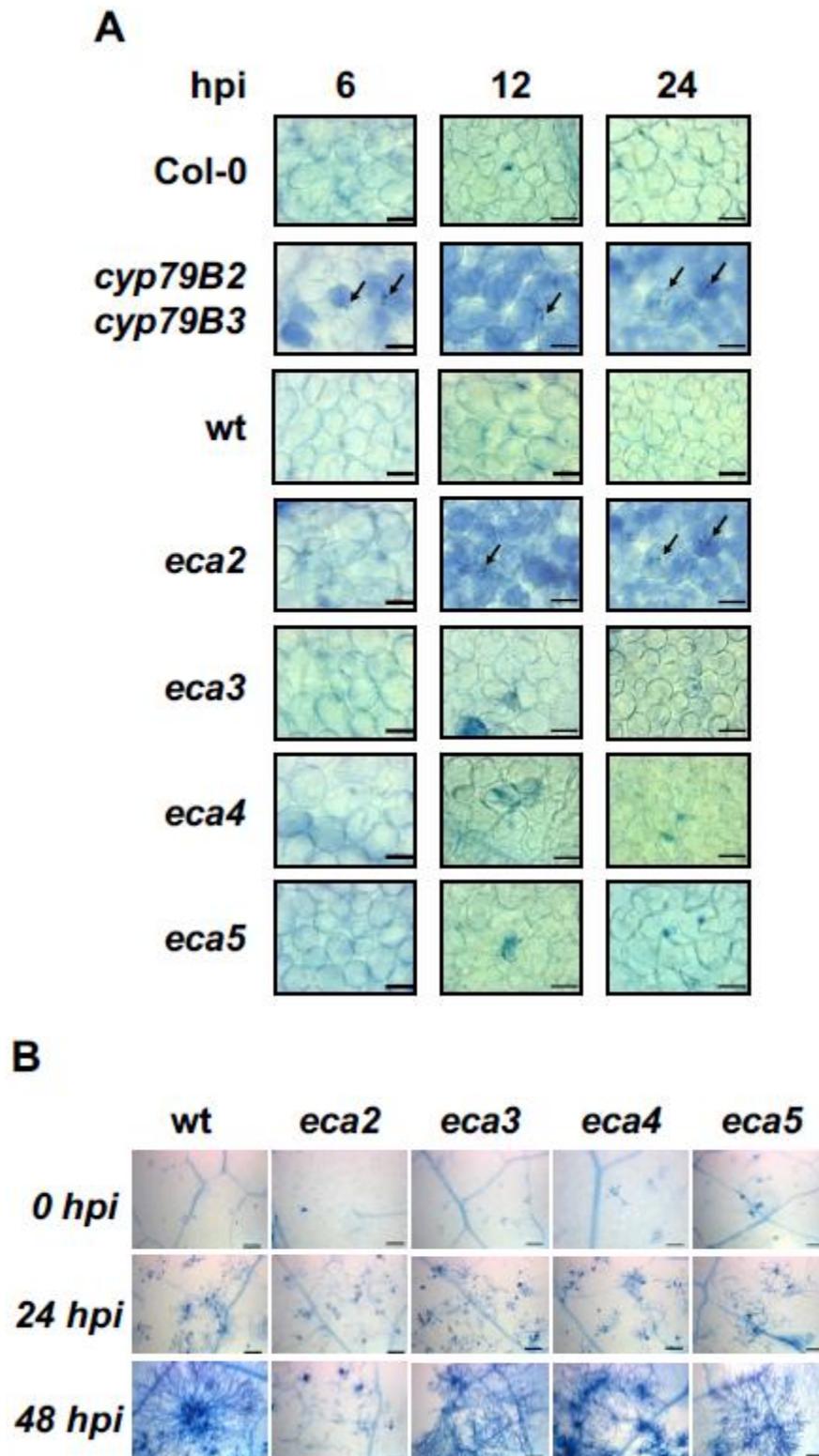
Supplementary Figure S2 presents a genome-wide transcriptomic analysis of unchallenged plants.

Supplementary Figure S3 illustrates the composition of aliphatic wax components.

Supplementary Figure S4 illustrates that *eca2* presents a novel cuticular-related set of differentially expressed genes.

Supplementary Table S1 lists the identified differentially expressed genes.

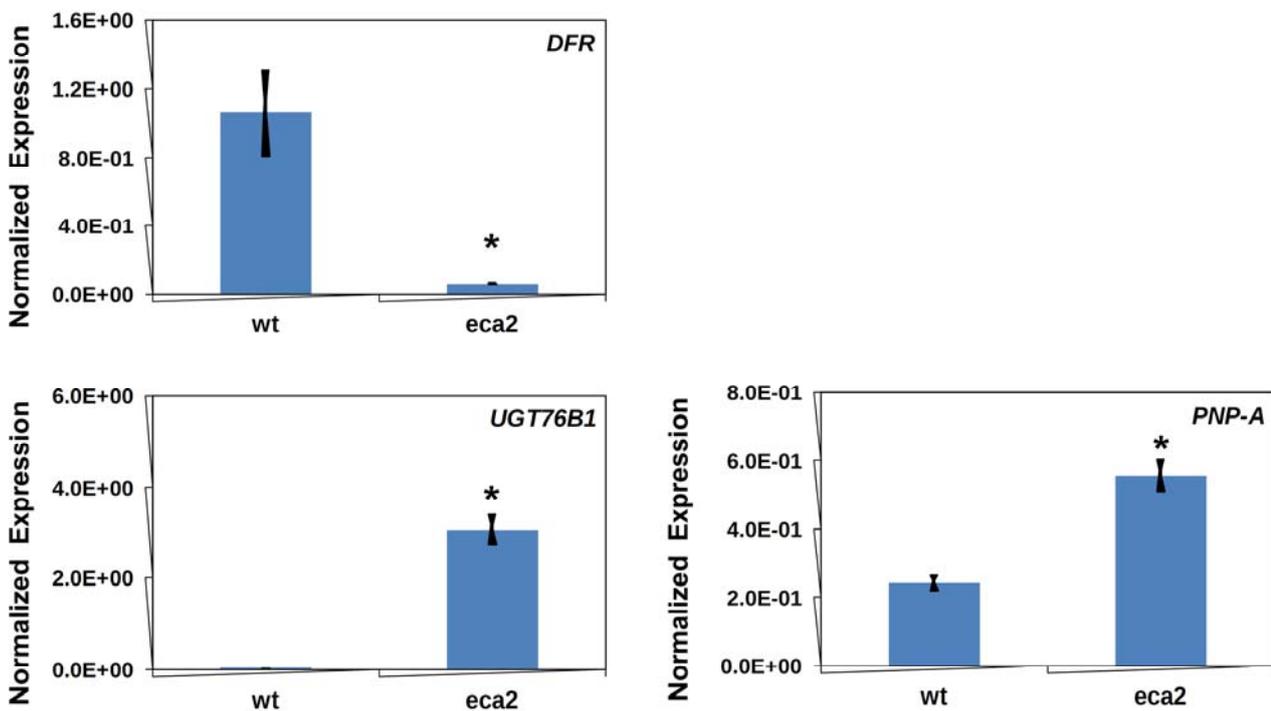
Supplementary Table S2 presents a Gene Ontology analysis of differentially expressed genes of unchallenged *eca2* plants.



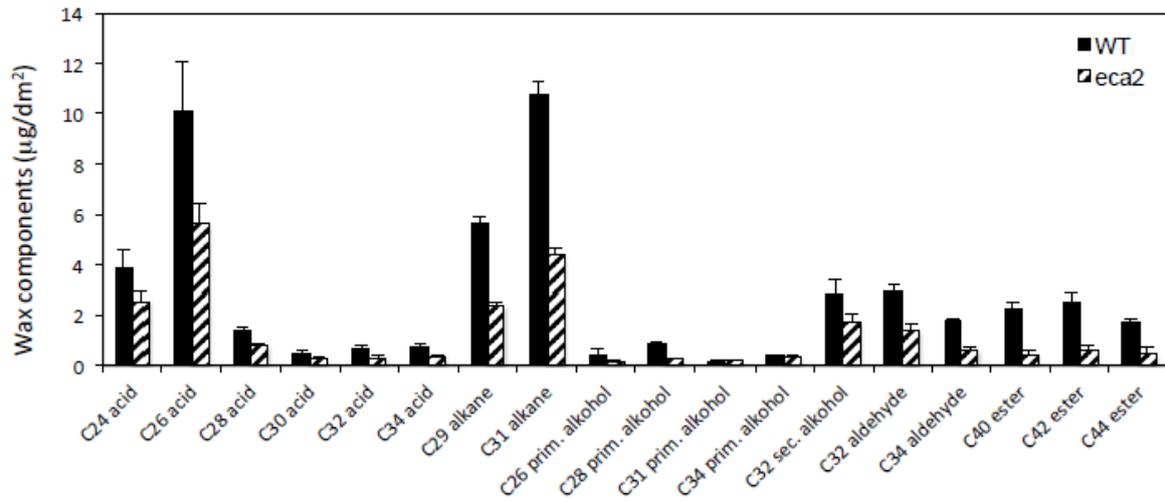
Supplementary Figure 1. Cytological characterization of *P. brassicae* and *B. cinerea* infection. **(A)** *P. brassicae* penetration and haustorium formation were determined by trypan blue staining at 6, 12 and 24 hpi. Arrows indicates cell death caused by the penetration and haustorium formation. **(B)** *B. cinerea* development over Arabidopsis infected leaves was determined by trypan blue staining at 0, 24 and 48 hpi. Representative images were selected as a visual illustration from three independent experiments.

A

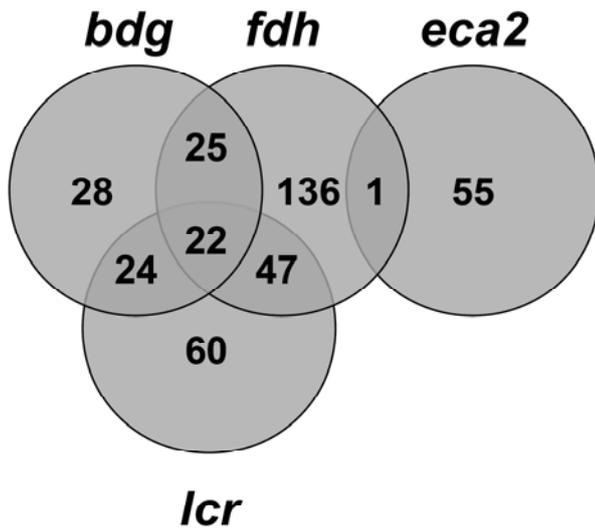
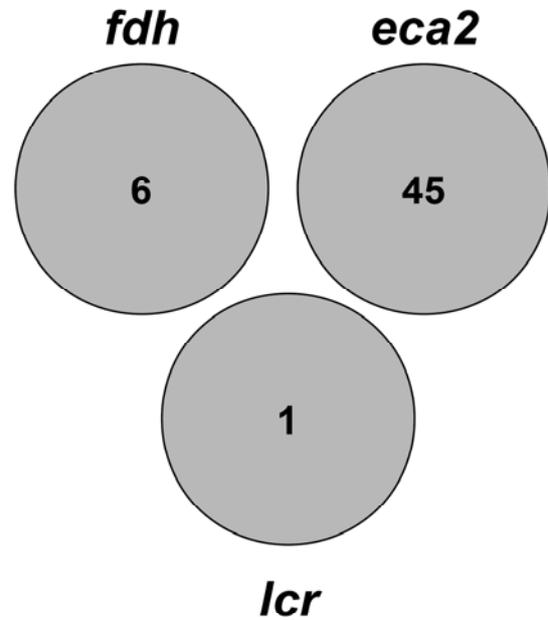
Locus	Description	Ratio <i>eca2</i> /wt
AT5G42800	DFR (DIHYDROFLAVONOL 4-REDUCTASE)	0.20
AT3G11340	UGT76B1 (UDP-glucuronosyl/UDP-glucosyl transferase family protein)	2.85
AT2G18660	PNP-A EXLB3 (EXPANSIN-LIKE B3 PRECURSOR)	4.27

B

Supplementary Figure 2. Genome-wide transcriptomic analysis of unchallenged plants. (A) Normalized gene expression ratio of down- and up-expressed genes are indicated in green and red color scale respectively. (B) Quantitative real-time PCR (qRT-PCR) analysis of differential expressed genes selected. The expression of selected genes was determined from non-infected leaves and normalized with respect to the reference gene AT4G26410, previously described as a stable reference gene (Czechowski et al., 2005). Histograms represent mean values (\pm SE) of three independent experiments with three technical replicates for each qRT-PCR assay. Asterisks indicate a statistical significant differences between wt and *eca2*, according to Student's *T*-test ($p < 0.05$).



Supplementary Figure 3. Composition of aliphatic wax components. Values are the mean of 5 to 6 replicate samples (\pm SD). Asterisks indicate a statistically significant difference compared to WT samples, according to the Student's *T*-test ($P \leq 0.01$).

A**B**

Supplementary Figure 4. *eca2* presents a novel cuticular-related set of DEGs. (A) and (B) Venn diagrams representing overlapping or non-overlapping gene sets of differential up- and down-regulated genes respectively, identified on *eca2*, *bdg*, *fdh* and *lcr*, compared to their corresponding wt plant.

(A)

locus	description	Ratio Values
		0 hpi Ratio eca2/wt
AT1G67810	SUFE2 (SULFUR E 2); enzyme activator	0.33
AT4G15210	BAM5 (BETA-AMYLASE 5); beta-amylase	0.48
AT4G16260	catalytic/ cation binding / hydrolase, hydrolyzing O-glycosyl compounds	0.22
AT4G17470	palmitoyl protein thioesterase family protein	0.34
AT4G14090	UDP-glucuronosyl/UDP-glucosyl transferase family protein	0.34
AT1G56600	AtGolS2 (Arabidopsis thaliana galactinol synthase 2); transferase, transferring glycosyl groups / transferase, transferring hexosyl groups	0.48
AT1G56650	PAP1 (PRODUCTION OF ANTHOCYANIN PIGMENT 1); DNA binding / transcription factor	0.39
AT1G73330	ATDR4; peptidase inhibitor	0.39
AT5G61160	AACT1 (anthocyanin 5-aromatic acyltransferase 1); transferase/ transferase, transferring acyl groups other than amino-acyl groups	0.19
AT5G54060	UF3GT (udp-glucose:flavonoid 3-O-glucosyltransferase); transferase, transferring glycosyl groups	0.47
AT5G42800	DFR (DIHYDROFLAVONOL 4-REDUCTASE); dihydrokaempferol 4-reductase	0.20
AT5G39610	ATNAC6 (ARABIDOPSIS NAC DOMAIN CONTAINING PROTEIN 6); protein heterodimerization/ protein homodimerization/ transcription factor	0.48
AT5G13220	JAZ10 (JASMONATE-ZIM-DOMAIN PROTEIN 10)	0.45
AT5G10520	RBK1 (Rop Binding protein Kinases 1); ATP binding / kinase/ protein kinase/ protein serine/threonine kinase	0.34
AT3G61990	O-methyltransferase family 3 protein	0.43
AT4G38540	monooxygenase, putative (MO2)	0.46
AT4G34590	[AT4G34590, GBF6 (G-BOX BINDING FACTOR 6); DNA binding / protein heterodimerization/ transcription factor];[AT4G34588, CPuORF2]	0.49
AT4G23990	ATCSLG3; cellulose synthase/ transferase/ transferase, transferring glycosyl groups	0.46
AT4G22870	[AT4G22870, leucoanthocyanidin dioxygenase, putative / anthocyanidin synthase, putative];[AT4G22880, LDOX (LEUCOANTHOCYANIDIN DI-OXYGENASE)]	0.29
AT4G12500	protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	0.42
AT4G12280	[AT4G12280, copper amine oxidase family protein];[AT4G12290, amine oxidase/ copper ion binding / quinone binding]	0.47
AT3G12500	ATHCHIB (ARABIDOPSIS THALIANA BASIC CHITINASE); chitinase	0.48
AT1G66690	[AT1G66690, S-adenosyl-L-methionine:carboxyl methyltransferase family protein];[AT1G66700, PXMT1; S-adenosylmethionine-dependent methyltransferase]	0.37
AT3G28270	FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: cellular_component unknown; ECA2: 0.47	0.47
AT3G26200	CYP71B22; electron carrier/ heme binding / iron ion binding / monooxygenase/ oxygen binding	0.48
AT3G23550	MATE efflux family protein	0.26
AT3G16360	AHP4 (HPT PHOSPHOTRANSMITTER 4); histidine phosphotransfer kinase/ transferase, transferring phosphorus-containing groups	0.47
AT1G72260	THI2.1 (THIONIN 2.1); toxin receptor binding	0.28
AT2G41800	FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; LOCATED IN: cell wall, plant-type cell wall; ECA2: 0.45	0.45
AT1G06160	ORA59 (OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59); DNA binding / transcription activator/ transcription factor	0.38
AT1G17380	JAZ5 (JASMONATE-ZIM-DOMAIN PROTEIN 5)	0.42
AT1G18710	AtMYB47 (myb domain protein 47); DNA binding / transcription factor	0.37
AT1G21100	O-methyltransferase, putative	0.47
AT1G73040	jacalin lectin family protein	0.36
AT1G59950	aldo/keto reductase, putative	0.22
AT1G24070	ATCSLA10; cellulose synthase/ transferase, transferring glycosyl groups	0.33
AT1G78460	SOUL heme-binding family protein	0.44
AT1G54020	myrosinase-associated protein, putative	0.49
AT2G36490	DML1 (DEMETER-LIKE 1); DNA N-glycosylase/ DNA-(apurinic or apyrimidinic site) lyase/ protein binding	0.49
AT1G61800	GPT2; antiporter/ glucose-6-phosphate transmembrane transporter	0.37
AT1G10070	ATBCAT-2 (ARABIDOPSIS THALIANA BRANCHED-CHAIN AMINO ACID TRANSAMINASE 2); branched-chain-amino-acid transaminase/ catalase	0.27
AT1G03940	[AT1G03940, transferase family protein];[AT1G03495, transferase/ transferase, transferring acyl groups other than amino-acyl groups]	0.36
AT1G62540	FMO GS-OX2 (FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 2); 3-methylthiopropyl glucosinolate S-oxygenase/ 4-methylthiopropyl glucosinolate S-oxygenase	0.40
AT2G18050	HIS1-3 (HISTONE H1-3); DNA binding / nucleosomal DNA binding	0.36
AT2G47670	invertase/pectin methyltransferase inhibitor family protein	0.50

Supplementary Table 1

(B)

locus	description	Ratio Values
		0 hpi Ratio eca2/wt
no_match	no_match	3.51
AT4G08010;	[AT4G08010, transposable element gene];[AT1G39190, transposable element gene];[AT3G42721, transposable element gene]	6.16
AT4G07523;	[AT4G07523, transposable element gene];[AT1G43205, transposable element gene];[AT5G27180, transposable element gene]	4.33
AT5G43800	transposable element gene	2.39
AT5G18430	GDSL-motif lipase/hydrolase family protein	2.12
AT5G03350	legume lectin family protein	2.26
AT1G30784;	[AT1G30784, Pseudogene of AT5G01080; beta-galactosidase];[AT5G01080, beta-galactosidase]	4.32
AT5G44415;	[AT5G44415, transposable element gene];[AT1G36080, transposable element gene];[AT1G23930, transposable element gene];[AT3G47330, transposable element gene];[AT3G42930, transposable element gene];[AT5G30545, transposable element gene]	2.32
AT3G42930;	[AT3G42930, transposable element gene];[AT5G30545, transposable element gene]	2.61
AT5G44415;	[AT5G44415, transposable element gene];[AT5G33230, transposable element gene];[AT4G28960, transposable element gene];[AT5G37390, transposable element gene];[AT4G25260, invertase/pectin methyltransferase inhibitor family protein]	2.96
AT4G25260	invertase/pectin methyltransferase inhibitor family protein	2.06
no_match	no_match	2.05
AT4G08000	transposable element gene	2.40
AT4G05640	transposable element gene	6.06
AT2G10140;	[AT2G10140, transposable element gene];[AT3G30680, transposable element gene];[AT4G03900, transposable element gene];[AT1G40123, transposable element gene];[AT4G03790, transposable element gene]	4.38
AT4G03790	transposable element gene	4.51
AT3G11340	UGT76B1 (UDP-glucuronosyl/UDP-glucosyl transferase family protein)	2.85
AT1G36190;	[AT1G36190, transposable element gene];[AT3G32230, transposable element gene]	3.58
AT3G30360	transposable element gene	3.61
AT3G30396	transposable element gene	5.62
AT2G06720;	[AT2G06720, transposable element gene];[AT3G31955, transposable element gene];[AT3G42720, transposable element gene]	4.40
AT1G36540;	[AT1G36540, transposable element gene];[AT3G33066, transposable element gene];[AT1G41700, transposable element gene]	5.52
AT1G33840	unknown protein	3.50
AT5G45082;	[AT5G45082, transposable element gene];[AT3G30670, transposable element gene];[AT1G35600, transposable element gene];[AT4G03910, transposable element gene];[AT3G09170, transposable element gene];[AT1G34610, transposable element gene]	3.24
AT3G09170;	[AT3G09170, transposable element gene];[AT1G34610, transposable element gene]	2.74
AT1G01560	ATMPK11; MAP kinase/ kinase	2.17
AT1G44060	transposable element gene	3.70
AT1G20400	myosin heavy chain-related	5.12
AT1G33960	AIG1 (AVRRPT2-INDUCED GENE 1); GTP binding	2.09
AT1G43840	transposable element gene	5.90
AT1G02450	NIMIN1 (NIM1-INTERACTING 1); protein binding	2.85
AT1G34530	transposable element gene	4.99
AT1G35710	leucine-rich repeat transmembrane protein kinase, putative	3.50
AT1G33130	transposable element gene	6.06
AT1G36470;	[AT1G36470, transposable element gene];[AT2G10630, transposable element gene];[AT1G50860, transposable element gene];[AT2G12260, transposable element gene];[AT5G45085, transposable element gene];[AT3G30680, transposable element gene];[AT4G03900, transposable element gene];[AT1G40123, transposable element gene];[AT1G37160, transposable element gene]	6.26
AT5G45085;	[AT5G45085, transposable element gene];[AT3G30680, transposable element gene];[AT4G03900, transposable element gene];[AT1G40123, transposable element gene];[AT1G37160, transposable element gene]	3.11
AT1G37160	transposable element gene	3.17
AT1G42410	transposable element gene	3.37
AT1G42370;	[AT1G42370, transposable element gene];[AT1G42360, transposable element gene]	3.37
AT2G04770	transposable element gene	5.31
AT2G06330	transposable element gene	2.23
AT2G10140	transposable element gene	5.03
AT1G36270;	[AT1G36270, transposable element gene];[AT3G30790, transposable element gene];[AT1G42510, transposable element gene];[AT2G13000, transposable element gene];[AT5G19015, transposable element gene];[AT1G42500, transposable element gene];[AT3G30780, transposable element gene];[AT2G12980, transposable element gene];[AT2G13310, transposable element gene]	2.27
AT5G19015;	[AT5G19015, transposable element gene];[AT1G42500, transposable element gene];[AT3G30780, transposable element gene];[AT2G12980, transposable element gene];[AT2G13310, transposable element gene]	6.21
AT2G13310	transposable element gene	2.74
AT2G14230	transposable element gene	2.17
AT1G52850;	[AT1G52850, transposable element gene];[AT2G13870, transposable element gene]	4.68
AT2G10640	transposable element gene	5.64
AT2G34130;	[AT2G34130, transposable element gene];[AT4G02314, transposable element gene];[AT2G13160, transposable element gene]	4.11
AT2G15090	KCS8 (3-KETOACYL-COA SYNTHASE 8); acyltransferase/ catalytic/ transferase, transferring acyl groups other than amino-acyl groups	3.55
AT2G18660	PNP-A EXLB3 (EXPANSIN-LIKE B3 PRECURSOR)	4.27
AT1G36460;	[AT1G36460, transposable element gene];[AT2G12240, transposable element gene];[AT3G29730, transposable element gene]	5.02
AT1G50850;	[AT1G50850, transposable element gene];[AT2G12250, transposable element gene]	2.12
AT2G12300	transposable element gene	4.93
AT2G19850;	[AT2G19850, FUNCTIONS IN: molecular_function unknown; INVOLVED IN: biological_process unknown; BEST Arabidopsis thaliana protein match is	3.93
no_match	no_match	5.03

Supplementary Table 1

Supplementary Table 1. List of identified differentially expressed genes (DEGs). **(A)** down-regulated and **(B)** up-regulated on *eca2* compared to WT. For each indicated condition, DEGs of normalized data was done using the software FiRe 2.2 (<http://www.unifr.ch/plantbio/FiRe/main.html>) (Garcion and Mettraux, 2006).

A

GO ID	Ontology	Description	Significant	p-Value
GO:0042221	Molecular function	response to chemical stimulus	14	8.30E-08
GO:0009753	Molecular function	response to jasmonic acid stimulus	6	2.50E-07
GO:0003824	Molecular function	catalytic activity	27	1.10E-06
GO:0050896	Molecular function	response to stimulus	17	1.90E-06
GO:0010033	Molecular function	response to organic substance	10	3.40E-06
GO:0006950	Biological process	response to stress	12	1.30E-05
GO:0009719	Molecular function	response to endogenous stimulus	8	3.50E-05
GO:0016740	Biological process	transferase activity	13	9.30E-05
GO:0070887	Molecular function	cellular response to chemical stimulus	5	2.10E-04
GO:0051716	Molecular function	cellular response to stimulus	6	4.80E-04
GO:0016757	Biological process	transferase activity, transferring glycosyl groups	5	4.80E-04
GO:0007242	Biological process	intracellular signaling cascade	5	1.10E-03
GO:0016491	Biological process	oxidoreductase activity	7	1.60E-03
GO:0005623	Biological process	cell	28	2.40E-03
GO:0044464	Biological process	cell part	28	2.40E-03
GO:0006355	Biological process	regulation of transcription, DNA-dependent	5	6.30E-03
GO:0051252	Molecular function	regulation of RNA metabolic process	5	6.50E-03
GO:0032774	Biological process	RNA biosynthetic process	5	7.80E-03
GO:0006351	Molecular function	transcription, DNA-dependent	5	7.80E-03
GO:0007165	Molecular function	signal transduction	5	1.50E-02
GO:0050794	Biological process	regulation of cellular process	9	1.70E-02
GO:0012505	Biological process	endomembrane system	9	1.80E-02
GO:0050789	Biological process	regulation of biological process	9	2.80E-02
GO:0009628	Biological process	response to abiotic stimulus	5	3.00E-02
GO:0031323	Biological process	regulation of cellular metabolic process	6	3.30E-02
GO:0005634	Molecular function	nucleus	7	3.40E-02
GO:0009058	Biological process	biosynthetic process	11	3.50E-02
GO:0019222	Biological process	regulation of metabolic process	6	4.60E-02
GO:0016070	Biological process	RNA metabolic process	5	4.70E-02

B

GO ID	Ontology	Description	Significant	p-Value
GO:0003677	Molecular function	DNA binding	20	2.40E-11
GO:0003676	Molecular function	nucleic acid binding	20	1.80E-07
GO:0070011	Molecular function	peptidase activity, acting on L-amino acid peptides	6	6.50E-05
GO:0005488	Molecular function	binding	26	9.00E-05
GO:0008233	Molecular function	peptidase activity	6	1.20E-04
GO:0006508	Biological process	proteolysis	6	3.90E-04
GO:0016787	Molecular function	hydrolase activity	9	2.00E-02
GO:0019538	Biological process	protein metabolic process	7	2.00E-01
GO:0003824	Molecular function	catalytic activity	14	2.40E-01
GO:0016740	Molecular function	transferase activity	5	3.60E-01
GO:0044238	Biological process	primary metabolic process	12	3.80E-01
GO:0043170	Biological process	macromolecule metabolic process	9	4.80E-01
GO:0008152	Biological process	metabolic process	13	5.10E-01
GO:0050896	Biological process	response to stimulus	5	5.40E-01
GO:0009987	Biological process	cellular process	5	1.00E+00
GO:0044464	Cellular component	cell part	8	1.00E+00
GO:0005623	Cellular component	cell	8	1.00E+00

Supplementary Table 2. GO analysis of differentially expressed genes of unchallenged *eca2* plants. Biological process identified to be (A) down-regulated and (B) up-regulated on *eca2* compared to wt plants.