

SUPPLEMENTARY MATERIAL

Expression of *Castanea crenata* Allene Oxide Synthase in *Arabidopsis* Improves the Defense to *Phytophthora cinnamomi*

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	Wild type	CcAOS1	CcAOS2	CcAOS3
Rosette area (cm ²)	0.61 ± 0.14	0.71 ± 0.19 *	0.79 ± 0.30 *	0.83 ± 0.22 *
Root (dry weight; g)	0.0006±0.0002	0.0009±0.0005	0.0007±0.0005	0.0005±0.0002
Aerial (dry weight; g)	0.0049±0.0009	0.0069±0.0006 *	0.0098±0.0034 *	0.0078±0.0006 *
Time of flowering	73±6.67	81±14.67	94±6.0	100±0.00 *
Primary root length (cm)	2.28±1.22	3.30±1.02 *	2.77±1.14	3.55±1.38 *
Lateral root number	6.4±3.5	11±4.08 *	7.7±6.2	10.8±5.5 *
Root hair number (between 5-10mm)	67.3±33.1	43.6±31.2	37.5±25.2 *	49.3±40.3
Root hair length (µm) (between 5-10mm)	243.24±47.79	209.13±47.7	226.27±38.22	228.46±33.14

Supplementary Table 1. Phenotypical parameters of transgenic *CcAOS* *Arabidopsis* plants.

Rosette area and dry weight were measured 1 month after germination. Time of flowering was measured 2 weeks after germination. The remaining root parameters were measured 13 days after germination. Measurements (means ± standard deviation) are from three independent assays, each using 5 plants per genotype. Asterisks refers to significant differences from the *Ler-0* wild type ($P < 0.05$, *t*-test).

Gene name, locus and relation to pathway (JA – jasmonate; SA – salicylate)	Forward (5'-3')	Reverse (5'-3')	Melting temperature (°C)
ALLENE OXIDE SYNTHASE AT5G42650.1 (JA)	CGACGGTGGGGAATAAACAA	TCGCCGAAAATCTCAATCA	60
LIPOXYGENASE 1 AT1G55020.1 (JA)	GATGGGCTTGAGGTTTGTA	TTCACGGGTTTGCATTTAGG	62
JASMONATE-ZIM-DOMAIN PROTEIN 10 AT5G13220.1 (JA)	CGATTTCTCGGACTTGAGA	GGAACCGAACGAGATTTAGC	60
PLANT DEFENSIN 1.2 AT5G44420.1 (JA) (Proietti et al., 2018)	CACCCTTATCTTCGCTGCTCTT	GCCGGTGCGTCGAAAG	58
PATHOGENESIS-RELATED GENE 5 AT1G75040.1 (SA)	ACTCCAGGTGCTTCCCGACA	ACTCCGCCCGGTTACATCTT	60
NONEXPRESSER OF PR GENES 1 AT1G64280.1 (SA)	TTATCTGGCCGCCGAACAAG	AATCATCCGGCGAGTCAAAG	60

Supplementary Table 2. Primers used in RT-qPCR analysis of jasmonic acid and salicylic acid pathway genes.

	5 15 25 35 45 55
Cc AOS	MASTSLAFP- SLQPKFQSSR KPSKPSTRRF IVRPITASVS EKPSVSVPPA TVVEQAEPTK
At AOS	MASISTPFPI SLHPKTVRS- ---KPLKFRV LTRPIKASGS E----- TPDL TVATRTGSKD
Consensus	MAS S FP SL PK S KP R RPI AS SE P TV

	65 75 85 95 105 115
Cc AOS	LPIKKIPGNY GLPFVGPIRD RFDFFYNQGR DEYFKSRAHK YQSTVFRANM PPGPLIASNP
At AOS	LPIRNIPGNY GLPIVGPDKD RWDYFYDQGA EEFFKSRIRK YNSTVYRVNM PPGAFIAENP
Consensus	LPI IPGNY GLP VGPI D RDFYQG E FKSR KYSTVRNM PPG IA NP

	125 135 145 155 165 175
Cc AOS	NVVVLLDGKS FVPLFDVTKV EKKDLFTGTF MPSTELTGGY RVLSYLDPSE PNHGKLRLL
At AOS	QVVALLDGKS FVPLFDVDKV EKKDLFTGTY MPSTELTGGY RILSYLDPSE PKHEKLNLL
Consensus	VV LLDGKS FVPLFDV KV EKKDLFTGT MPSTELTGGY R LSYLDPSE PHKLRLL

	185 195 205 215 225 235
Cc AOS	FFHLKARRDH VIPEFHSSYT ELFEGLENEL ATKGKAAPGE PSDLAAFNFL ARSLYGTNPV
At AOS	FFLLKSSNR IFPEFQATYS ELFDSLEKEL SLKGKADFGG SSDGTAFNFL ARAFYGTPA
Consensus	FF LK R PEF Y ELF LE EL KGKA FG SD AFNFL AR YGTNP

	245 255 265 275 285 295
Cc AOS	DTKGLDAPK MIDKWLQVQI SPLFSLGLPK HLDDLLLRV RLPPALVKAD YQKLYDFFYA
At AOS	DTKLKADAPG LITKWVLFNL HPLLSIGLPR VIEEPLIHTF SLPPALVKSD YQRLYEFFLE
Consensus	DTKL DAP I KW PL S GLP L T LPPALVK D YQ LY FF

	305 315 325 335 345 355
Cc AOS	SSGFVLDEAE RLGISREEAC HNLLFATCFN SFGGMKFLFP NMVKLIGRAG VKLHTQLAEE
At AOS	SAGEILVEAD KLGISREEAT HNLLFATCFN TWGGMKILFP NMVKRIGRAG HQVHNRLAEE
Consensus	S G L EA LGISREEA HNLLFATCFN GGMK LFP NMVK IGRAG H LAEE

	365 375 385 395 405 415
Cc AOS	IRSVIRSNGG NITMAAMEQM PLMKSVVYES LRIEPPVALQ YGKAKKDLVI ESHDAAFVK
At AOS	IRSVIKSNGG ELTMGAIEKM ELTKSVVYEC LRFEPVTAQ YGRAKKDLVI ESHDAAFVK
Consensus	IRSVI SNGG TMAEM L KSVVYE LR EPPV Q YG AKKDLVI ESHDAAF VK

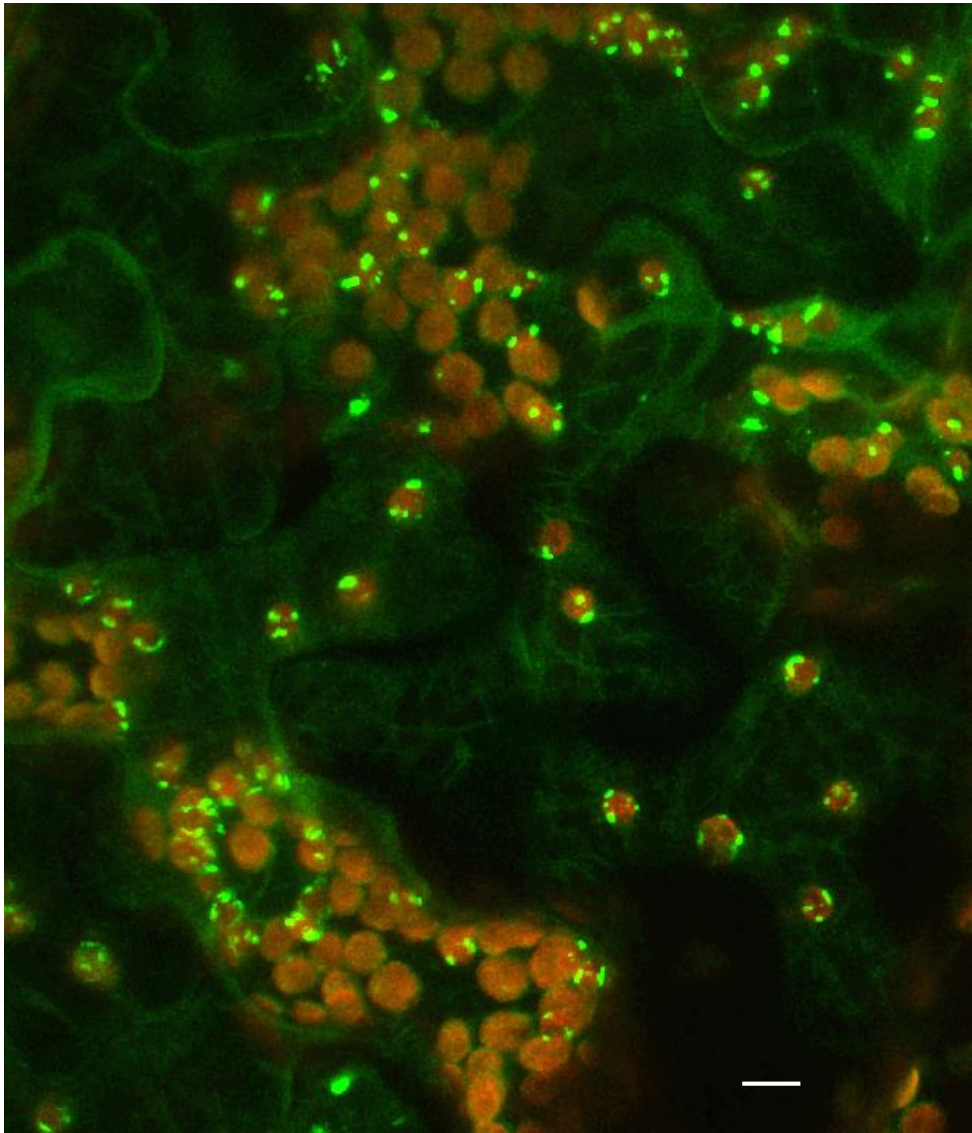
	425 435 445 455 465 475
Cc AOS	EGEMLFYQF FATKDPKIFE RAEFVADRF VGEEGKLLK HVLWSNGPES ESPSVGNKQC
At AOS	AGEMLYGYQF LATRDPKIFD RADEFVPERF VGEEGKLLR HVLWSNGPET ETPTVGNKQC
Consensus	GEML GYQF AT DPKIF RA EFV RF VGEEGKLL HVLWSNGPE E P VGNKQC

	485 495 505 515 525
Cc AOS	AGKDFVVLVA RLLVVELFLR YDSIEIEVDS SPLGAALTVT SLKKASF
At AOS	AGKDFVVLVA RLFVIEIFRR YDSFDIEVGT SPLGSSVNFS SLRKASF
Consensus	AGKDFVVLVA RLVEFR YDS IEV SPLG SL KASF

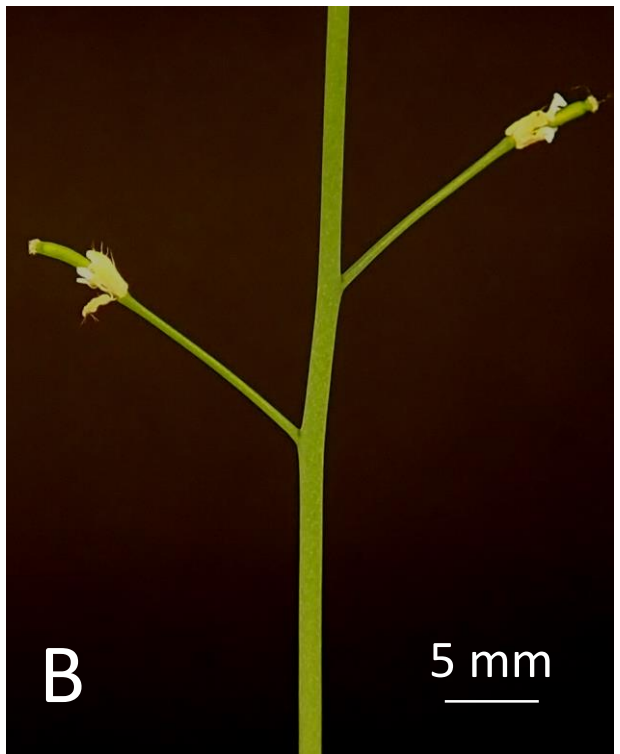
Supplementary Figure 1. Alignment of amino acid sequences of *Castanea crenata* (CcAOS) and *Arabidopsis thaliana* (AtAOS) allene oxide synthase.

Clustal W alignment was conducted using the BioEdit Sequence Alignment Editor.

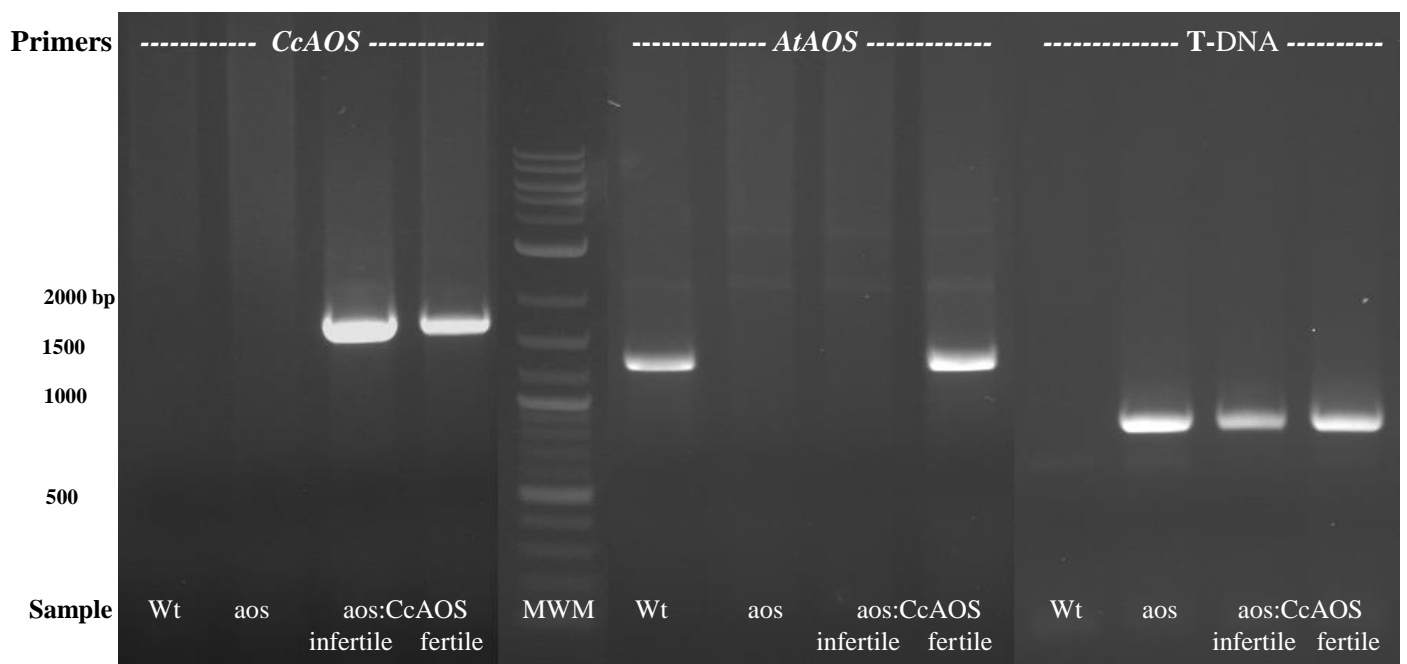
Numbers refer to the amino acid position.



Supplementary Figure 2. 2D stack of subcellular imaging upon transient CcAOS-eGFP expression in leaves of *N. benthamiana*. Although CcAOS clearly accumulates at chloroplasts, a reticulate-like signal in the cytosol suggests that trafficking of the protein may involve the endomembrane compartment. Scale Bar = 10 μm .



Supplementary Figure 3. Two-month old *Arabidopsis thaliana aos-GK624b02* mutant plant (A) and detail of siliques (B). The site of the T-DNA insertion in the mutant (5'UTR) is described in <https://www.gabikat.de/db/visualization.php?lineid=624B02&genecode=At5g42650> and is also reported in <https://www.arabidopsis.org/servlets/TairObject?id=503215774&type=polyallele>



Supplementary Figure 4. Genotyping of plants after expression of *CcAOS* gene in *aos-GK624b02* mutants. Genomic DNA of F2 *aos:CcAOS*-infertile and *aos:CcAOS*-fertile plants was screened for *CcAOS*, *AtAOS* and T-DNA insertion and further compared with wild-type Col-0 (Wt) and *aos* plants (used as controls). The expected band sizes are 1581 bp for the amplification of *CcAOS* ORF, 1345 bp for the amplification of a fragment of non-disrupted *AtAOS* and 850 bp for the amplification of a fragment of the T-DNA left border fused to *AtAOS* (T-DNA). MWM: molecular weight marker N3200L (New England Biolabs, Ipswich, MA, USA).