Jasmonic acid is a downstream component in the modulation of somatic embryogenesis by the Arabidopsis class 2 phytoglobin

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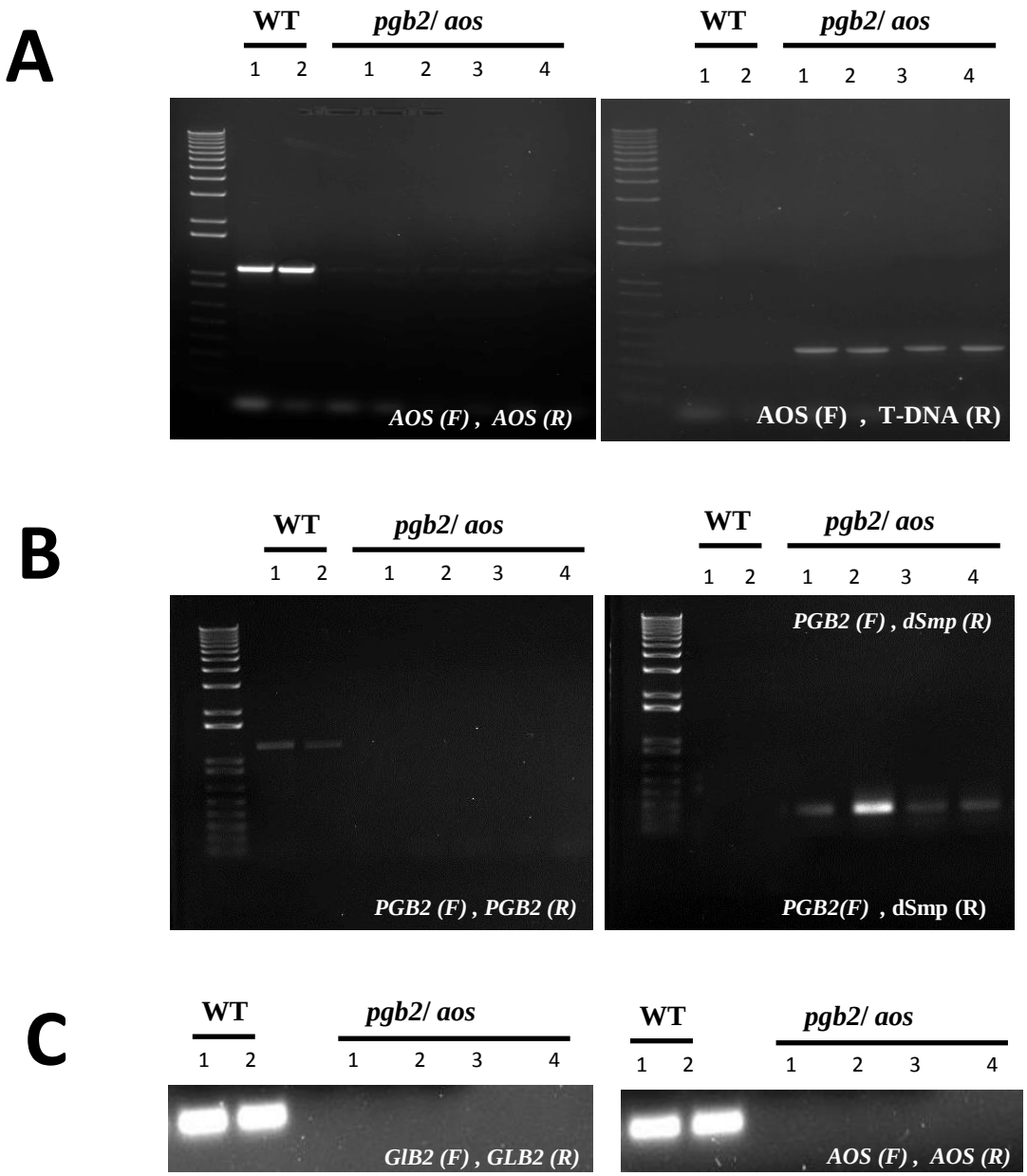
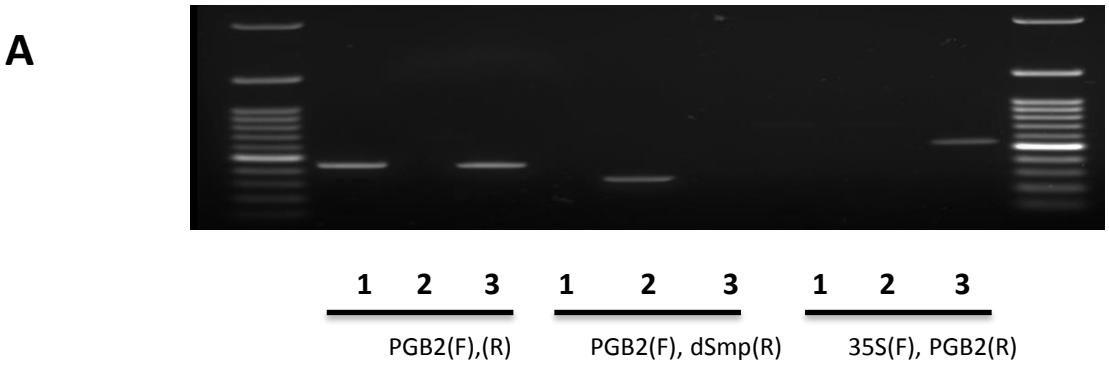
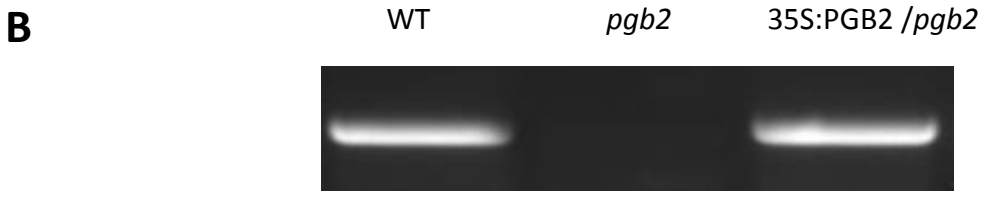
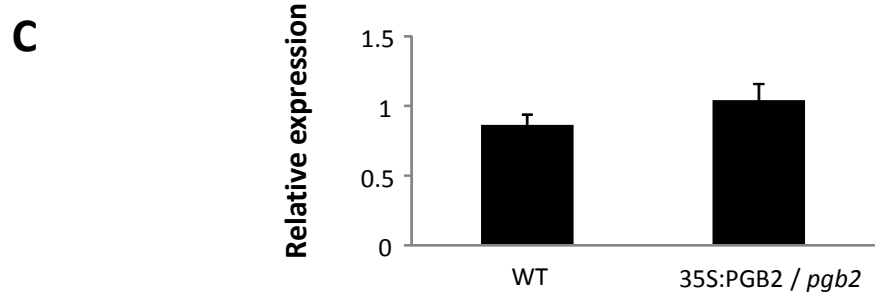
Supplemental Figures and Tables



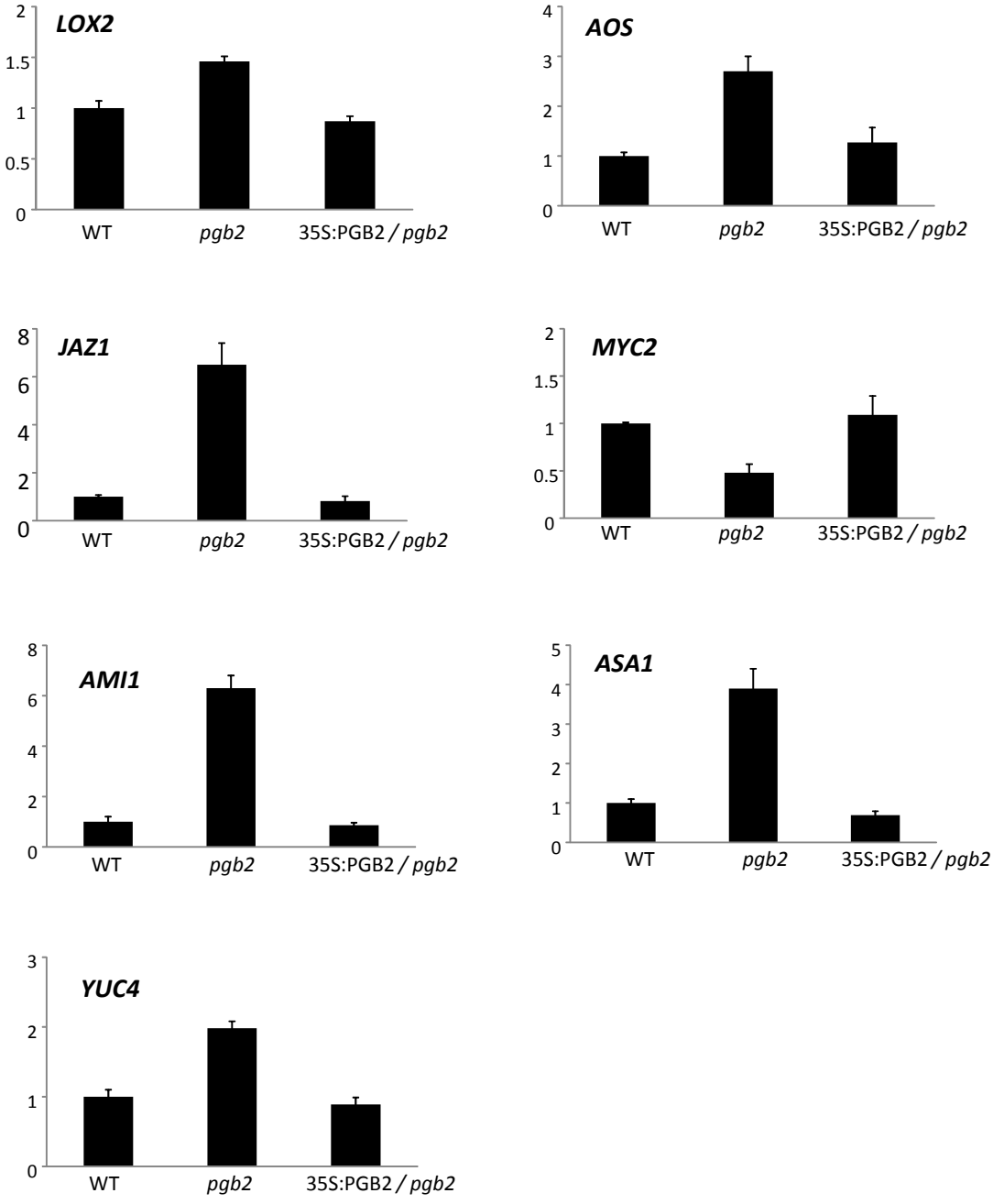
Supplemental Fig.1 . Characterization of *pgb2/ aos* double mutant lines produced by crossing the *pgb2* T-DNA line and the *aos* T-DNA line (SALK 017756). **(A)** PCR analysis of DNA to detect *AOS* (left panel) and the T-DNA insertion disrupting *AOS* (right panel). **(B)** PCR analysis of DNA to detect *PGB2* (left panel) and the T-DNA insertion disrupting *Pgb2* (dSmp) (right panel). **(C)** PCR analysis of cDNA synthesized from RNA of tissue cultured for 7 days on induction medium. Primers are shown at the bottom right corner of each gel. Primers used for amplification: AOS(F), 5'-CGAGAAATTAACGGAGCTTCC-3'; AOS(R) , 5'-CTAACCGGAGGCTACCGTATC-3'; TDNA(R), 5'-ATT TTG CCG ATT TCG GAAC-3'; PGB2(F), 5'-CTCAAAGCTCATGCTGTTAAAGTC-3'; PGB2(R), TGTGTCAGCCACTACCACCT-3'; dSmp(R), 5'-ACCGTCGACTACCTTTTTTCTTGTAGTG-3.





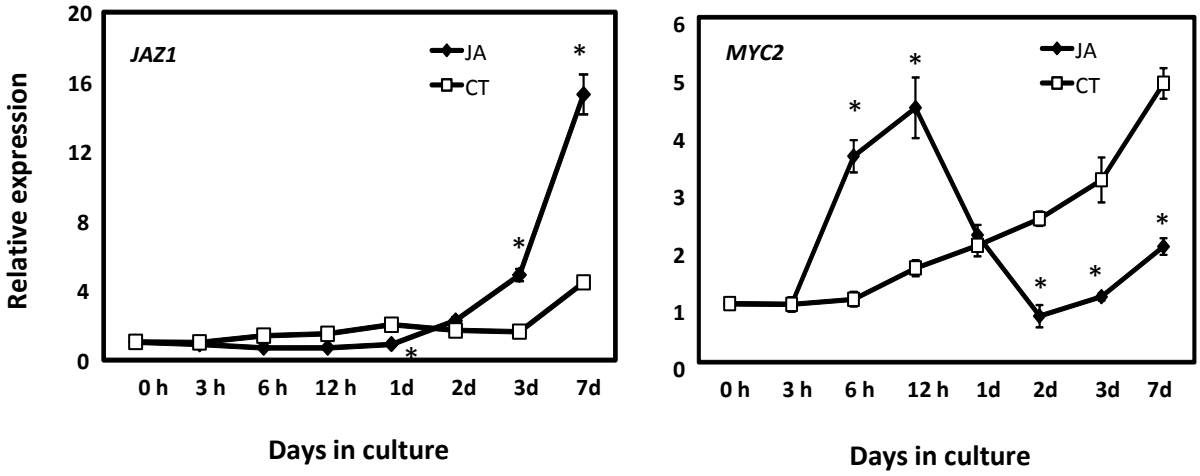


Supplemental Fig.2 . Characterization of the 35S:PGB2 / *pgb2* line produced by introducing *PGB2*, driven by the 35S promoter, into the *pgb2* line. **(A)** PCR analysis of DNA to detect the presence of PGB2 [PGB2 (F), (R) primers]; the T-DNA insertion disrupting *PGB2* (dSmp) [PGB2(F), dSmp(R) primers], and the 35S:PGB2 insert [35S (F), PGB2(R) primers]. 1, WT; 2 *pgb2;* 3, 35S:PGB2 / *pgb2*. Primers used for amplification : PGB2(F), 5'-ATGGGAGAGATTGGGTTTACAG -3'; PGB2(R), 5'-TTATGACTCTTCTTGTTTCATCTCG-3'; 35S(F), 5'-CTATCCTTCGCAAGACCCTTC-3'; and dSmp(R), 5'-ACCGTCGACTACCTTTTTTCTTGTAGTG-3. **(B)** Amplification of PGB2 by PCR from cDNA prepared from samples at 7 days on induction medium. Primers used: 5'-CTCAAAGCTCATGCTGTTAAAGT-3' and 5'- TGTGTCAGCCACTACCACCT-3'. **(C)** Relative expression of PGB2 in WT and 35S:PGB2 \ *pgb2*tissues from samples at 7 days on induction medium. Primers used: 5'- CTCAAAGCTCATGCTGTTAAAGT-3' and 5'- TGTGTCAGCCACTACCACCT-3'.



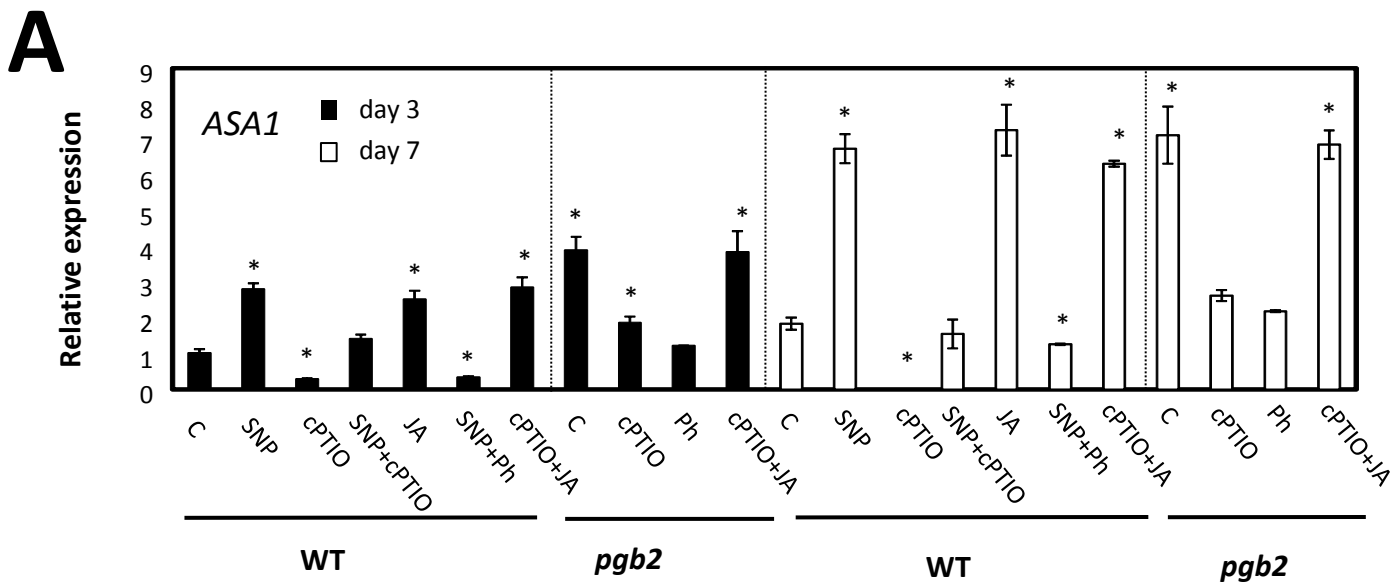
Supplemental Fig. 3. Expression level by quantitative (q)RT-PCR of several genes participating in jasmonic acid and auxin synthesis and signaling (see text for the names of the genes) in the WT, *pgb2,* and 35S:*PGB2* / *pgb2* lines at day 7 on induction medium. Values are means ± SE of at least three biological replicates and are normalized to the value of WT set at 1.

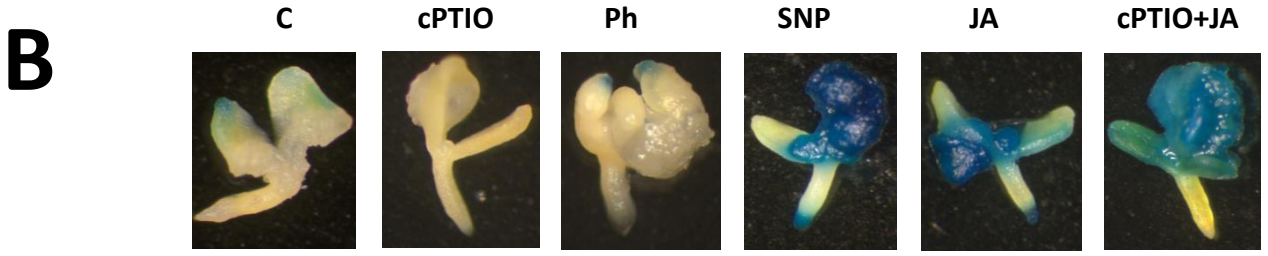
Supplemental Fig. 3

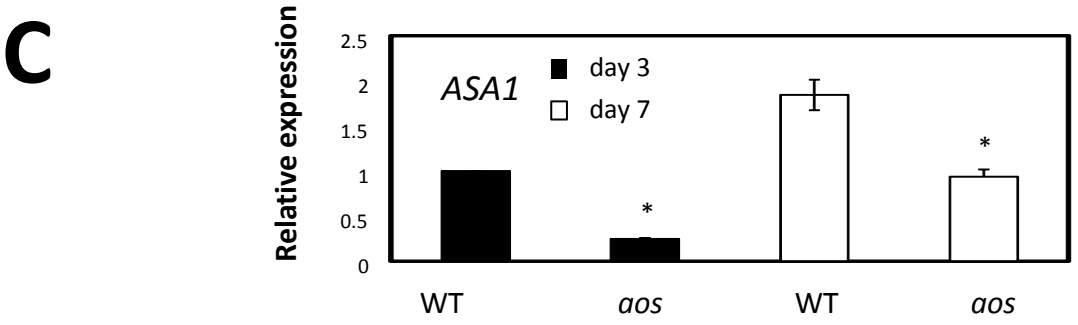


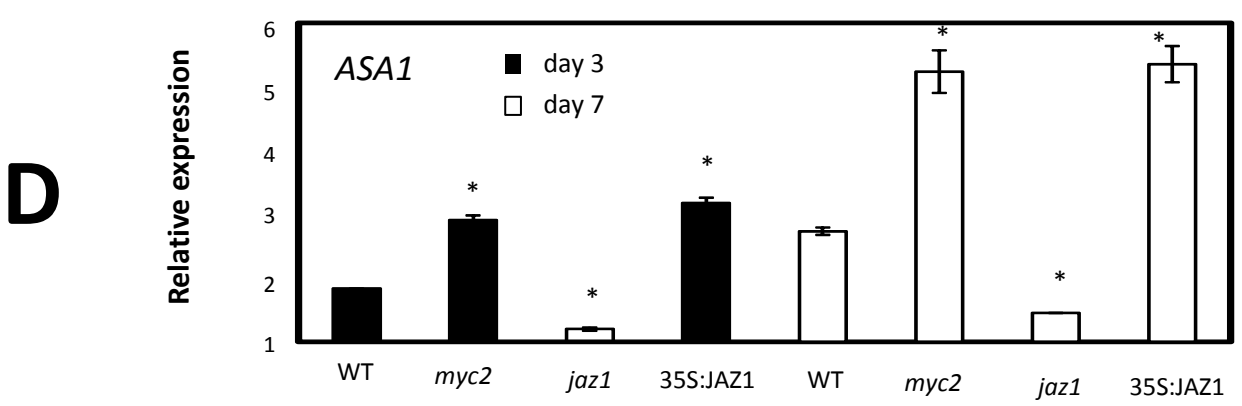
Supplemental Fig. 4. Effects of JA on the expression level of *JAZ1* and *MYC2* . Explants were cultured for 7 days on induction medium and then exposed to JA for the following 7 days. Values are means ± SE of at least three biological replicates and are normalized to the value of control at time 0 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the control value of the respective time in culture.

Supplemental Fig. 4



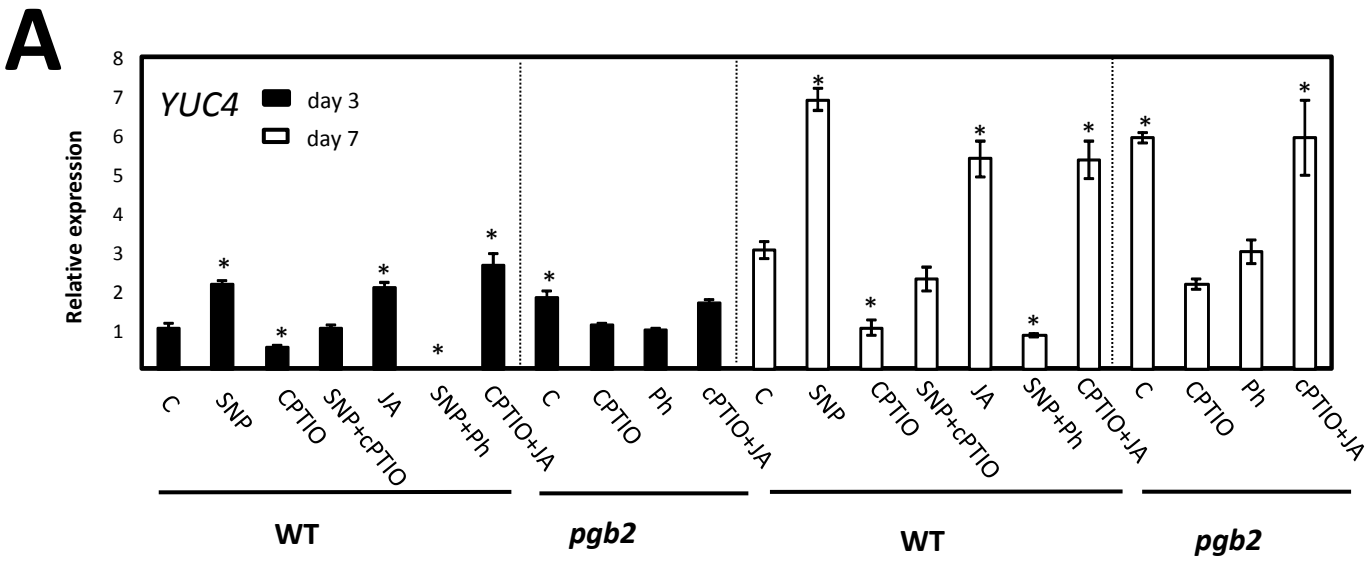


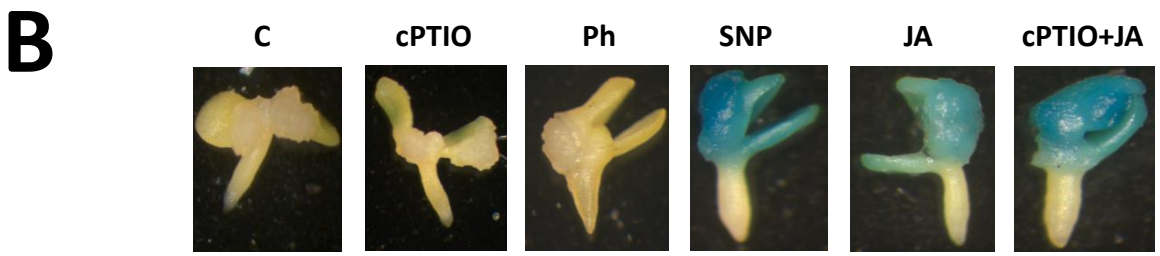


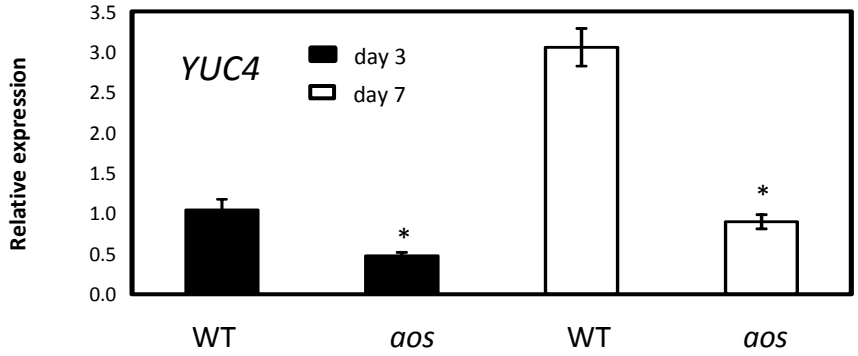


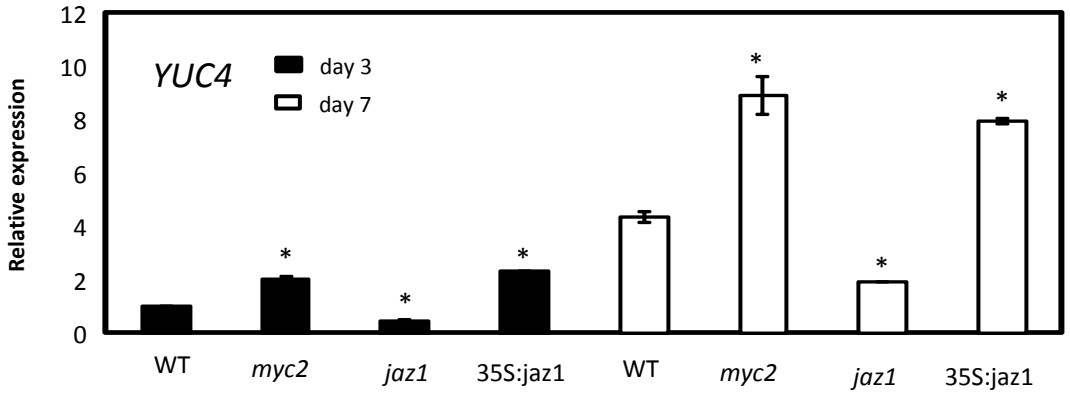
Supplemental Fig. 5

Supplemental Figure 5. Effects of jasmonic acid (JA) and nitric oxide (NO) on *ASA1* expression and localization. **(A)** Expression level by quantitative (q)RT-PCR of the auxin biosynthetitic gene *anthranilate synthase-α subunit* (*ASA1*) at day 3 and 7 in the induction medium of somatic embryogenesis. WT and *pgb2* tissues were cultured in media with altered levels of NO and JA using pharmacological treatments described in Fig. 2. Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT(C) of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT(C) value of the respective day in culture. **(B)** Localization patterns of *ASA1* by GUS staining at day 7 on induction medium. Tissue was subjected to the indicated pharmacological treatments. **(C)** Expression level by quantitative (q)RT-PCR of *ASA1* in WT and *aos* lines at day 3 and 7 on induction medium. Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT value of the respective day in culture. **(D)** Expression level by quantitative (q)RT-PCR of *ASA1* at day 3 and 7 in the induction medium of somatic embryogenesis in the WT, *myc2*, *jaz1*, and 35S:JAZ1 lines. Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT value of the respective day in culture.









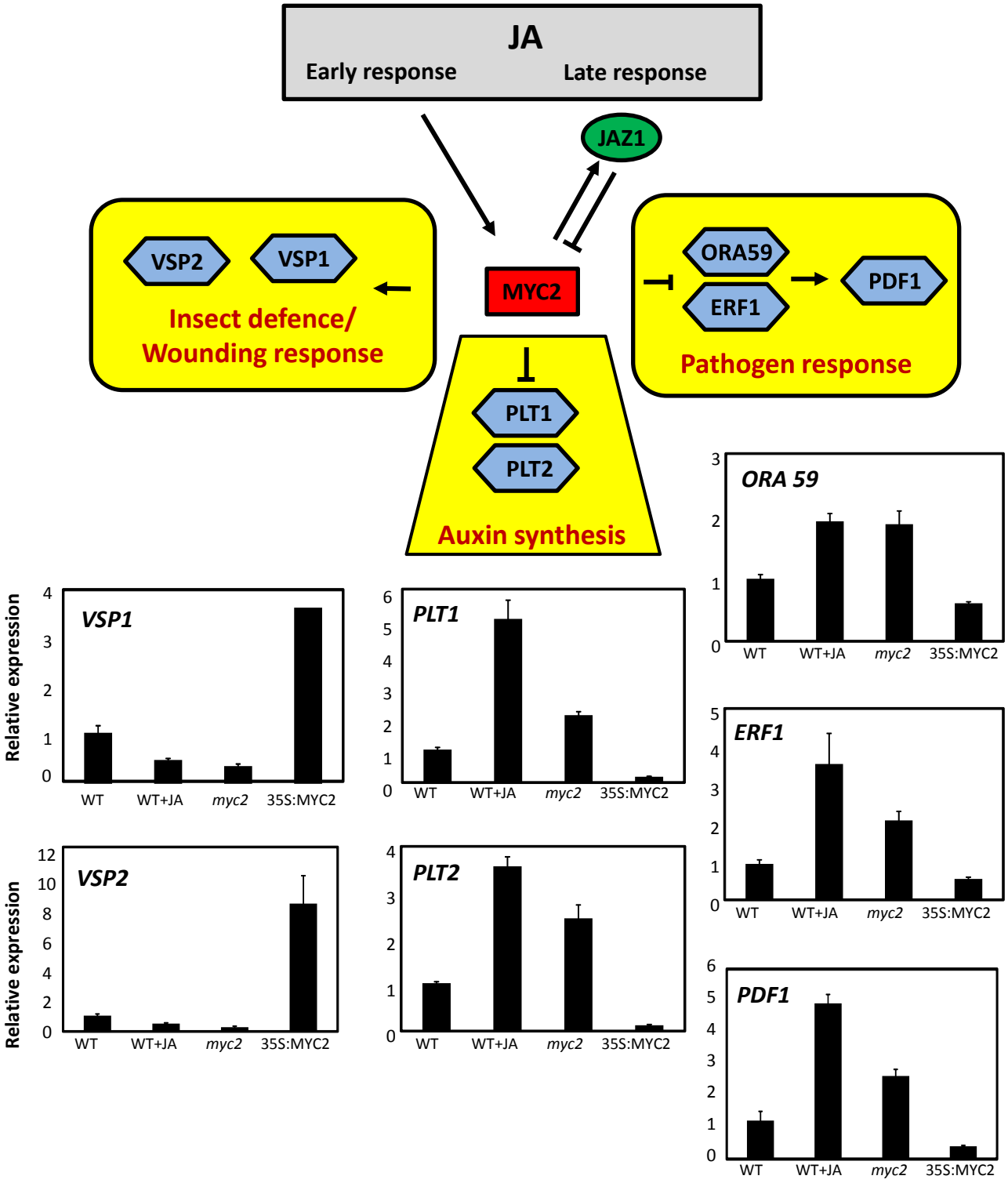
Supplemental Fig. 6

Supplemental Fig. 6. Effects of jasmonic acid (JA) and nitric oxide (NO) on *YUC4* expression and localization. **(A)** Expression level by quantitative (q)RT-PCR of *YUC4* at day 3 and 7 in the induction medium of somatic embryogenesis. WT and *pgb2* tissues were cultured in media with altered levels of NO and JA using pharmacological treatments described in Fig. 2. Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT(C) of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT(C) value of the respective day in culture.

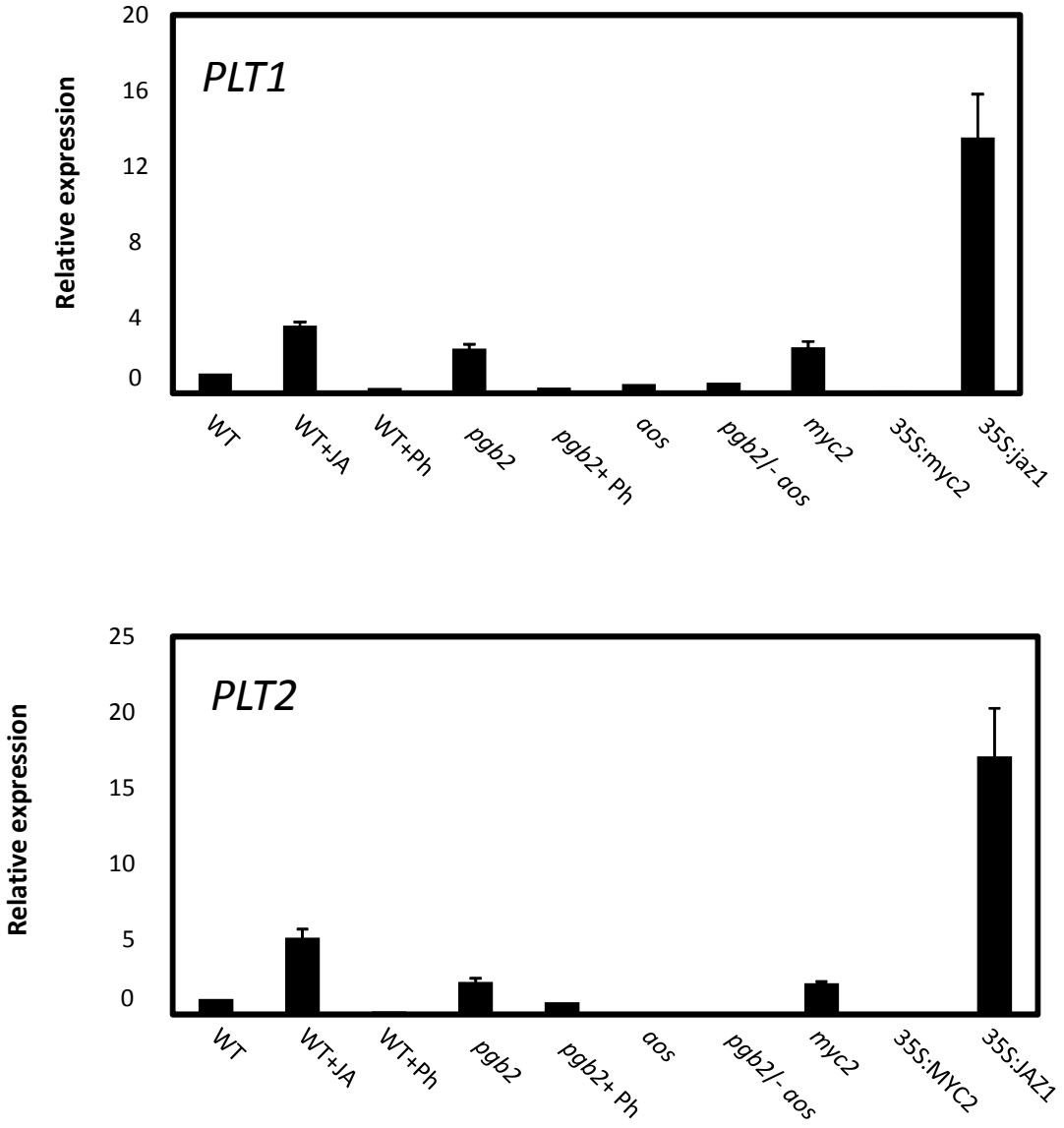
(B) Localization patterns of *YUC4* by GUS staining at day 7 on induction medium. Tissue was subjected to the indicated pharmacological treatments.

(C) Expression level by quantitative (q)RT-PCR of *YUC4* in the WT and *aos* line at day 3 and 7 on induction medium. Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT value of the respective day in culture. **(D)** Expression level by quantitative (q)RT-PCR of *YUC4* at day 3 and 7 in the induction medium of somatic embryogenesis in the WT, *myc2*, *jaz1*, and 35S:JAZ1 lines.

Values are means $\pm$ SE of at least three biological replicates and are normalized to the value of WT of day 3 set at 1. * indicates statistically significant differences ($P \leq 0.005$) from the WT value of the respective day in culture.



Supplemental Fig. 7. Diagram showing the effects of the "early" and "late" JA response on genes participating in insect defense/wounding response, pathogen response, and auxin synthesis. The expression of the genes participating in these responses (see the text for details) was measured at day 7 on induction medium in the WT line treated with JA and in lines with suppressed (*myc2*) or increased (35S:MYC2) levels of MYC2. Expression values are means of three biological replicated and are normalized to the value of WT set at 1.



Supplemental Fig. 8. Expression levels of *PLETHORA 1* and *2* in day 7 explants. Gene expression was measured by (q)RT-PCR in the WT , *pgb2, aos*, myc2 and 35S:MYC2 lines. Values are means of three biological replicated and are normalized to the value of WT set at 1.

Supplemental Table 1. List of primers used for genotyping and for expression studies

primers for genotyping		
AOS-F	CGAGAAATTAACGGAGCTTCC	AT5G42650.1	ALLENE OXIDE SYNTHASE
AOS-R	CTAACCGGAGGCTACCGTATC	AT5G42650.1	ALLENE OXIDE SYNTHASE
LBb1-3	ATT TTG CCG ATT TCG GAA C		T-DNA insertion
PGB2-F	CTCAAAGCTCATGCTGTTAAAGTC	AT3G10520	hemoglobin-2
PGB2-R	TGTGTCAGCCACTACCACCT	AT3G10520	hemoglobin-2
dSmp-R	5-ACCGTCGACTACCTTTTTTCTTGTAGTG-3		
primers for RT-PCR			
AtUBQ10-F	AACTTTGGTGGTTTGTGTTTTGG	AT4G05320	UBIQUITIN 10
AtUBQ10-R	TCGACTTGTCATTAGAAAGAAAGAGATAA	AT4G05320	UBIQUITIN 10
MYC2-F	ACCACGTCGAAGCAGAGAGACAAA	AT1G32640.1	JASMONATE INSENSITIVE 1
MYC2-R	TGTAAGCGATTGCGTCACCGAGTA	AT1G32640.1	JASMONATE INSENSITIVE 1
JAZ1-F	CCGGTTCTTGGAGAAGAGAAAG	AT1G19180.1	JASMONATE-ZIM-DOMAIN PROTEIN 1
JAZ1-R	CCTGTGGTTTGAGGGTTTGA	AT1G19180.1	JASMONATE-ZIM-DOMAIN PROTEIN 1
LOX2-F	ATGCTACGTCATGCTGGCTATGGA	AT3G45140	LIPOXYGENASE 2
LOX2-R	TGCCGCTATTATGTATGGCTCCGT	AT3G45140	LIPOXYGENASE 2
AOS-F	ACGACGCGGCGTTTAAAGTCAAAG	AT5G42650	ALLENE OXIDE SYNTHASE
AOS-R	ACGAATCTCTCCGGCACAAACTCA	AT5G42650	ALLENE OXIDE SYNTHASE
PDF1.2-F	GTTCTCTTTGCTGCTTTCGAC	AT5G44420	PLANT DEFENSIN 1.2
PDF1.2-R	GCAAACCCCTGACCATGT	AT5G44420	PLANT DEFENSIN 1.2
ASA1-F	ACAAGGATGCTAACAAACGGCGTG	AT1G19920	ATP SULFURYLASE ARABIDOPSIS 1
ASA1-R	TCTGGCACTCACAGTGTTCGTCTT	AT1G19920	ATP SULFURYLASE ARABIDOPSIS 1
Yuc4-F	CTAACGGATGGAAAGGAGAGAAG	AT4G32540.1	YUCCA 4
Yuc4-R	GCGATCTTAACGGCGTCATA	AT4G32540.1	YUCCA 4
AMI1-F	ATCTCGTCGGTGAAGCCAGAGTTT	AT1G08980	AMIDASE 1
AMI1-R	CCGAGCAAAGTTGAAAGAGCCGTT	AT1G08980	AMIDASE 1
PGB2-F	CTCAAAGCTCATGCTGTTAAAGTC	AT3G10520	Hemoglobin-2
PGB2-R	TGTGTCAGCCACTACCACCT	AT3G10520	Hemoglobin-2