

Role of *ARABIDOPSIS A-FIFTEEN* to regulate leaf senescence involves response to reactive oxygen species and is dependent on *ETHYLENE INSENSITIVE2*

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Table S1. Prediction of chloroplast targeting peptides of AAF orthologues by TargetP1.1.

AAF othologues	Signal peptide	Specificity ^a
ABN08080 (<i>Medicago truncatina</i>)	1-21	0.95
ABK95826 (<i>Populus trichocampa</i>)	1-61	0.66
CAO17159 (<i>Vitis vinifera</i>)	1-58	0.98
SPA15 (<i>Ipomoea batatas</i>)	1-68	0.70
NTA15 (<i>Nicotiana tabacum</i>)	1-69	0.65
AAF (<i>Arabidopsis thaliana</i>)	1-36	0.52
ACG28979 (<i>Zea mays</i>)	-	
OSA15 (<i>Oryza sativa</i>)	-	
XP-001763895 (<i>Pyscomitrella patens</i>)	-	

^a Specificity on the TargetP test sets

Table S2. Primers used for genotyping , RT-PCR and cloning

Primer	Sequence (5'→ 3')
AAF-F	GGCGCTTAGAGGCTGATTAAGGCG
AAF-R	TTCAGCGAGTCTGTAAATGCTCCC
18S rRNA-F	GGGATGGGTCGGCCGGTCCGCCTT
18S rRNA-R	CTCCACTCCTGGTGGTGCCCCCTCCG
SAG12-F	GTCAGAACACAGCTTGTTGATTGC
SAG12-R	GACATCAATCCCACACAAACATACAC
SAG13-F	GGAGTCTTGGAGGCAATGACCGCTC
SAG13-R	ATGCCGTCCATCCGGAGAGTGAAC
aaf-a	AGCTCGGGCAAGAGAAGATA
aaf-b	AACGTCCGCAATGTGTTATTAAGTTGTC
aaf-c	CTGGAGAGCTTGAGCATTCA
aaf-d	CCCATTGGACGTGAATGTAGACAC
aaf-e	CTGTCTTCAGCGAGTCTCTGC
ATA15SpelF1	GGACTAGTATGGCTTAAACGTGAGCAAGGTGG
ATA15PmII R2	GGCACGTGTCAGATCTCATCAAGGTCACTGTG
ATA15-GFP-f1	CCCGAATT CCTTCTTCTCGTTCTTA
ATA15-GFP-r1	CTTGCTCACGTTAGGATCCCAT
ATA15F8	ATGGCTCTAACGTGAGCAAGGTTG
ATA15R9	GATCTCATCAAGGTCACTGTC
ATA15-41F	GGTCATCTATTGAAGAGTTAAAGC
ATA15FL-attB1F	AAAAAGCAGGCTCCACCATGGCTTAAACGTGAGCAAG
ATA1540-attB2R	AGAAAGCTGGTCCGGATCMTACTTATTAGCAGCTAAAGGATGAT
SPA15-attB1F	AAAAAGCAGGCTCCACCATGGCAAACCTAATGGGATTATC
SPA15-attB1R	AGAAAGCTGGTCCGGATCGCTATCTACATCGTCATCTTCTTG

Table S3. Primers used for quantitative PCR

Primer	Sequence (5' → 3')
qAAF-F	TGCAGGAATGGGAGCATTACAGAG
qAAF-R	TTCAGCGCATTGATCGCTCTCG
qSAG12-F	ACAAAGGCGAAGACGCTACTTG
qSAG12-R	ACCGGGACATCCTCATAACCTG
qSAG13-F	GCTTTCCATCTCTCACAGCTTGCC
qSAG13-R	GCCAGCTGATTGATGGCTCCTTG
qAt5g59310-F	TCCTGGAAAGTGCAGGTGTTAGC
qAt5g59310-R	TTCACTTGATGGTGGCGCAGTTG
qAt2g26020-F	CGCTGCTCTGTTCTCTTGCA
qAt2g26020-R	TCTCGCACAACTTCTGTGCTTC
qAt5g44420-F	ACACAACACATACATCTATACTT
qAt5g44420-R	TATATTATTGTAACAAACAACGGGA
qAt4g17500-F	ATGTCGATGACGGCGGATTCTC
qAt4g17500-R	CGGCTCCGATTCTCTTAGTAAGTG
qAt3g04720-F	GCGGCAAGTGTAAAGGGTGAAG
qAt3g04720-R	TCCAATCCAAGCCTCCGTTGC
qAt2g43570-F	TTCGGTGCTTCCATCTCAAACG
qAt2g43570-R	TGAACCTGTTCTGGCTACG
qAt1g06290-F	ATGTATTGATGCAGCAGGTGAGC
qAt1g06290-R	TCATGTGCTCCAATCCCAGTCC
qSEN1-F	GCCATCGACGAGAGACTCAAAGAG
qSEN1-R	CCTCAGCACCTTCCATTCCAC
qAt5g37940-F	GGATCCATGGCGGTTCTAGTCAG
qAt5g37940-R	CCAGAGATTGGCTTGCCGATAGTG
qAt1g02920-F	AGCCTTCATCTCCGCAACCC
qAt1g02920-R	TTGGAGCCAAGGGAGACAAGTTGG
qAt4g37520-F	TCGTTAATCTGCGGGTGGAC
qAt4g37520-R	CGCTAGCCGCTGTTGATGATAG
qAt5g58390-F	TGGGTGCTCTCCTCGTTG
qAt5g58390-R	AGTAAGGATCCGTACACCCATTG
qACTIN-F	ACCTTGCTGGACGTGACCTTACTGAT
qACTIN-R	GTTGTCTCGTGGATTCCAGCAGCTT
qAtEm1-F	TTGCTGAAGGAAGGAGCAAGG
qAtEm1-R	G ACCCATCTCCTGATAACCCTCGTG
qAtPR1-F	ACACGTGCAATGGAGTTGTGG
qAtPR1-R	TTGGCACATCCGAGTCTCACTG

ACG28979 -----
 AAK59984 -----
 AAQ15125 -----
 AAP41026 -----
 ABN08080 -----
 ABK95826 -----
 CAO17159 -----
 AAF -----
 XP_001763895 MSCEKVMBGHAIPLCDEQSFKRQEESRTLTHAIHPGRGIFSQCQGFTCFGRSVMSLSCGRIPLLPALSFLICNAGCGILKPSASDLVLFVLGVAVHLVRLCCKPFGSLVIQRACAV

ACG28979 CYRDQYRPPR-----DFTECARKESEARRWGNGVHHIP-RLCQWKPRG---SKCYRDQYRPPR-----DFTECARKESEARRWGNGVHHIP-RLCQWKPRG---SK
 AAK59984 YYNDQYRMPCKL-----KG1HCMLNC1P0KAKVRKCMNGYQSTF-RFCVNEKENQQTIGOYYNDQYRMPCKL-----KG1HCMLNC1P0KAKVRKCMNGYQSTF-RFCVNEKENQQTIGQ
 AAQ15125 -----PKSPQHPK1YAKTQVIEHSTQSNGYLSML-RLKLSNKGFWRAYR-----PKSPQHPK1YAKTQVIEHSTQSNGYLSML-RLKLSNKGFWRAYR
 AAP41026 -----AKSPQHPK1YAKTQVIEHSTQSNGYLSML-RLKLSNKGFWRAYR-----AKSPQHPK1YAKTQVIEHSTQSNGYLSML-RLKLSNKGFWRAYR
 ABN08080 TNRATLCSRSHG-----SSSPR1NR1QFSKGRLENGHLLNDSVLNE-RSTLSNDWFRFVNNTNRATLCSRSHG-----SSSPR1NR1QFSKGRLENGHLLNDSVLNE-RSTLSNDWFRFVN
 ABK95826 TQRKLITSTSHG1ICSFKSFKQNKLNHPTHOGIELQQLSSKHLTA-KLAFSCELOGIHGTQQRKLITSTSHG1ICSFKSFKQNKLNHPTHOGIELQQLSSKHLTA-KLAFSCELOGIH
 CAO17159 TDRTS1SKSQGTISSLFSKS1KHCGLHSKNOQVELRRQSSGHLTA-RYRFNSKGFWHICGTDRTS1SKSQGTISSLFSKS1KHCGLHSKNOQVELRRQSSGHLTA-RYRFNSKGFWHICG
 AAF -----SPILVKSVNASRSRERVLLAYVHHPLAANK-GSS1IEELKQGLCCT-----SPILVKSVNASRSRERVLLAYVHHPLAANK-GSS1IEELKQGLCCT
 XP_001763895 SFTWF1FEWFKYYPLPHSGFDAL1TTTALFSLSPLFPELML1ANSVNFDEQKFDTMQSFTWF1FEWFKYYPLPHSGFDAL1TTTALFSLSPLFPELML1ANSVNFDEQKFDTMFQ***

 ACG28979 SDGSLLGDGH0-----GRDARCHCSGSHNSCEC-----ETRDCDAMEDAAASSYRDFKOHSGRNQPSDQVSLK-NKSAYASQGLAEACKFVYNDAKFVN-----ERAQN
 AAK59984 SNGSLIQQ-----GQNFRCHSYGHSNSSE-----TKBCS-LEDGTDs-YRDFEEHRSRGASQFSDMVQAk-KKSVSSQGLAEACKFVYNDAKFVN-----ERAQN
 AAQ15125 AFSGVIATR-----GSSLRCHSAETRAHET-----EFCV-----REYRDSSTDSMGGDKDFASLGG-----KSGTPSPGLAEACKFVYNDAKFVN-----ERAKN
 AAP41026 KLSNRVIFR-----GSPVLCRSTETHDTE-----KDFS-----RNSSGDYSN1SSVQ-EDDHAITVG--KVP1SSQAI1AEACKFAYNDAKFVN-----ERAKN
 ABN08080 RNPVSLISK-----TSSVCKSTGANTEE-----KECV-----TYDDVDSLTRHAEDEMDKR-----ARSVRGLSEAYRFACNDAKFL-----
 ABK95826 KFVSL1ISR-----RSTTLQSTHRTHTEEE-----KECT-----RPYSDSDSSRAVGEKEDEHQJLMSGRTIHSCHALAEACRVYNDAKFVN-----ERARN
 CAO17159 KTGHYFSSR-----GSSVLCWLTHTRTEA-----KOCV-----EHYSDRDPFVSOLDEEDEENLVMPERI1HSNQGLAEACKFVYNDAKFVN-----ERARN
 AAF KTVTFVSSR-----RCSTLCFVGKQSDTET-----NSQV-----VQKEGEKQVMPRR-----KSSNSSQLLVEVWSNDAKFVN-----ERARN
 XP_001763895 GGEGBFVPTEAOPHTHSEKMSL1AETHLREACTQ1LMGAQGGSKDRKGKRNMV1GVGCRFEMKGDAKVDKEVKCMMSDKKTWVPL-PARNVGGPAEARCAFAMLVRATVQNI
 DERARS ***

 ACG28979 D1LLLSRG1ITRLNKRACQDAAVLGLGFLKLDARARDTQK1DHTVKERAA1LNHFARAFKERAQSDLKKAADKHWSGDAL-----EADLRLRADLVKKRAMEADFM
 AAK59984 D1LLLSRG1ITRLNKRACQDVAVLGSGLFLKLDARARDTQK1DHSVAKERAA1LNHFARAFKERAQSDLKKAADKHWSGDAL-----EADLRLRADSVVKKRAMEADFM
 AAQ15125 D1VLLSRG1IMRLDARARQDVAVLGSGLFLKLDARAREDTKE1DNDVVKRAERLHHVAT1LNKAQSRLKNAADRHWSGDAL-----EADLRLRADFAAKRAMEADLM
 AAP41026 D1VLLSRG1IMMDARARQDVAVLGSGLFLKLDARAREDTKE1DTEWDHDVVKRAERLHHVAT1LNKAQSRLKNAADRHWSGDAL-----EADLRLRADFAK
 ABN08080 D1VLLSRG1IMMDARARQDVAVLGSGLFLKLDARAREDTKE1DNGVKEKAKRLN1IAT1LKD1AQ0TRLKSAADEDHWSGDAL-----EADLRLRADFAK
 ABK95826 D1ILLLSRG1ISRLDARARQDVAVLGSGLFLKLDARAREDTKE1IDRVKEKAKRLN1IAT1KDRAQTKLRTAADKHWSGDAL-----EADLRLADFRAKRAMEADLM
 CAO17159 DMVLLSRG1IMRLDARARQDVAVLGSGLFLKLDARAREDTKE1IDRVVKRAERLHH1IAT1LKDQSRLKNAADKHWSGDAL-----EADLRLRADFC
 AAF DFVLLSRG1IMRLDARARQDVAVLGSGLFLKLDARAREDTKE1IDRVVKRAERLHH1IAT1FKN1IAESKLKNAADKHWSGDAL-----EADLRLRADFR
 XP_001763895 DFHLLSRQ1ILRDLRVRVEDVALLGSGFLKLDARAREDVVKLDTNARQKMRHL1ALGLTESASMELSNAAEEHWSDGALDVGSLLSFNCLASLVLLDADLRLV
 LARRRAMEADLY ***

 ACG28979 ALKFVQD1HDMMVNRLYEQLP-----KGSSRSTNSTGF1ITLEKNGKALE1FLPGEVSDQ1IYA1EEAYQSMASAFSEADG1DYTDPEEELLVAT1LDDAMDGKRSVSL1AE
 AAK59984 ALKFVRD1HDMMANRLQEQFA-----KGSSPANSRSF1ITLEKNGNTFELPFHEVSTDQ1ITA1EQAYWSMASALSEADG1DYTDPEEELLVAT1LDDAMDGK
 AAQ15125 ALEFKVN1HDMMWSKMCNLK-----RSSLNFMNMTER1ITLEKNGMLNFLGEVSAER1SA1EQAYWD1AAALSEADG1DYTDPEEELLVAT1LDDAMDG
 AAP41026 ALEFKVN1HDMMWSKMCNLK-----RSSVD-SEKTRH1ITLEKNGKTLEFLPGEVSADRT1IA0AYWD1AAALSEADG1DYTDPEEELLVAT1LDDAMDG
 ABN08080 SLELIKNI1HDMMWSKTYNPF1FRDKGSLSENNVRGE1MLEKNGRTTNSPGDVTAAER1ITALQ1EQAYWSMASALSEADG1DYTDPEEELLVAT1LDDAMDG
 ABK95826 ALEFKVN1HDMMWSKMYKYL-----KKGTPSSNDTMGH1MLEKNGKTLDFPGEVSTDRT1IA1EQVYLSMASALSEADG1DYTDPEEELLVAT1LDDAMDG
 CAO17159 ALEFKVN1HDMMWSKMYKYL-----LVTSETGTTDR1ITLEKNG1ALGFPGEVSSDR1IA1EEAYWSMASALSEADG1DYTDPEEELLVAT1LDDAMDG
 AAF ALEFKVN1HDMMWSKMYKYL-----LVTSETGTTDR1ITLEKNG1ALGFPGEVSSDR1IA1EEAYWSMASALSEADG1DYTDPEEELLVAT1LDDAMDG
 XP_001763895 ALEVVKNVRDALVNTLVRVRSNKP1AQDVGVGVSEGAAADDSDQFRSYLRKSNMPMDRLLTA1QDACLKMASALVEAEGMECTDPDELEF1VA
 ALDMEEVDDGSGALLVTEASSPDVATR ***

 ACG28979 KALANALATAPSMWTLGNAGMGALQRLAQDPNYPAYVARAASRAIDEKLKQWELEE-----GDSLRFVMNQNLASGDTDDNSAADDAA-----
 AAK59984 KALANALAAFP1SMWTLGNAGMGALQRLAQDPNYPAYVARAASRAIDEKLKQWELEE-----GDSLRFVLNMQNVMSKETADDSSAADDTR-----
 AAQ15125 KALANALAAFP1SMWTLGNAGMGALQRLAEDTNPA1AAAASKT1NELKQWE1EE-----GDSWRFVMVNEKSPEDDVDS-----
 AAP41026 KALANALAAFP1SMWTLGNAGMGALQRLAEDDNPA1AAAASKT1NELKQWE1EE-----GDSWRLMVEEESP-DDWGS-----
 ABN08080 RALAKA1AAP1SMWTLGNAGMGALQRLAEDSNPA1AAAASKT1NELKQWE1EE-----GDSWRFMMEGSTKEE NET-----
 ABK95826 QALANALAAFP1SMWTLGNAGMGALQRLAEDDNPA1AAAASKT1NELKQWE1EE-----GDSWRFMNMQKPMVEEDF1SQEDNNADTG-----
 CAO17159 QALANALAAFP1SMWTLGNAGMGALQRLAEDSNPA1AAAASKA1NELKQWE1EE-----GDSWRFMNMQKPMCEADDVETD-----
 AAF KALANALAAFP1SMWTLGNAGMGALQRLAEDSNPA1AAAASKA1NELKQWE1EE-----GDSLRFMMNFERPNDDVDSDLDE1-----
 XP_001763895 LALAGALAD1SMWTLGNAGMGALQRLSTDNSPEVAAATA1NELKQWE1EE-----GDSLRFMMNFERPNDDVDSDLDE1-----
 ELCHNYS1S1SEVL1SDHVTL1VIELATQELEKTAI ***

 ACG28979 -----
 AAK59984 -----
 AAQ15125 -----
 AAP41026 -----
 ABN08080 -----
 ABK95826 -----
 CAO17159 -----
 AAF -----
 XP_001763895 -----

Figure S1. Protein alignment of AAF orthologues. ClustalW (v1.81) was used for multiple sequence alignment of the deduced amino acid sequences of AAF's orthologues from *Zea mays* (ACG28979), *Oryza sativa* (OSA15; AAK59984), *Ipomoea batatas* (SPA15; AAQ15125), *Nicotiana tabacum* (NTA15; AAP41026), *Medicago truncatula* (ABN08080), *Populus trichocarpa* (ABK95826), *Vitis vinifera* (CAO17159), *Arabidopsis thaliana* (AAF), and *Physcomitrella patens* (XP_001763895). A colon (:) represents amino acid homologous substitutions. A period (.) identifies amino acid non-homologous substitutions. Asterisks indicate identical amino acids conserved in all sequences.

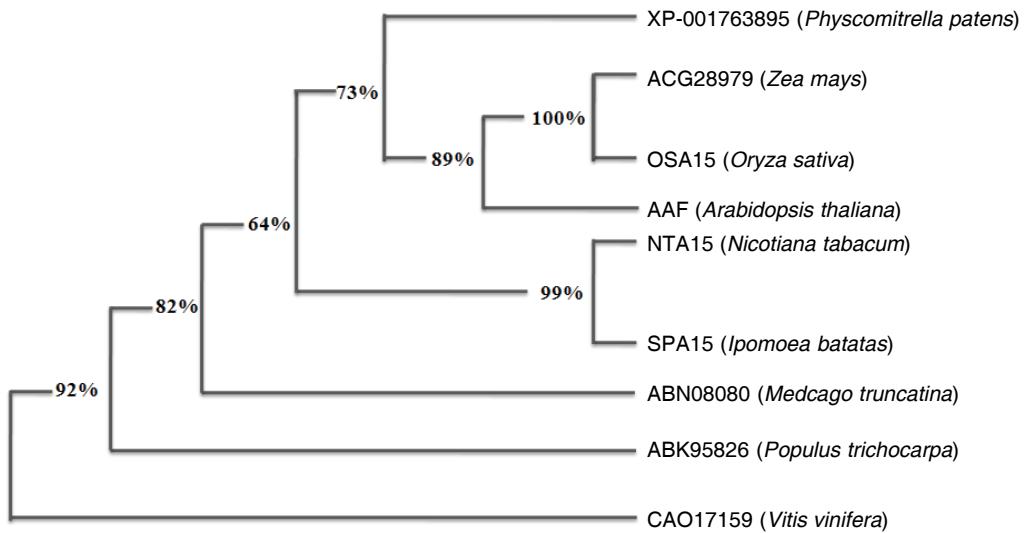


Figure S2 . Phylogenetic tree analysis of AAF orthologues. Different orthologues of AAF were analyzed by the web-based service, POWER (<http://power.nhri.org.tw/power/home.htm>). Multiple protein sequence alignment based on well-established methods, *ClustalW* and *PHYLIP* in POWER.

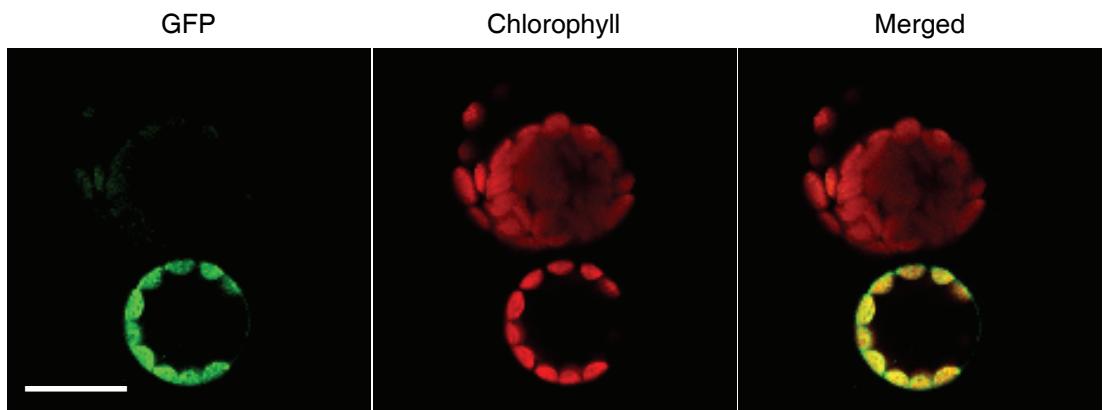


Figure S3. SPA15 is targeted to chloroplasts in *Arabidopsis* mesophyll protoplasts. *Arabidopsis* mesophyll protoplasts were transfected with 35S:SPA15-GFP. The fluorescence images were taken by using laser confocal confocal laser scanning microscopy (Zeiss LSM 510 Meta) with excitation at 488 nm and emission at 500-530 nm. Bar = 20 μ m

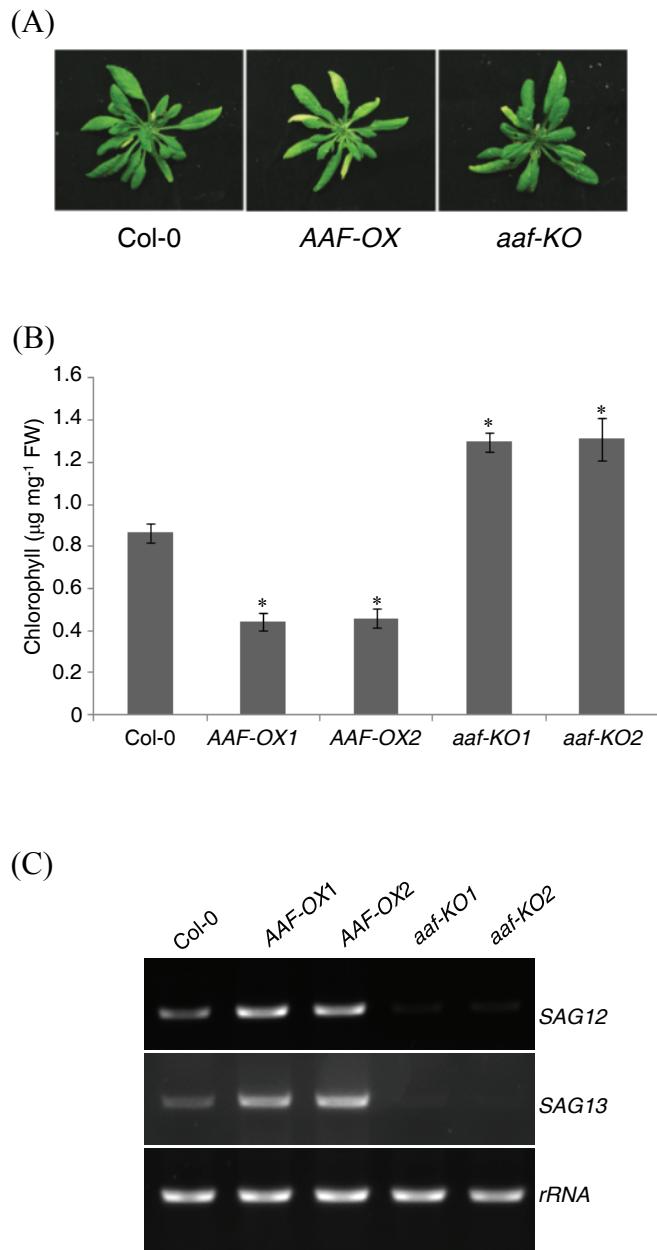


Figure S4. Age-dependent leaf senescence and expression of *SAG12* and *SAG13* in 7-week-old *AAF-OX* and *aaf-KO* plants. (A) Rosette leaves of 7-week-old plants. Inflorescences were removed for photograph. (B) Chlorophyll contents in rosette leaves of 7-week-old plants. * $P < 0.01$ vs wild type, Col-0 (n=6). Data represent means \pm SE. (C) Expression of 2 representative *SAG* genes in *AAF-OX* and *aaf-KO* plants by RT-PCR analysis.

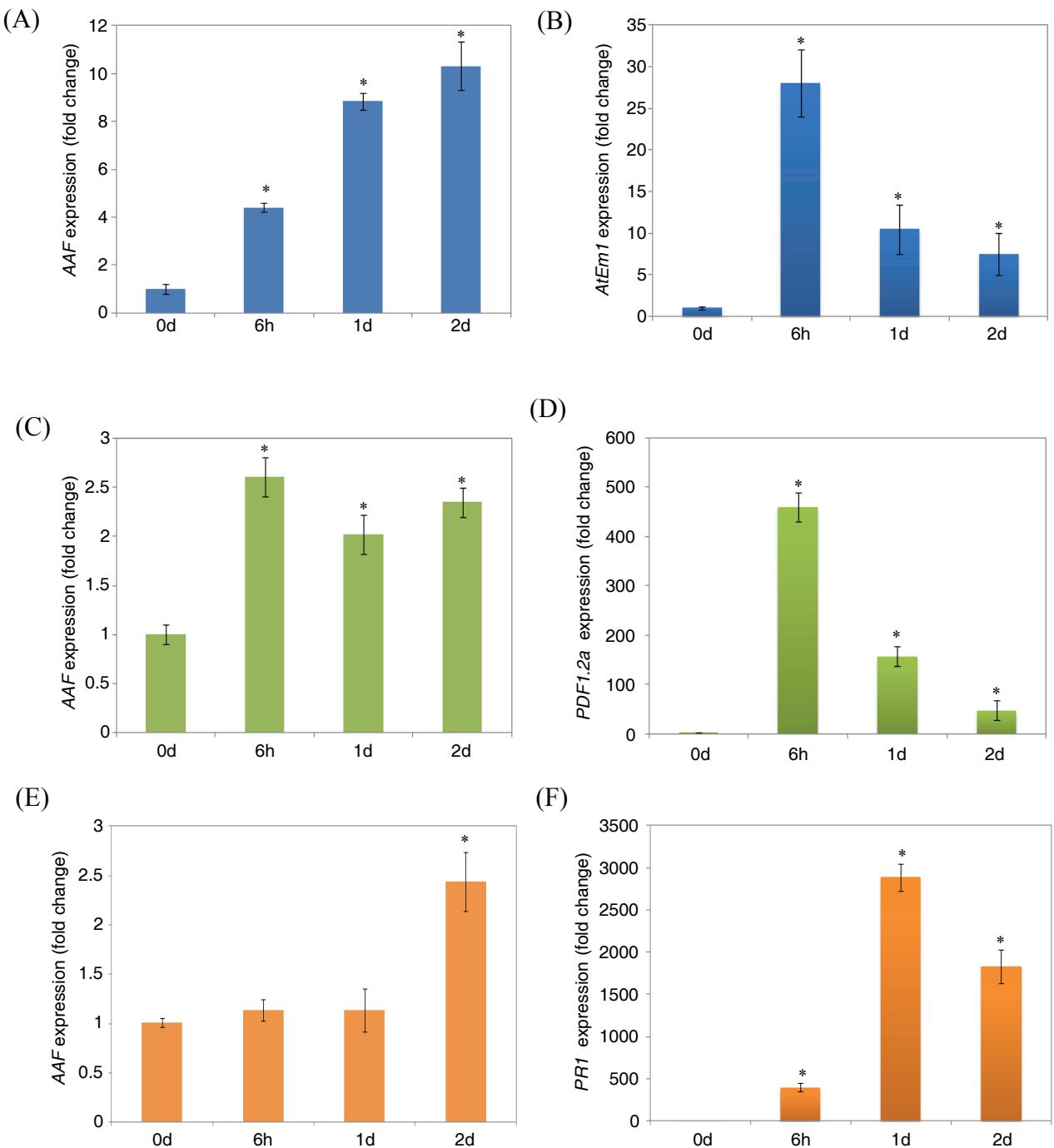


Figure S5. ABA, MeJA and SA can induce *AAF* expression in light-grown *Arabidopsis* seedlings. The expression level of *AAF* and hormone-responsive genes were detected by real-time PCR with 7-day-old seedlings treated by 100 μ M ABA (A, B), 100 μ M MeJA (C, D) and 100 μ M SA (E, F) in the indicated time. Fold change of Col-0 at 0 d was set as 1. * $P < 0.01$ vs wild type, Col-0 at 0 d. Data represent means \pm SE ($n = 3$). The whole experiment was repeated three times and one representative set of data is shown. Expression of *AtEm1* (At3g51810), *PDF1.2a* (At5g44420), and *PR1* (At2g14610) was used as control to validate the induction by ABA, MeJA and SA, respectively.