

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 orthologues	Signal peptide	Specificity ^a
ABN08080 (<i>Medicago truncatina</i>)	1-21	0.95
ABK95826 (<i>Populus trichocarpa</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>Piscomitrella 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	GGCGCTTTAGAGGCTGATTTAAGGCG
AAF-R	TTCAGCGAGTCTCTGTAATGCTCCC
18S rRNA-F	GGGATGGGTCGGCCGGTCCGCCTT
18S rRNA-R	CTCCACTCCTGGTGGTGCCCTTCCG
SAG12-F	GTCAGAACAACAGCTTGTTGATTGC
SAG12-R	GACATCAATCCCACACAAACATACAC
SAG13-F	GGAGTCTTGGAGGCAATGACCGCTC
SAG13-R	ATGCGGTCCATCCGGAGAGTGAACT
aaf-a	AGCTCGGGCAAGAGAAGATAC
aaf-b	AACGTCCGCAATGTGTTATTAAGTTGTC
aaf-c	CTGGAGAGCTTGAGCATTGAG
aaf-d	CCCATTTGGACGTGAATGTAGACAC
aaf-e	CTGTCTTCAGCGAGTCTCTGC
ATA15SpeIF1	GGACTAGTATGGCTCTTAACGTGAGCAAGGTGG
ATA15PmlIR2	GGCACGTGTCAGATCTCATCAAGGTCACTGTG
ATA15-GFP-f1	CCCGAATTCCTTCTTCTTCGTTCTTA
ATA15-GFP-r1	CTTGCTCACGTTAGGATCCCAT
ATA15F8	ATGGCTCTTAACGTGAGCAAGGTTG
ATA15R9	GATCTCATCAAGGTCACTGTC
ATA15-41F	GGTTCATCTATTGAAGAGTTAAAGC
ATA15FL-attB1F	AAAAAGCAGGCTCCACCATGGCTCTTAACGTGAGCAAG
ATA1540-attB2R	AGAAAGCTGGGTCCGGATCMTACTTATTAGCAGCTAAAGGATGAT
SPA15-attB1F	AAAAAGCAGGCTCCACCATGGCCAAACCTAATGGGATTATC
SPA15-attB1R	AGAAAGCTGGGTCCGGATCGCTATCTACATCGTCATCTTCTTG

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	GCCAGCTGATTCATGGCTCCTTTG
qAt5g59310-F	TCCTGGAAAGTGCGGTGTTAGC
qAt5g59310-R	TTCACCTTGATGGTGGCGCAGTTG
qAt2g26020-F	CGCTGCTCTTGTTCTCTTTGCAG
qAt2g26020-R	TCTCGCACAACTTCTGTGCTTC
qAt5g44420-F	ACACAACACATACATCTATACATT
qAt5g44420-R	TATATTATTGTAACAACAACGGGA
qAt4g17500-F	ATGTCGATGACGGCGGATTCTC
qAt4g17500-R	CGGCTCCGATTCTCCTAGTAAGTG
qAt3g04720-F	GCGGCAAGTGTTTAAGGGTGAAG
qAt3g04720-R	TCCAAATCCAAGCCTCCGTTGC
qAt2g43570-F	TTCGGTGCTTCCATCTCCAAACG
qAt2g43570-R	TGAACCCTGTTTCTTGGGCTACG
qAt1g06290-F	ATGTATTGATGCAGCAGGTGAGC
qAt1g06290-R	TCATGTGCTCCAATCCCAGTCC
qSEN1-F	GCCATCGACGAGAGACTCAAAGAG
qSEN1-R	CCTCAGCACCTTTCCATTTCCAC
qAt5g37940-F	GGATCCATGGCGTTCTAGTCAAG
qAt5g37940-R	CCAGAGATTGGCTTGCCGATAGTG
qAt1g02920-F	AGCCTTTCATCTTCCGCAACCC
qAt1g02920-R	TTGGAGCCAAGGGAGACAAGTTGG
qAt4g37520-F	TCGTTAATCTTGCGGGTGGAC
qAt4g37520-R	CGCTAGCCGCTGTTGATGATAG
qAt5g58390-F	TGGGTGCTTCTCTCCTTCGTTTG
qAt5g58390-R	AGTAAGGATCCGTCACACCCATTG
qACTIN-F	ACCTTGCTGGACGTGACCTTACTGAT
qACTIN-R	GTTGTCTCGTGGATTCCAGCAGCTT
qAtEm1-F	TTGCTGAAGGAAGGAGCAAGG
qAtEm1-R	G ACCCATCTCCTGATAACCCTCGTG
qAtPR1-F	ACACGTGCAATGGAGTTTGTGG
qAtPR1-R	TTGGCACATCCGAGTCTCACTG

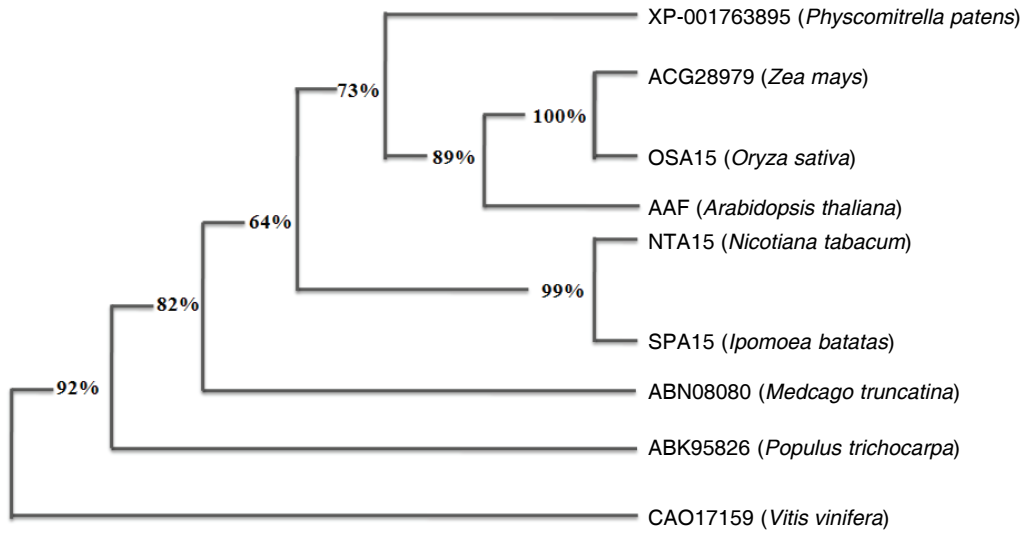


Figure S2 . Phylogenetic tree analysis of AAF orthologues. Different orthologues of AAF were analyzed by the web-based service, POWER (<http://power.nhri.org.tw/power/home.htm>). Multiple protein sequence alignment based on well-established methods, *ClustalW* and *PHYMLIP* in POWER.

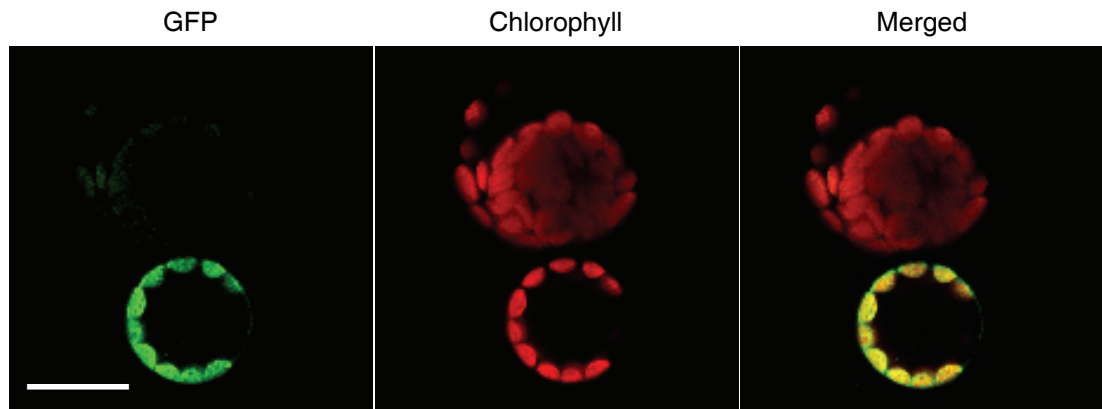


Figure S3. SPA15 is targeted to chloroplasts in Arabidopsis mesophyll protoplasts. Arabidopsis mesophyll protoplasts were transfected with 35S:SPA15-GFP. The fluorescence images were taken by using laser confocal confocal laser scanning microscopy (Zeiss LSM 510 Meta) with excitation at 488 nm and emission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

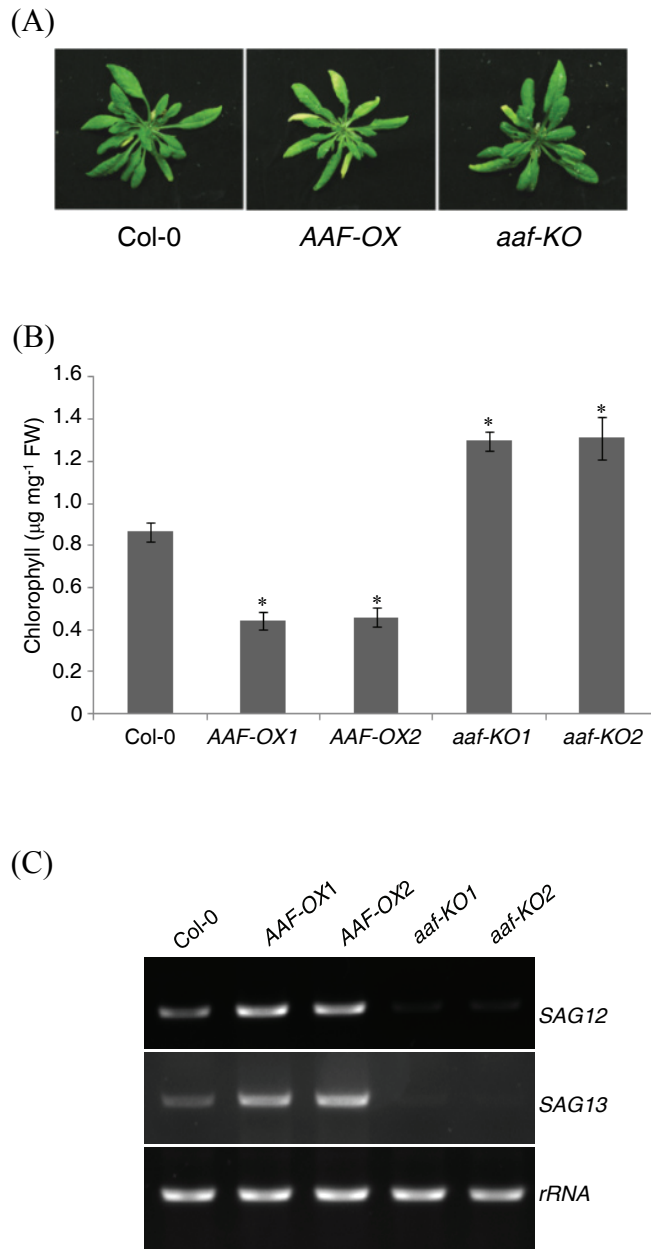


Figure S4. Age-dependent leaf senescence and expression of *SAG12* and *SAG13* in 7-week-old *AAF-OX* and *aaf-KO* plants. (A) Rosette leaves of 7-week-old plants. Inflorescences were removed for photograph. (B) Chlorophyll contents in rosette leaves of 7-week-old plants. * $P < 0.01$ vs wild type, Col-0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

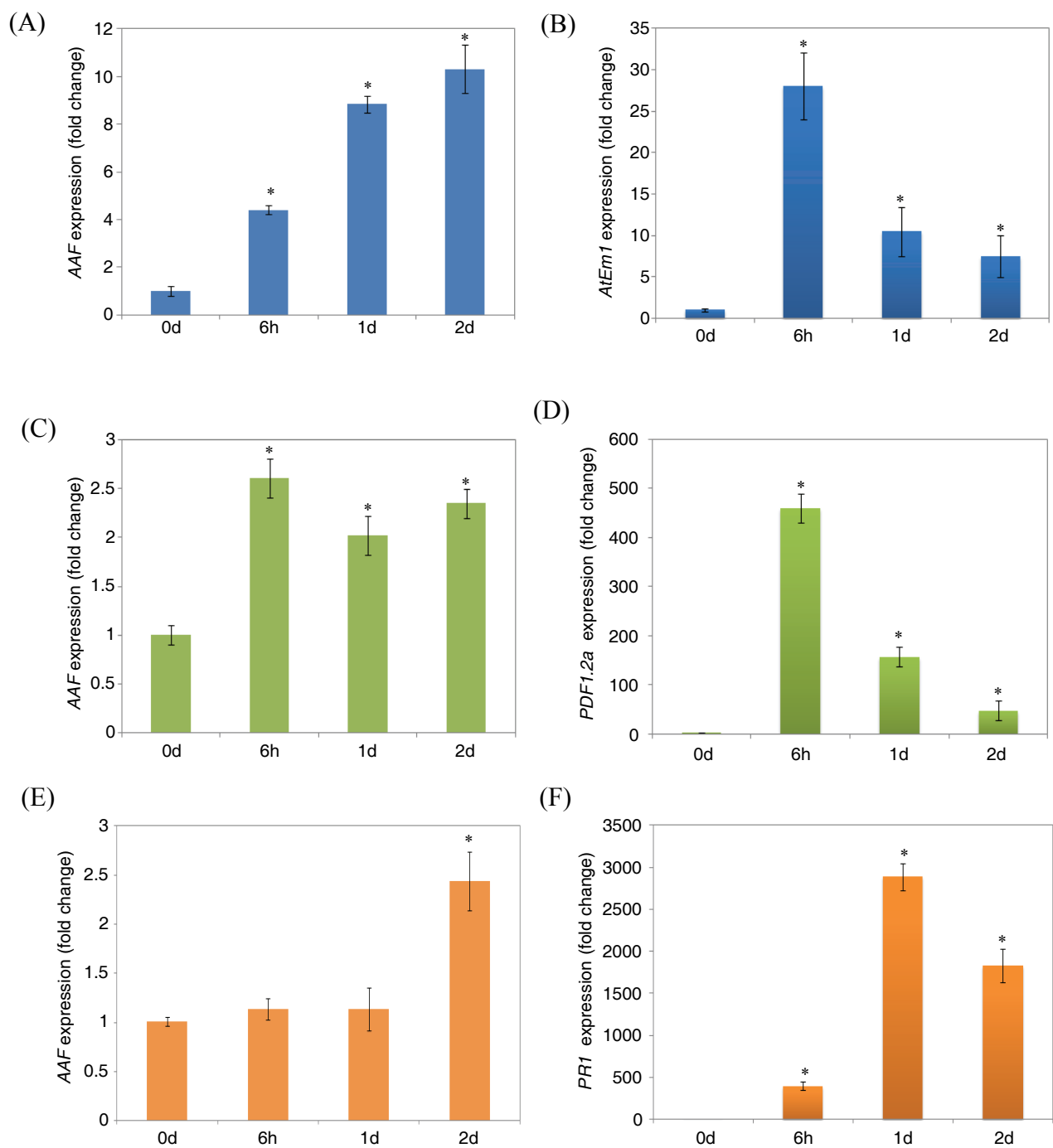


Figure S5. ABA, MeJA and SA can induce *AAF* expression in light-grown *Arabidopsis* seedlings. The expression level of *AAF* and hormone-responsive genes were detected by real-time PCR with 7-day-old seedlings treated by 100 μ M ABA (A, B), 100 μ M MeJA (C, D) and 100 μ M SA (E, F) in the indicated 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 *PRI* (At2g14610) was used as control to validate the induction by ABA, MeJA and SA, respectively.