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***Original Paper***

# The ERF transcription factor EPI1 is a negative regulator of dark-induced and jasmonate-stimulated senescence in *Arabidopsis*

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**Abstract** Identification of the factors involved in the regulation of senescence and the analysis of their function are important for both a biological understanding of the senescence mechanism and the improvement of agricultural productivity. In this study, we identified an *ERF* gene termed *"ERF gene conferring Postharvest longevity Improvement 1"* (EPI1) as a possible regulator of senescence in *Arabidopsis*. We found that EPI1 possesses transcriptional repression activity and that the transgenic plants overexpressing *EPI1* and expressing its chimeric repressor, *EPI1-SRDX*, commonly suppressed the darkness-induced senescence in their excised aerial parts. These transgenic plants additionally maintained a high level of chlorophyll, even after the methyl jasmonate (MeJA) treatment, which stimulated senescence in the dark. In addition, we found that senescence-induced and -reduced genes are down- and upregulated, respectively, in the MeJA-treated transgenic plants under darkness. Our results suggest that EPI1 functions as a negative regulator of the dark-induced and JA-stimulated senescence.

**Key words:** ERF, jasmonate, senescence, transcription factor.

Senescence is the final stage of development and is not only induced by aging but also by diverse environmental factors including unfavorable light intensity, temperature, water and nutrient states (Lim et al. 2003; Woo et al. 2010). Several reports have suggested that senescence functions as an active defense response, which is regulated by genetic and physiological processes. For example, leaf senescence is involved in transferring and recycling the limited nutrients from senescent leaves to young developing leaves under stress conditions (Himelblau and Amasino 2001). By contrast, stress-induced senescence is a major reason for the reduction in the final productivity and postharvest quality of plants. Therefore, the identification of factors involved in the regulation of senescence and the analysis of their function are essential for not only understanding the regulatory mechanisms driving senescence but also further biotechnological applications to produce plants with enhanced longevity through molecular manipulations.

Jasmonate (JA) is involved in the regulation of senescence. To date, many studies have demonstrated that JA is an important hormone involved in not only stress responses but also diverse developmental processes in plants (Demianski et al. 2011; Feys et al. 1994; Koo and Howe 2009; Staswick et al. 1992). It has been also demonstrated that JA is a positive regulator of senescence. Exogenous JA accelerates chlorophyll degradation depending on its receptor, CORONATINE INSENSITIVE 1 (COI1), in the dark; thus, the loss

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Abbreviations: CRES-T, Chimeric Repressor Gene-Silencing Technology; EPI1, ERF gene conferring Postharvest longevity Improvement1; GAL4B, GAL4-Binding Site; GAL4DB, GAL4-DNA Binding domain; JA, Jasmonate; LUC, Luciferase; MeJA, Methyl Jasmonate; PCR, Polymerase Chain Reaction; WT, Wild Type.
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of function of COI1 reduces JA-induced chlorophyll degradation (Shan et al. 2010). Moreover, JA-biosynthetic genes are highly expressed during senescence (He et al. 2002). Recently, well-known JA-signaling transcription factors such as MYC2 and JASMONATE-ASSOCIATED MYC2-LIKE 1 (JAM1) have been shown to be involved in the regulation of JA-dependent senescence (Qi et al. 2015).

The ERF family is a large transcription factor family consisting of 122 genes in *Arabidopsis*, which are classified into 12 subgroups based on their conserved amino acid motifs (Nakano et al. 2006). To date, an increasing number of *ERF* genes have been characterized in diverse processes including stress responses and developmental processes (Licausi et al. 2013). For example, ERF1 (At3g23240) and ORA59 (At1g06160) redundantly and positively regulate JA- and ethylene-dependent defense responses to the necrotrophic pathogens by activating *PDF1.2* genes (Lorenzo et al. 2003; Pre et al. 2008). ERF109 has been shown to be involved in JA-induced lateral root formation by the expression of two genes for key auxin biosynthesis enzymes, *ASA1* (*ANTHRANILATE SYNTHASE ALPHA SUBUNIT 1*) and *YUC2* (*YUCCA2*), via its binding to GCC-boxes in the promoters (Cai et al. 2014). Furthermore, several *ERF* genes are known to be involved in the regulation of senescence. For instance, Koyama et al. (2013) have reported that ERF4 (AT3g15210) and ERF8 (At1g53170) positively regulate senescence by suppressing its target gene, which is a negative regulator of leaf senescence such as *EPITHIOSPECIFIER PROTEIN/EPITHIOSPECIFYING SENESCENCE REGULATOR* (*ESP/ESR*). Chen et al. (2015) have additionally reported that an *ERF* gene termed *FYF UP-REGULATING FACTOR 1 (FUF1*) is a negative regulator of senescence of the floral organs. However, many *ERF* genes remain uncharacterized.

In the present study, we performed the functional analysis of an *ERF* gene [AT1g44830, designated as *ERF gene conferring Postharvest longevity Improvement 1* (*EPI1*)]. *EPI1* is a member of group II in the ERF family (Nakano et al. 2006). The group II consists of three subgroups, IIa, IIb, and IIc, and EPI1 belongs to the group IIb (Nakano et al. 2006). Previously, several genes in the group IIa were suggested to be involved in diverse JA-related stress responses (Brown 2003; Maruyama et al. 2013; Tsutsui et al. 2009). *EPI1* was reported to be affected by the mutation of *MAP kinase 4* gene, which regulates salicylic acid, jasmonic acid, and ethylene-dependent responses (Brodersen et al. 2006). However, the function of EPI1 and the relationship between EPI1 and the JA signaling pathway remain unknown. Here we demonstrated that the ectopic expression of *EPI1*, which may function as a transcriptional repressor, and the expression of its chimeric repressor *EPI1-SRDX* conferred the reduction of leaf senescence in dark- and methyl jasmonate (MeJA)-treated plants.

## Materials and methods

### *Plant materials and growth conditions*

Seeds of wild type (WT, *Arabidopsis thaliana* accession Col-0) and transgenic plants were surface-sterilized and then plated on one-half strength Murashige and Skoog (0.5× MS) (Murashige and Skoog 1962) supplemented with 0.5% sucrose and solidified with 1% (w/v) agar. After incubating the plates at 4°C in the dark for at least 2 days, plates were placed in a growth chamber at 22°C under constant light.

To grow plants in the soil pots, seeds were soaked in water before incubation for 2 days at 4°C in the dark, sowed in the soil pots, and then incubated in the plant room at 22°C with a photoperiod of 16/8-h light/dark.

### *Plasmid construction and transformation of plants*

All primer sets used for plasmid construction are listed in the Supplemental Table 1.

For preparing the plasmid for overexpression of *EPI1*, the coding region of *EPI1* was amplified by polymerase chain reaction (PCR) and introduced into the Gateway entry vector, pENTR (Thermo Fisher Scienctific, MA, USA). After confirming the sequences, the *EPI1* conding region was recombined into a destination vector, pK2GW7 (Karimi et al. 2002). Using the resultant plasmid, *Agrobacterium tumefaciens* LBA4404 was transformed according to the method previously described by Walkerpeach and Velten (1994).

For preparing the plasmid expressing a chimeric EPI1 repressor, *EPI1-SRDX*, the coding region without a stop codon was amplified by PCR and then introduced into the *Sma1* site of p35SSRDXG vector as described previously (Mitsuda et al. 2006, 2011). After confirming the insert sequences, the cassette region was transferred into the T-DNA destination vector, pBCKH (Mitsuda et al. 2006), containing a hygromycin resistance gene by Gateway LR reaction (Thermo Fisher Scienctific, MA, USA). The completed plasmid was transformed into the A*grobacterium tumefaciens* GV3101 strain by electroporation.

Transgenic plants were generated by *Agrobacterium*-mediated transformation of *Arabidopsis* Col-0 plants using the floral dip method (Clough and Bent 1998).

### *Treatments with darkness and MeJA*

For dark-induced senescence, aerial parts of 3–4-week-old plants grown in soil were detached and then placed on Petri dishes containing two layers of wet filter paper. The Petri dishes were enclosed and maintained in the dark at 22°C for 5 days.

For assaying MeJA-induced senescence, we followed the previously described method by Shan et al. (2010) with certain modifications. The aerial parts of the 11-day-old seedlings were detached and floated on 3 mM MES buffer (pH 5.8) with 50 $\mu$M MeJA or 0.1% methanol as a mock treatment for 3 and 5 days in

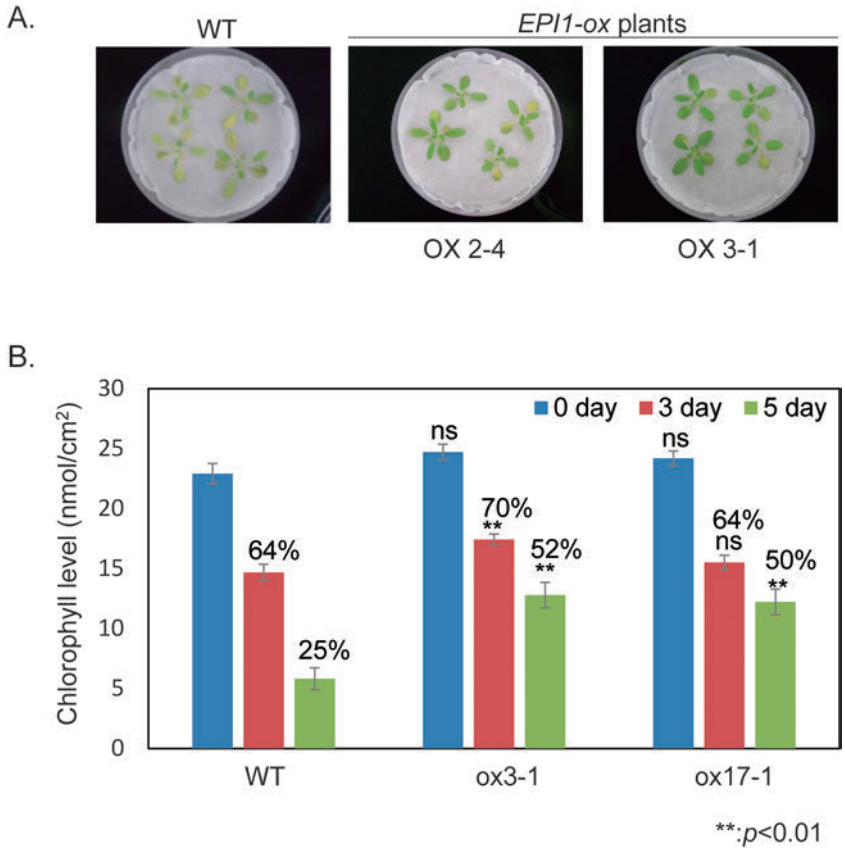


Figure 1. Overexpression of *EPI1* reduces chlorophyll degradation during dark-induced senescence. A. Leaf phenotypes of wild type (WT) and *EPI1*-overexpressing plants (*EPI1-ox* plants, OX) after the darkness treatment for 5 days. B. Each bar indicates the chlorophyll content that was converted from the SPAD values. The SPAD value was measured from three leaves of similar developmental stage from each of the three plants at days 0, 3, and 5 after shifting to the dark. Each number above the bar represents the percentage (%) of the average of SPAD values at each time point relative to that at the start of the experiment (day 0). Error bars represent standard deviation of the mean. Asterisks represent the significant difference between each value and that of the WT on the same day (Welch's *t* test, ** $p<0.01$, ns: no significance)

continuous light or darkness for 5 days.

### *Measurement of chlorophyll content*

For measuring the chlorophyll content, we used two methods depending on the experiment. For darkness-treated aerial parts of plants, we measured the SPAD meter values using a portable chlorophyll meter (the SPAD-502, Konica-Minolta, Japan) according to the time course, following which chlorophyll values were converted into absolute chlorophyll concentration following the equation adjusted to *Arabidopsis* by Ling et al. (2011). For measuring the chlorophyll content of MeJA or mock-treated seedling samples, we extracted chlorophyll by incubating frozen powder of leaves in 80% aceton (v/v) overnight. Absorbance was measured at 663 and 647 nm, and the chlorophyll level was calculated by following a previously described method (Lichtenthaler and Buschmann 2001).

### *Transient expression assay*

The detailed description of the method used is previously described (Hiratsu et al. 2002; Ohta et al. 2001). To prepare the effector plasmid expressing the GAL4 DNA binding domain (*GAL4DB*)-fused EPI1 driven by CaMV35S promoter, the coding region of *EPI1* without a stop codon was amplified using PCR and introduced into the pDONR207 vector plasmid by the BP reaction (Thermo Fisher Scienctific, MA, USA). After confirming the sequences, the *EPI1* coding region was transferred into the pDEST430T1.2 vector plasmid by LR reaction (Thermo Fisher Scienctific, MA, USA). The *Pro35S:GAL4DB-SRDX* effector plasmid has been previously described (Mitsuda et al. 2005).

Each 800 ng of effector plasmid was transiently co-introduced with 400 ng of the reporter plasmid for *pro35S-5XGAL4 Binding Site (GAL4BS)-Luciferase* (Hiratsu et al. 2002) and 400 ng of reference plasmid expressing modified *Renilla luciferase* (pRL plasmid) (Mitsuda et al. 2005) into rosette leaves of 3-week-old plants by particle bombardment. After bombardment, the leaves were incubated in darkness for 12 h. Each experiment was replicated at least three times.

### *RNA extraction and qRT-PCR*

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Hilden, Germany). RNA (1 $\mu$g) was reverse transcribed using the PrimeScript RT reagent kit (Takara Holdings Inc, Japan) according to the manufacturer's instructions. Real time qRT-PCR was performed using the ABI7300 real-time PCR system (Thermo Fisher Scienctific,

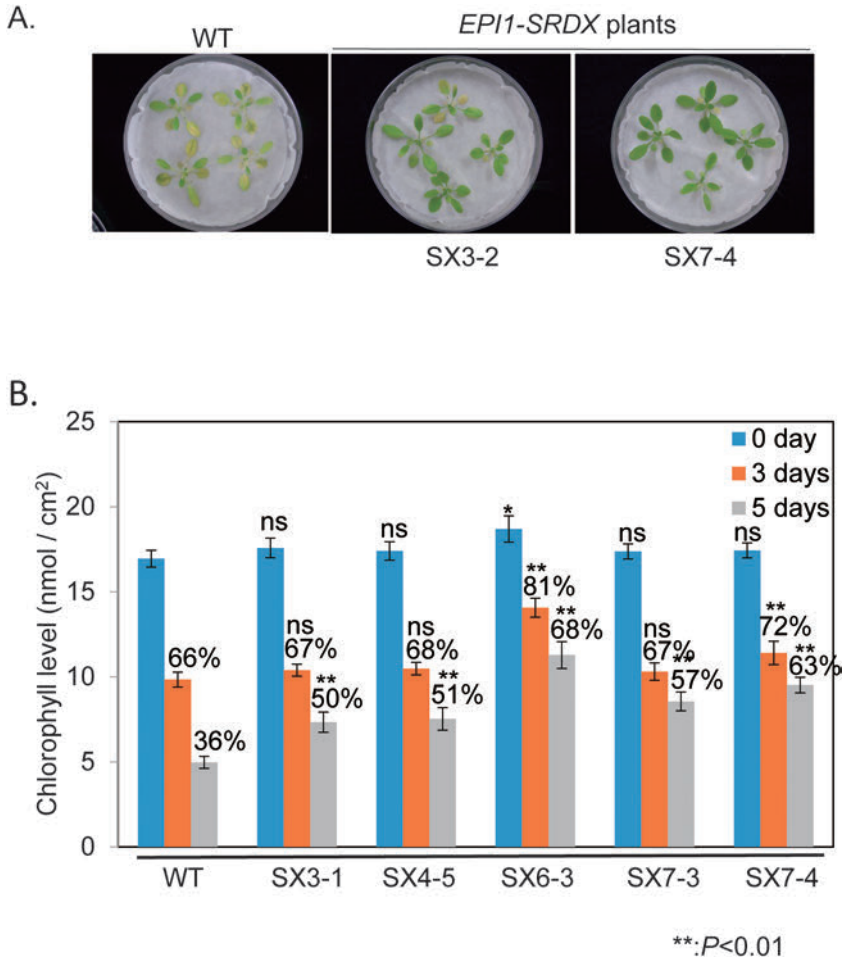


Figure 2. Expression of *EPI1-SRDX* reduces the chlorophyll degradation during dark-induced senescence, leading to similar phenotypes as shown in the *EPI1*-overexpressing plants. A. The phenotypes of wild type (WT) and two representative *EPI1-SRDX*-expressing plants (*EPI1-SRDX* plants, SX) after darkness treatment for 5 days. B. Chlorophyll content was calculated from the SPAD values at days 3 and 5. Each bar represents the average chlorophyll in each line. Each number above the bar represents the percentage of the average SPAD values at each time point relative to that at the start of the experiment (day 0). Error bars represent standard deviation of the mean. Asterisks represent the significant difference between each value and that of the WT on each day (Welch's *t* test, ** $p<0.01$, * $p<0.05$, ns: no significance).

MA, USA). Gene specific primers used for qRT-PCR are shown in Supplemental Table 2. The expression value of each gene was normalized with that of the *PP2AA3* gene as an internal control. Each experiment included at least three biological replicates.

## Results and discussion

### *EPI1 negatively regulates dark-induced senescence*

For the functional analysis of ERF transcription factors, we generated transgenic *Arabidopsis* plants overexpressing various *ERF* genes and assessed the changes in multifaceted phenotypes under diverse stress conditions. Finally, we found the delay of dark-induced senescence in a transgenic plant overexpressing an *ERF* gene, At1g44830 (*EPI1-ox*). As shown in Figure 1A, most of the leaves in the aerial portions of *EPI1-ox* plants still exhibited a green color, in contrast to those of WT plants which turned yellow under continuous darkness for 5 days. The total chlorophyll levels in WT plants were rapidly decreased in darkness treatment, finally reaching about 25% of the level in the start of the 5-day darkness treatment (Figure 1B). On the other hand, *EPI1-ox* plants retained more than 50% of their original total chlorophyll levels after the same treatment. These results suggest that the increased expression of *ERF* (AT1g44830) has a suppression effect on dark-induced senescence. Therefore, we designated this gene as *ERF gene conferring Postharvest longevity Improvement 1* (*EPI1*).

### *EPI1-SRDX plants are also resistant to dark-induced senescence*

The expression of a transcription factor fused to a plant-specific repression domain termed SRDX is a useful technique for the functional analysis of transcription factors because SRDX-fused transcriptional activators can function as transcriptional repressors, resulting in phenotypes opposite to their overexpressing plants and

similar to their loss-of-function mutants. By contrast, SRDX-fused transcriptional repressors still function as transcriptional repressors, and their transgenic plants display the same phenotypes as shown in their over-expressing plants (Hiratsu et al. 2003; Ikeda and Ohme-Takagi 2009; Matsui et al. 2008; Mitsuda et al. 2011; Nakata et al. 2013).

We generated transgenic plants expressing the chimeric repressor of *EPI1*, *EPI1-SRDX* (*EPI1-SRDX* plants), and examined the effect of its expression on dark-induced senescence. The *EPI1-SRDX* plants retained dark green leaves similar to *EPI1*-ox plants, whereas WT plants displayed light green or yellow leaves after the 5-day darkness treatment (Figure 2A). The chlorophyll levels in the *EPI1-SRDX* plants were also significantly higher than those in WT plants (Figure 2B). Thus, *EPI1-SRDX* plants were resistant to dark-induced senescence. These results suggest that EPI1-SRDX negatively regulates the dark-induced senescence.

### *EPI1 acts as a transcriptional repressor*

Because *EPI1-ox* and *EPI1-SRDX* plants displayed similar phenotypes in the dark-induced senescence, we hypothesized that EPI1 possesses transcriptional repression activity. To confirm this hypothesis, we examined the transcriptional activity of EPI1 using a transient expression assay (Figure 3A). We fused the coding region of *EPI1* with the yeast GAL4 DNA binding domain (*GAL4DB*) and performed transient expression assays using the luciferase reporter driven by the CaMV 35Spromoter (*Pro35S:GAL4BS-LUC*). As a positive control for a transcriptional repression, we used the SRDX fused GAL4DB (*GAL4DB-SRDX*), which strongly represses the activity of the reporter gene as previously reported (Hiratsu et al. 2003). As shown in Figure 3B, GAL4DB-EPI1 significantly reduced the reporter activity as compared to GAL4DB. These results suggest that EPI1 is a possible transcriptional repressor. This result may explain the reason for *EPI-ox* and *EPI1-SRDX* plants displaying similar phenotypes. EPI1 may possess a novel repression domain because it has not been shown to possess any known repression domain, including an EAR (ERF-associated amphiphilic repression) motif (Nakano et al. 2006).

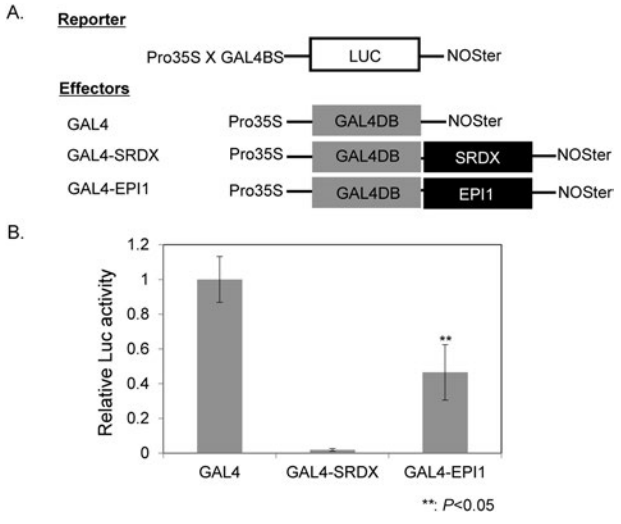


Figure 3. EPI1 possesses transcriptional repression activity. A. Schematic representation of the constructs used for the transient expression assay. The reporter construct consists of the CaMV 35S promoter, GAL4 DNA binding site (GAL4 BS), firefly LUC coding sequence, and Nos terminator (NOS ter). Effector constructs consist of GAL4 DNA binding domain (GAL4DB)-fused proteins under the CaMV 35S promoter. B. The mean of values for vector control, GAL4, was set to 1, and relative values were calculated. Error bars indicate standard deviation of results from three replicates. This experiment is representative of three independent experiments. Asterisks indicate significant difference from vector control (Welch's *t* test, ** $p<0.05$).

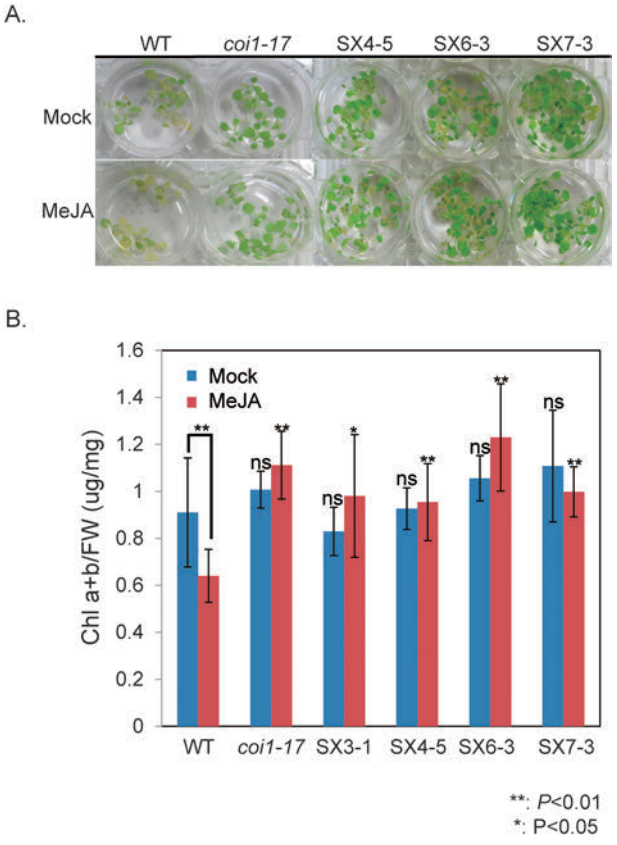


Figure 4. *EPI1-SRDX* plants are insensitive to the stimulation effect of MeJA on dark-induced chlorophyll degradation. A. The aerial parts of wild type (WT), *coi1-17*, and *EPI1-SRDX*-expressing plants (SX) plants were excised from 11-day-old plants grown on the one-half strength Murashige and Skoog (0.5×MS) medium plate, and then soaked in 50 $\mu$M methyl jasmonate (MeJA) or 0.1% methanol (mock) solution in the dark for 5 days. B. Chlorophyll content was measured after MeJA or mock treatment under the dark for 5 days. Bars represent the average chlorophyll values in leaves of each plant. Similar results were obtained in three independent experiments. Error bars represent standard deviation of the results ($n>6$). Asterisks represent the significant difference between each value and that of WT plants in the same condition. In WT plants, the asterisks above the line represent the difference between the chlorophyll values of mock- and MeJA-treated WT plants (Welch's *t* test, ** $p<0.01$, * $p<0.05$, ns: no significance).

### *The stimulation effect of JA on dark-induced senescence is suppressed in EPI1-ox and EPI1-SRDX plants*

The JA-dependent signaling pathway is known to have an acceleration effect on dark-induced senescence (He et al. 2002). We assumed that *EPI1-ox* and *EPI1-SRDX* plants might additionally display an altered response to MeJA treatment. Therefore, we observed the effect of MeJA treatment on darkness-treated aerial parts of *EPI1-ox*, *EPI1-SRDX*, and WT plants. Before the treatment, the chlorophyll levels in *EPI1-ox* and *EPI1-SRDX* plants did not considerably differ from those of WT plants (Supplemental Figure 1). In a 50 $\mu$M MeJA solution, most of the leaves of the darkness-treated WT plants turned yellow, whereas the *coi1-17* mutant plants, which are defective in JA perception, retained green leaves as previously reported (He et al. 2002). In the case of the *EPI1-SRDX* plants, green leaves were retained and displayed a similar level of chlorophyll to that in the mock solution, even in the presence of MeJA (Figure 4). This phenotype was also observed in *EPI1-ox* plants (data not shown). Therefore, we suggest that EPI1-SRDX and EPI1 negatively regulate the JA-induced acceleration of senescence in darkness.

### *The expressions of senescence-associated genes are altered in the MeJA-treated EPI1-SRDX plants under constant darkness*

Because the present results suggest that EPI1 and EPI1-SRDX negatively affect MeJA-stimulated senescence, we examined whether the expression levels of the well-known senescence-induced genes *SENESCENCE-ASSOCIATED GENE* (*SAG*) *12*, *SAG13*, and *SAG29* (Otegui et al. 2005; Park et al. 1998; Shi et al. 2015; Weaver et al. 1998) are also affected by MeJA treatment in WT and *EPI1-SRDX* plants under the constant dark condition. Because *EPI1-ox* and *EPI1-SRDX* plants displayed similar responses in all experiments, further data shown here were obtained by using *EPI1-SRDX* plants. As shown in Figure 5, the MeJA-induced upregulation of *SAG12*, *SAG13*, and *SAG29* expression was highly suppressed in most of the *EPI1-SRDX* plants, except for the lines 4–5, which displayed lower level of *EPI1-SRDX* expression as compared with that of other lines (Supplemental Figure 2). We also tested the expression of the *RIBULOSE BISPHOSPHATE CARBOXYLASE SMALL CHAIN (RBCS)* gene whose expression is known to be downregulated during senescence. The expression level of *RBCS* was decreased

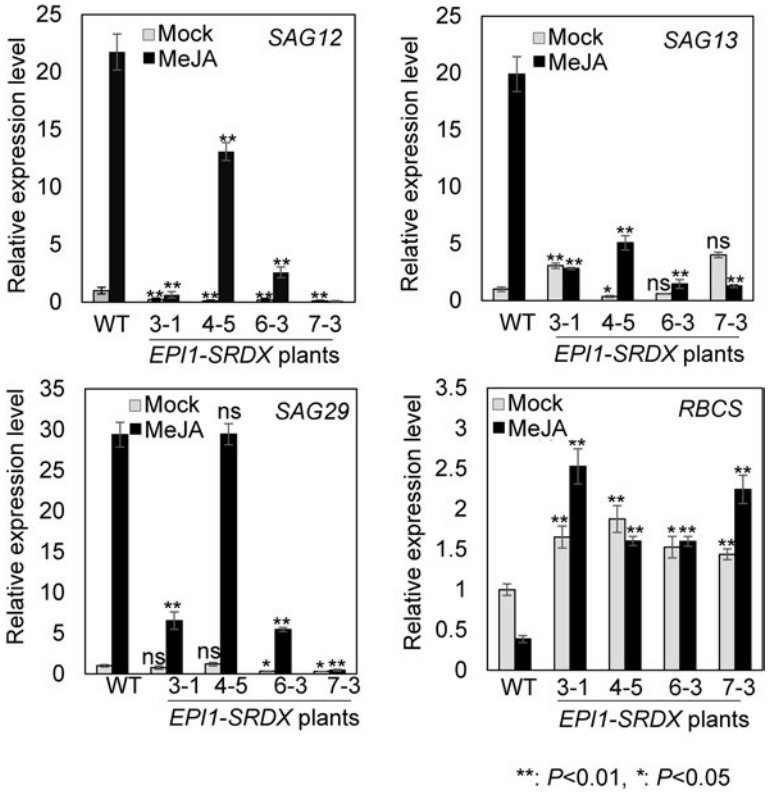


Figure 5. Expressions of senescence-associated genes are altered in *EPI1-SRDX*-expressing plants. Gene expressions in 50 $\mu$M methyl jasmonate (MeJA)- and 0.1% methanol (mock)-treated wild type (WT) and *EPI1-SRDX* expressing transgenic plants (*EPI1-SRDX* plants) were calculated relative to the reference gene *PP2AA3* (AT1g13320) and then normalized to that of mock-treated WT plants. The average value for each respective gene in mock-treated WT was set to 1. All data were generated from three biological replicates. Error bars represent the standard deviations of the mean. Asterisks above the bars represent significant difference between each gene expression level and that of WT in the same condition (Welch's *t* test, ** $p<0.01$, * $p<0.05$, ns: no significance).

by MeJA treatment in WT plants, whereas it was increased or remained at a high level in *EPI1-SRDX* plants. These results suggest that *EPI1-SRDX* and possibly EPI1 reduce MeJA-stimulated senescence by affecting the expression of the diverse senescence-related genes.

### *Expression of EPI1 is upregulated by darkness and JA treatments*

Because our results suggested the possible involvement of EPI1 in the negative regulation of dark-induced senescence and JA signaling, it could be hypothesized that the expression of *EPI1* is downregulated in WT plants under those conditions. To test this hypothesis, we evaluated the expression level of *EPI1* in aerial parts excised from WT plants in mock and MeJA solution in the dark. The expression of *VEGETATIVE STORAGE PROTEIN 1* (*VSP1*) was also examined as a positive control for JA response. As shown in Figure 6, the *VSP1* transcript was markedly increased under darkness, and it was further increased in response to the MeJA treatment. This result is consistent with a previous report (Benedetti et al. 1995), confirming that our JA treatment experiment was rigorously performed. On the other hand, the transcript level of *EPI1* was increased by not only MeJA but also the mock treatment. Although this result is incompatible with our hypothesis, a possible explanation for this is that the activity of EPI1 might be suppressed via post-translational regulatory mechanisms under darkness and JA treatment. Furthermore, the dark-induced increase of the *EPI1* transcript level was decreased after 3-h in the presence of MeJA. Therefore, it is possible that this decrease might be a reason for the MeJA-induced stimulation of the dark-induced senescence in the WT plants.

### *Concluding remarks*

In the present study, we found that dark-induced senescence and the stimulation effect of MeJA are suppressed in *EPI1-ox* and *EPI1-SRDX* plants (Figures 1, 2, 4). Furthermore, senescence-induced and -reduced genes are suppressed and enhanced in the MeJA-treated transgenic plants, respectively, in contrast to WT plants under darkness treatment (Figure 5). These results suggest that EPI1 may induce an activity that interferes with the stress-induced senescence through the transcriptional regulation of senescence-related genes in plants. Furthermore, we found that EPI1 has a transcriptional repression activity in the transient assay (Figure 3). Consequently, all of our findings provide important clues to understand physiological and molecular function of EPI1, describing the involvement of EPI1 in the senescence of *Arabidopsis*. Thus, further studies are warranted to understand the underlying molecular mechanism of EPI1 involvement, including the identification of its direct or indirect downstream genes by analyzing *EPI1* knockout mutants and/or RNAi plants. It would additionally be important to determine the mechanism behind the cooperation between EPI1 and other *ERF* genes in the same group for the regulation of senescence.

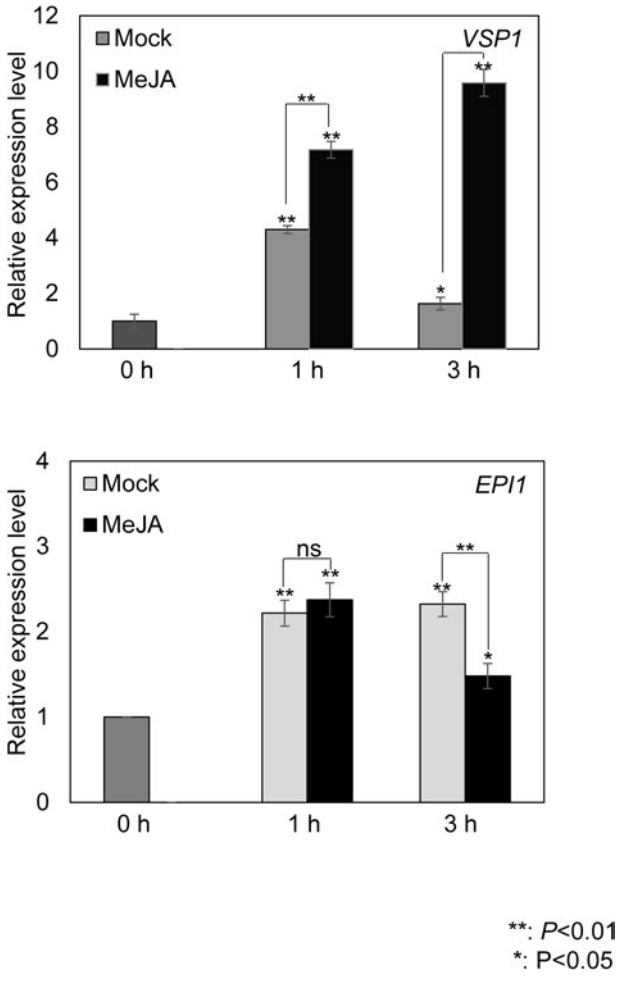


Figure 6. *EPI1* transcript is induced by darkness and JA treatments. The aerial parts of 11-day-old wild type (WT) plants were soaked in 50 $\mu$M methyl jasmonate (MeJA) or 0.1% methanol (mock) in the dark for 0, 1, and 3 h. Each gene expression value was calculated relative to the reference gene *PP2AA3* (AT1g13320), and then normalized to that of each gene in untreated WT plants (0 h), which was set to 1. Upper panel shows the expression of *VSP1*, and lower panel indicates the expression of *EPI1*. Data bars represent the average of three biological replicates, and error bars represent the standard deviation of the mean. Asterisks above the lines represent the significant difference of the gene expression level between mock- and MeJA-treated samples. (Welch's *t* test, ** $p<0.01$, * $p<0.05$).

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Supplemental Table 1. Primers for cloning used in this study

| Amplified region | Forward primer | Reverse primer |
|---|---|---|
| EPI1 CDS for cloning into pEntry vector | 5`CACCATGGTGAAAACACTTCAAAAGACAC3′ | 5`TCAGCAGAAGTTCCATAATCTGATA3′ |
| EPI1 CDS for cloning into p35SSRDXG vector | 5`ATGGTGAAAACACTTCAAAAGACAC3′ | 5`GCAGAAGTTCCATAATCTGATATC3′ |
| EPI1 CDS for cloning into pDONR207 vector | 5`CAAGTTTGTACAAAAAAGCAGGCTTCATGGTGAAA ACACTTCAAAAGACAC3 | 5`GGGGACCACTTTGTACAAGAAAGCTG GGTTGCAGAAGTTCCATAATCTGATATC3′ |

Supplemental Table 2. Primers for qRT-PCR used in this study

| Amplified gene ( gene locus) | Forward primer | Reverse primer |
|---|---|---|
| EPI1 (At1g44830) | 5`-GAAGTTGGGGTTCATGGGTTTCA-3` | 5`-CGGCTTCAGCAGTTGAGTAAGA-3` |
| PP2AA3 (At1g13320) | 5`-GACCAAGTGAACCAGGTTATTGG-3` | 5`-TACTCTCCAGTGCCTGTCTTCA-3` |
| SAG12 (At5g45890) | 5`-GGCGTTTTCAGCGGTTGCGG-3` | 5`-CCGCCTTCGCAGCCAAAATCG-3` |
| SAG13 (At2g29350) | 5`-AGGAAAACTCAACATCCTCGTC-3` | 5`-GCTGACTCGAGATTTGTAGCC-3` |
| SAG29 (At5g13170) | 5`-GCCACCAGGGAGAAAAGG-3` | 5`-CCACGAAATGTGTTACCATTAGAA-3` |
| SEN4 (At4g30270) | 5`-GACTCTTCTCGTGGCGGCGT-3` | 5`-CCCACGGCCATTTCCCCAAGC-3` |
| RBCS (At1g67090) | 5`-CGCTCCTTTCAACGGACTTA-3` | 5`-AGTAATGTCGTTGTTAGCCTTGC-3` |
| CAB1 (At1g29930) | 5`-GCAAGGAACCGTGAACTAGAA-3` | 5`-TCCGAACTTGACTCCGTTTC-3` |

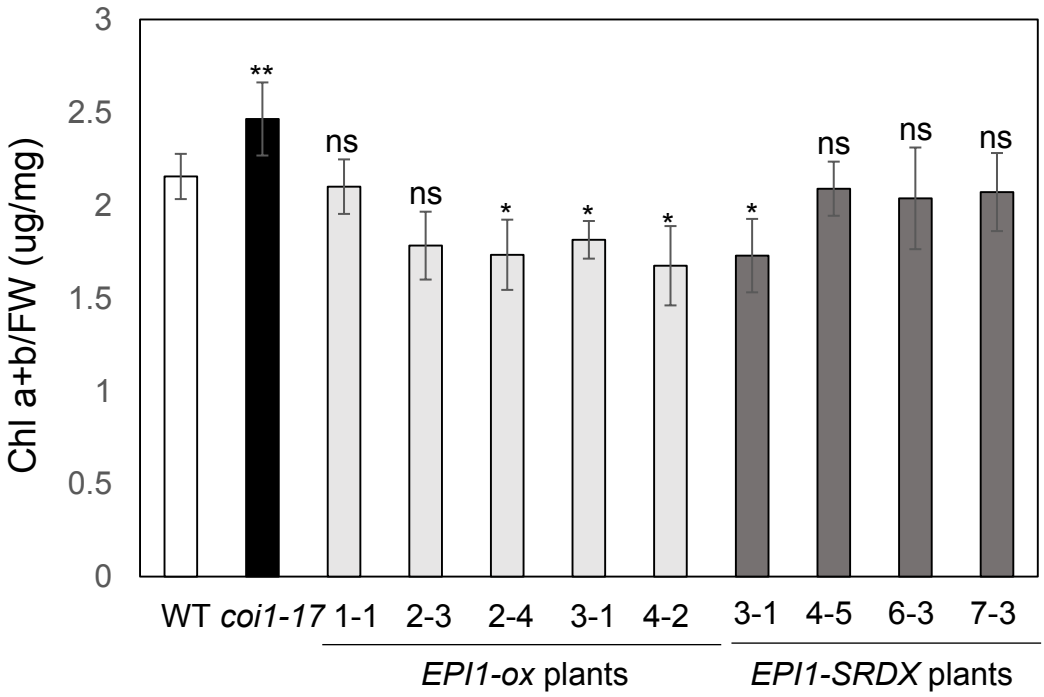
**Supplemental figure 1. Comparison of total chlorophyll levels among WT, *coi1-17*, *EPI1-ox* and *EPI1-SRDX* plants**

The aerial parts of wild-type (WT), *coi1-17*, *EPI1*-overexpressing plants (*EPI1-ox* plants), and *EPI1-SRDX* expressing plants (*EPI1-SRDX* plants) were excised from 11-day-old seedlings grown on the one-half strength Murashige and Skoog (0.5× MS) medium plate, and then the chlorophyll content of each sample was measured. Data bars represent the average of six biological replicates, and error bars represent the standard deviation of the results (n > 6). Asterisks above the bars represent significant difference in the chlorophyll content as compared to that of the WT plants (Welch's *t* test, **: $p < 0.01$, *: $p < 0.05$, ns: no significance).

**Supplemental figure 2. Expression level of total *EPI1* transcripts including both endogenous and transgene in WT and transgenic plants**

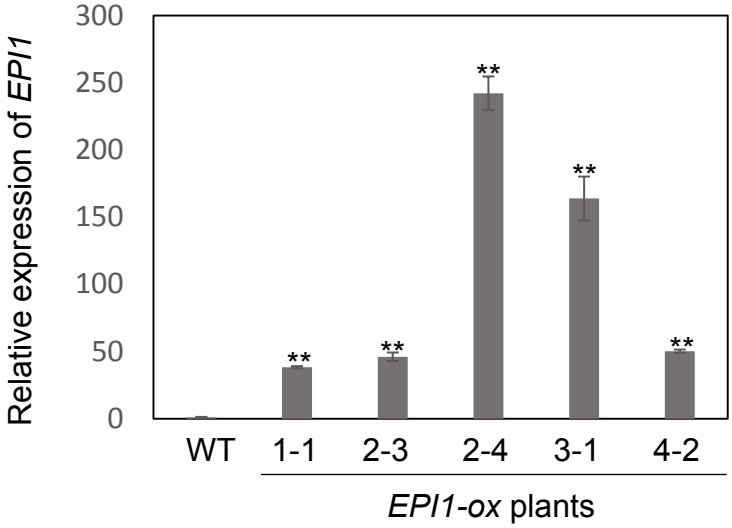
A. Total *EPI1* transcript level was determined using quantitative reverse transcription polymerase chain reaction (qRT-PCR) in wild-type (WT) and *EPI1* overexpressing plants (*EPI1*-ox plants). Total Gene expression was calculated relative to the reference gene *PP2AA3* (AT1g13320). All data were normalized to the WT value, which was set to 1. Data bars represent the average of three technical replicates. Error bars represent the standard deviation of the mean.

B. Total *EPI1* transcript level was determined using qRT-PCR in WT and *EPI1-SRDX*-expressing plants (*EPI1-SRDX* plants). Total Gene expression was calculated relative to the reference gene *PP2AA3* (AT1g13320), and all data were normalized to the WT value, which was set to 1. Data bars represent the average of three technical replicates and the numbers over the bars for line 4-5 and line 4-8 represent the relative expression level of *EPI1*. Error bars represent the standard deviation of the mean for three technical replicates. Asterisks above the bars represent significant difference in the gene expression level as compared to that of the WT plants (Welch's *t* test, **: $p<0.01$).

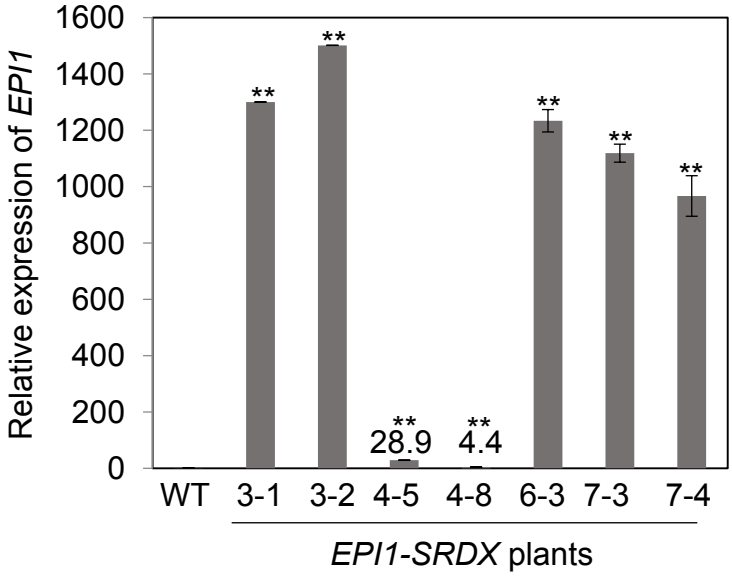



Supplemental Figure 1. Chung et al.

A




B




Supplemental Figure 2. Chung et al.