



Research paper

AtWRKY75 positively regulates age-triggered leaf senescence through gibberellin pathway

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ABSTRACT

WRKY transcription factors play essential roles during leaf senescence. However, the mechanisms by which they regulate this process remains largely unknown. Here, we identified the transcription factor WRKY75 as a positive regulator during leaf senescence. Mutations of *WRKY75* caused a delay in age-triggered leaf senescence, whereas overexpression of *WRKY75* markedly accelerated this process. Expression of senescence-associated genes (SAGs) was suppressed in *WRKY75* mutants but increased in *WRKY75*-overexpressing plants. Further analysis demonstrated that *WRKY75* directly associates with the promoters of *SAG12* and *SAG29*, to activate their expression. Conversely, *GAI* and *RGL1*, two DELLA proteins, can suppress the *WRKY75*-mediated activation, thereby attenuating *SAG* expression during leaf senescence. Genetic analyses showed that *GAI* gain-of-function or *RGL1* overexpression can partially rescue the accelerated senescence phenotype caused by *WRKY75* overexpression. Furthermore, *WRKY75* can positively regulate *WRKY45* expression during leaf senescence. Our data thus imply that *WRKY75* may positively modulate age-triggered leaf senescence through the gibberellin-mediated signaling pathway.

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1. Introduction

Plants undergo developmental and physiological changes throughout their life history, ending with senescence and death (Lim et al., 2005). Leaf senescence is an important part of plant development and increases reproductive success. During senescence, plants relocate mobilizable nutrients from older leaves to

other developing organs, including seeds, stems and roots (Lim et al., 2005). Under optimal conditions, the onset of leaf senescence is normally initiated in an age-dependent manner; however, it can also be triggered by environmental changes that are integrated into the developmental aging program (Buchanan-Wollaston et al., 2005; Lim et al., 2007). Plant senescence represents one of the adaptive mechanisms that plants possess that may help to increase survival in a given ecological niche.

Leaf senescence is a highly coordinated and sophisticated cellular process during which leaf cells undergo active degenerative alterations. Furthermore, this biochemical process is closely associated with the increased expression of numerous senescence-associated genes (SAGs), including *SAG12* and *SAG29*, but reduced expression of photosynthetic genes, such as *CAB1* and *RBCS1A* (Gan and Amasino, 1995; Hortensteiner, 2006; Park et al., 1998; Qi et al., 2015; Weaver et al., 1998). Studies have also

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demonstrated that a distinct set of phytohormones play critical roles during leaf senescence, with jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), salicylic acid (SA), brassinosteroids accelerating senescence, while auxin and cytokinins delay leaf aging (Jibrán et al., 2013). Interestingly, gibberellin (GA) was recently revealed to be a crucial growth regulator that positively modulates leaf senescence in *Arabidopsis* (Chen et al., 2014, 2017).

Temporal profiling has revealed that leaf senescence involves the coordinated expression of thousands of genes. Notably, numerous *WRKY* genes are strongly expressed in senescing leaves, suggesting that *WRKY* genes play a role in leaf senescence (Guo et al., 2004). Recent studies have provided evidence that *WRKY* proteins function as critical components in senescence-associated regulatory pathways. For example, *WRKY57* was shown to function as a common component of JA- and auxin-mediated signaling pathways to modulate JA-induced leaf senescence (Jiang et al., 2014). In addition, *WRKY75*, together with SA and reactive oxygen species (ROS), has been shown to form a tripartite amplification loop to promote leaf senescence (Guo et al., 2017). Furthermore, our recent study demonstrated that *WRKY45* directly binds several *SAG* promoters to activate their expression, thereby activating GA-mediated leaf senescence (Chen et al., 2017).

Here, we show that *WRKY75* transcript and protein levels increased during leaf senescence. We found that age-triggered leaf senescence was delayed in *WRKY75* mutants, but accelerated in plants overexpressing *WRKY75*. Further investigation indicated that *WRKY75* participates in GA-mediated leaf senescence by directly regulating the expression of several downstream *SAGs*. We also show that the ability of *WRKY75* to activate transcription can be repressed by both *GAI* and *RGL1*, and that *GAI* gain-of-function or *RGL1* overexpression can partially rescue the early-senescence phenotype caused by *WRKY75* overexpression. Our findings thus demonstrate that *WRKY75* may participate in GA-mediated leaf senescence in *Arabidopsis*.

2. Materials and methods

2.1. Materials and plant growth conditions

All mutant and transgenic plants were in the Col-0 background. The *WRKY* mutants (*wrky75-1* and *wrky75-25*), *WRKY75* and *RGL1* transgenic over-expressing plants, and *Myc-WRK75/RGL1* plants have been described in our previous studies (Chen et al., 2017; Zhang et al., 2018). *Arabidopsis* plants were grown at 22 °C under a 16-h-light/8-h-dark cycle. All chemicals were purchased from Takara Biotechnology (Dalian, China).

2.2. qRT-PCR analysis

Quantitative RT-PCR was conducted as described in Chen et al. (2013). The gene-specific primers for qRT-PCR were *WRKY75-F* (5'-ATATGGCCAAAAGGCCGTCA-3') and *WRKY75-R* (5'-TGCTCGAAGTTTCGGTGGA-3'), *SAG12-F* (5'-ATCCAAAAGCAACTTCTATTACAGG-3') and *SAG12-R* (5'-CCACTGCCTTCATCAGTGC-3'), *SAG29-F* (5'-GCCACCAGGGAGAAAAGG-3') and

SAG29-R (5'-CCACGAAATGTGTATACATTAGAA-3'), *ACTIN2-F* (5'-TGTGCCAATCTACGAGGGTTT-3') and *ACTIN2-R* (5'-TTTCCCGCTCTGCTGTGT-3').

2.3. Measurements of chlorophyll content and ion leakage

Chlorophyll was extracted from detached leaves with 80% acetone. Chlorophyll content was determined at 663 and 645 nm according to Lichtenthaler (1987).

To measure ion leakage, we incubated detached leaves in deionized water for at least 2 h (less than 10 h) and then determined conductivities (C1) of the solutions. The samples were then boiled in the same deionized water for 15 min. After cooling, the conductivities (C2) of the solutions were measured again (Li et al., 2013). The degree of ion leakage was calculated as the ratio of C1:C2.

2.4. ChIP assays

WRKY75:YFP-WRK75:3'-WRKY75, *Myc-WRK75/RGL1* and Col-0 leaves were harvested for ChIP experiments as described in Saleh et al. (2008). The GFP or Myc antibody was used to immunoprecipitate the protein–DNA complex, and the precipitated DNA was purified using a PCR purification kit for real-time qPCR analysis. The ChIP experiments were performed three times. Chromatin precipitated without antibody was used as the negative control, while isolated chromatin before precipitation was used as the input control. ChIP results are presented as a percentage of input DNA.

2.5. Transient expression assays

Transient expression assays were performed in *Nicotiana benthamiana* leaves as described by Chen et al. (2017). Briefly, NLS was fused with a GFP reporter gene behind the native promoter of *SAG12* or *SAG29*. The *CaMV* 35S promoter was used to drive full-length coding sequences of *RGL1*, *GAI*, *GUS*, and *WRKY75*. These constructs were then introduced into *Agrobacterium tumefaciens* (strain EHA105). Expression of GFP was determined 48 h after infiltration. Experiments were repeated three times with similar results.

2.6. Determination of YFP

Senescing leaves of *WRKY75:YFP-WRK75:3'-WRKY75* plants were detached and then YFP was observed under a confocal laser scanning microscope (Olympus).

2.7. Treatment of GA

We treated seedlings with GA₃ as described in Chen et al. (2017). *Myc-WRK75/RGL1* seedlings were harvested after treatment with 50 μM GA₃ or mock for 8 h.

2.8. Accession numbers

The following genes were detected in this work: *WRKY75* (At5G13080), *WRKY45* (At3G01970), *RGL1* (At1G66350), *GAI* (At1G14920), *SAG12* (At5G45890), *SAG29* (At5g13070), and *ACTIN2* (At3G18780).

3. Results

3.1. Expression of *WRKY75* in senescing leaves

WRKY75 was previously found to play important roles in phosphate starvation, defense responses, flowering initiation, and root hair initiation (Devaiah et al., 2007; Encinas-Villarejo et al., 2009; Zhang et al., 2018; Rishmawi et al., 2014). An *Arabidopsis* microarray database indicates that *WRKY75* is highly expressed in senescent leaves (Winter et al., 2007; Fig. S1). Our data further confirm these results (Fig. 1), and imply that *WRKY75* may function as a *SAG* that is up-regulated during senescence. *WRKY75* expression increased greatly in leaves during early senescence (ES), and showed even stronger expression at late senescence (LS) (Fig. 1A

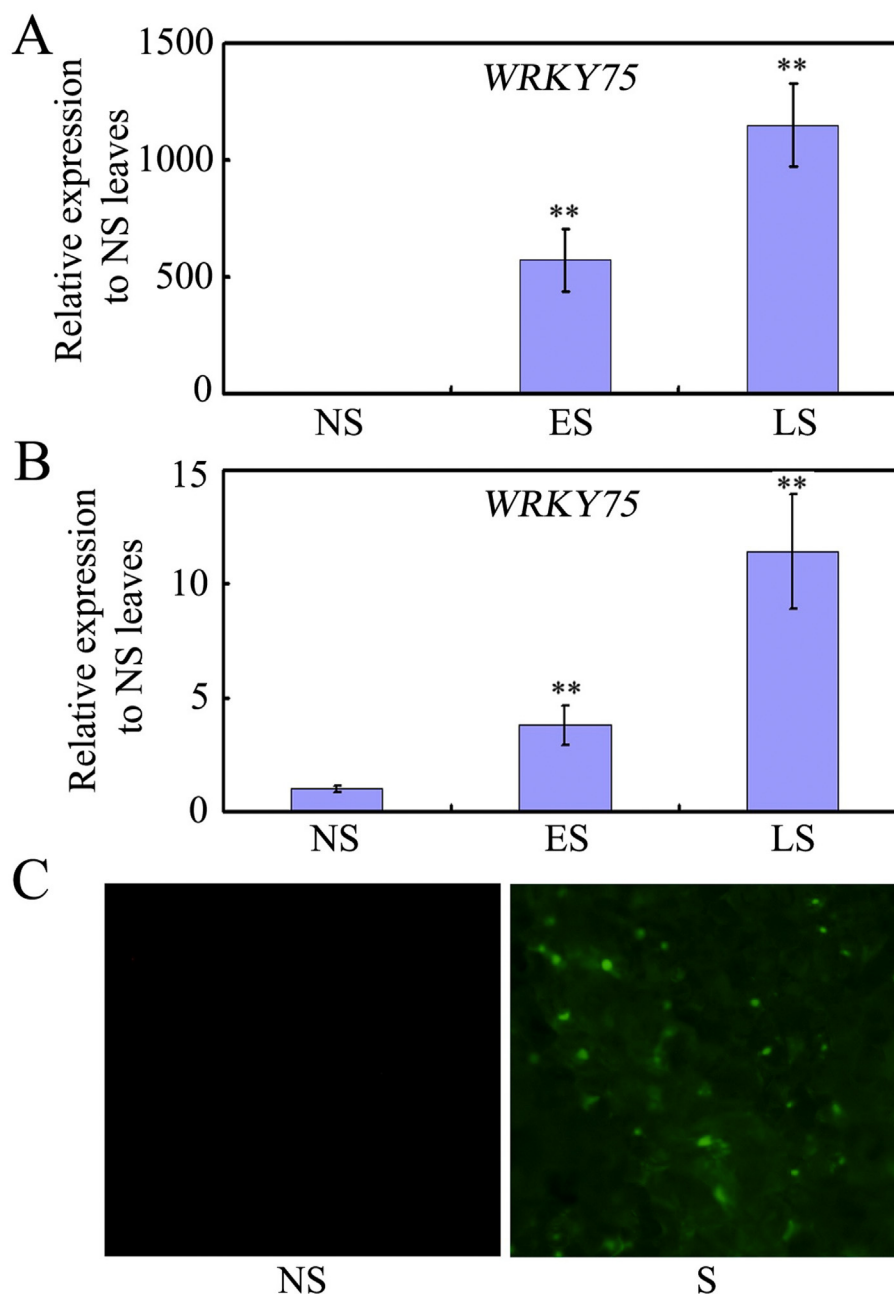


Fig. 1. Expression of WRKY75 in senescing leaves. (A) qRT-PCR analysis of WRKY75 transcript levels in wild-type leaves at different developmental stages. Transcript levels of WRKY75 in non-senescent (NS) leaves were arbitrarily set to 1. Values are means \pm SD of three independent biological replicates. ** $P < 0.01$, Student's t-test compared with NS leaves. (B) qRT-PCR analysis of WRKY75 transcript levels in different parts of a senescing wild-type leaf. Transcript levels of WRKY75 in NS leaves were arbitrarily set to 1. Values are means \pm SD of three independent biological replicates. ** $P < 0.01$, Student's t-test compared with NS parts of a senescing wild-type leaf. (C) YFP detection of WRKY75 in *wrky75-25* mutant background that harbors the *WRKY75::YFP-WRK75:3'-WRKY75*. YFP signals were observed in senescing leaves of the *WRKY75::YFP-WRK75:3'-WRKY75* transgenic plants. These experiments were performed three times with similar results.

and Fig. S2A). This same pattern of WRKY75 expression was observed across the senescence gradient from the tip to the base of leaves (Fig. 1B and Fig. S2B).

To further understand WRKY75 expression patterns, we measured WRKY75 protein accumulation during leaf senescence by visualizing yellow fluorescent protein (YFP) in leaves of *WRKY75::YFP-WRK75:3'-WRKY75* plants. No YFP signal was observed in non-senescent leaves, whereas strong YFP signals were observed in senescing leaves (Fig. 1C). These findings show that both transcript and protein levels of WRKY75 increase during leaf senescence, suggesting that WRKY75 plays a role in leaf senescence.

3.2. Altered age-triggered leaf senescence resulting from knock-down or ectopic expression of WRKY75

High expression levels of WRKY75 in senescing leaves prompted us to examine the role of WRKY75 in senescence using WRKY75 mutants (*wrky75-1* and *wrky75-25*) (Zhang et al., 2018). Analysis of the rosettes of 7-week-old plants revealed that leaf senescence was delayed in WRKY75 mutants compared to in WT plants (Fig. 2A). This finding is consistent with that of two recent reports (Guo et al., 2017; Zhang et al., 2017). Chlorophyll content in these plants indicated that chlorophyll was degraded more slowly in

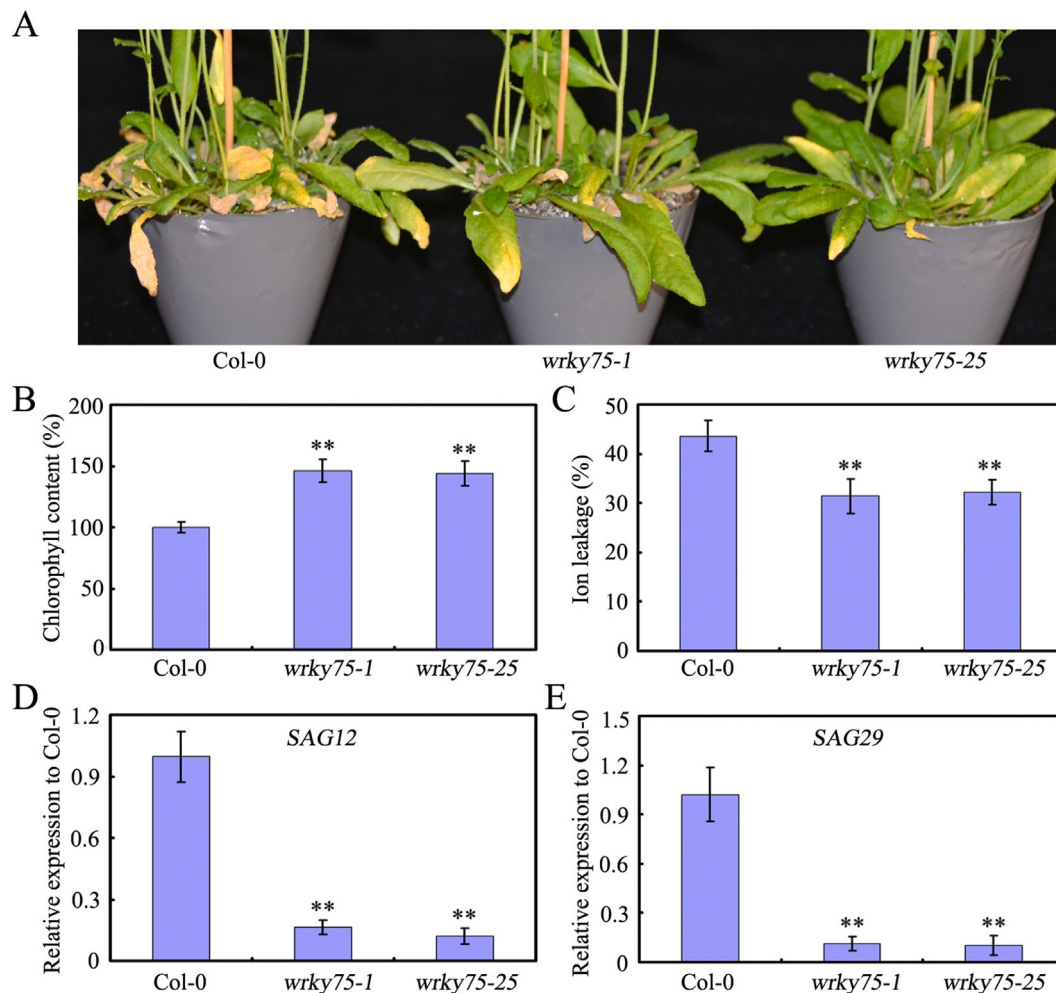


Fig. 2. WRKY75 mutation delays age-triggered leaf senescence. (A) The senescence phenotypes of 7-week-old Col-0 and *wrky75* mutant plants. (B) Relative chlorophyll content of the Col-0 and *wrky75* mutant plants. (C) Membrane ion leakage of the Col-0 and *wrky75* mutant plants. (D) and (E) qRT-PCR analysis of transcript levels of senescence marker genes in age-triggered senescing leaves of Col-0 and *wrky75* mutant plants. For B–E, values are means \pm SD of three independent biological replicates. ** $P < 0.01$, Student's *t*-test compared with Col-0. These experiments were performed three times with similar results.

WRKY75 mutants than in WT plants (Fig. 2B). Correspondingly, both ion leakage and expression of representative senescence-induced SAGs (e.g., *SAG12* and *SAG29*) were lower in *WRKY75* mutants than in WT plants (Fig. 2C–E). These findings indicate that disruption of *WRKY75* delays the senescence process of plants.

To further determine the role of *WRKY75* in leaf senescence, we again used 35S:*WRKY75* transgenic *Arabidopsis* plants (Zhang et al., 2018). Compared with WT plants, 35S:*WRKY75* transgenic plants showed early onset senescence (Fig. S3A). Consistent with this finding, transgenic plants had lower chlorophyll contents, but higher ion leakage and stronger SAG expression (Fig. S3B–E). Together, these observations suggest that over-expression of *WRKY75* promotes leaf senescence.

3.3. In vivo interactions between *WRKY75* and its target promoters

Previous studies have revealed that *WRKY* proteins function by directly binding to a *cis*-acting DNA element, namely W-box (T/CTGACC/T), present in promoters of their target genes (Eulgem et al., 2000; Ulker and Somssich, 2004). We provide evidence that *WRKY75* functions as an activator in age-triggered leaf

senescence by promoting the expression of senescence-associated genes. We found several putative W-box elements in promoters of both *SAG12* and *SAG29* (Fig. 3A), indicating that *WRKY75* may directly regulate their expression, thus promoting leaf senescence. To determine whether *WRKY75* can directly regulate *SAG12* or *SAG29* expression, chromatin immunoprecipitation (ChIP) experiments were performed using *WRKY75*:YFP-*WRKY75*:3'-*WRKY75* plants (Rishmawi et al., 2014). These experiments showed that *WRKY75* can interact with the *SAG12* and *SAG29* promoters (*pSAG12*-3, *pSAG29*-2, and *pSAG29*-3) via the W-box sequence (Fig. 3B), suggesting that *WRKY75* directly regulates their transcription.

To further determine the regulatory function of *WRKY75*, we performed transient expression assays in tobacco (*N. benthamiana*) leaves. We fused the promoters of *SAG12* and *SAG29*—both direct targets of *WRKY75*—to a reporter construct, the nuclear localization signal (NLS)-GFP gene (*SAG12*:NLS-GFP and *SAG29*:NLS-GFP) (Fig. 3C). The effector plasmids had a *GUS* or *WRKY75* gene under the control of the *CaMV* 35S promoter (35S:*GUS* and 35S:*WRKY75*) (Fig. 3C). Compared to controls, co-expression of *WRKY75* in the effector plasmids greatly increased GFP reporter expression

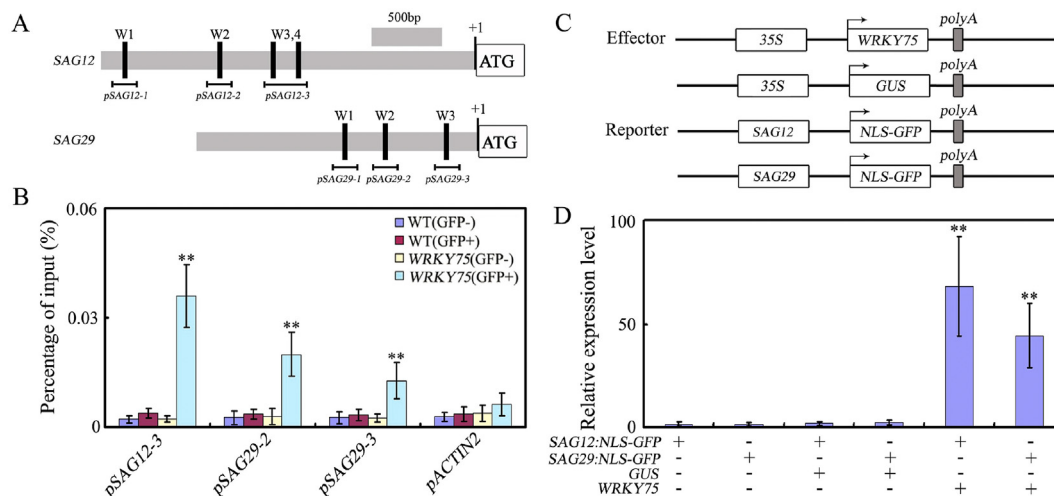


Fig. 3. WRKY75 directly regulates the expression of *SAG12* and *SAG29*. (A) The promoter structure of the *SAG12* and *SAG29* genes and fragment used in the ChIP assay. (B) WRKY75 directly binds to the promoters of *SAG12* and *SAG29*. ChIP assays were performed with chromatin prepared from *WRKY75::YFP-WRKY75::3'-WRKY75* transgenic plants, using an anti-GFP antibody (IP). ChIP results are presented as a percentage of input DNA. Values are mean \pm SD of three independent biological replicates. Asterisks indicate Student's t-test significant differences as compared to controls, ** $P < 0.01$. (C) Schematic of the *SAG12::NLS-GFP* and *SAG29::NLS-GFP* reporters and *WRKY75* and *GUS* effectors. (D) Transient expression assays showed that *WRKY75* activates the expression of *SAG12* and *SAG29* determined by qRT-PCR analysis. Values are means \pm SD of three independent biological replicates. ** $P < 0.01$, Student's t-test compared with controls. These experiments were performed three times with similar results.

(Fig. 3D), suggesting that *WRKY75* functions as an activator during age-triggered leaf senescence.

3.4. *RGL1* and *GAI* inhibit *WRKY75*-mediated transcriptional activation

We previously reported that *WRKY75* can physically interact with DELLA proteins (Zhang et al., 2018), prompting us to hypothesize that *WRKY75* may participate in senescence through the GA pathway. We also speculated that physical interactions between *WRKY75* and DELLA proteins inhibit the ability of *WRKY75* to activate transcription of target genes. To test these hypotheses, we again performed transient expression assays in tobacco (*N. benthamiana*) leaves with 35S:*WRKY75*, 35S:*RGL1*, 35S:*GAI* and 35S:*GUS* as effectors and *SAG12::NLS-GFP* as a reporter (Fig. 4A). We found that *WRKY75* expression greatly increases the expression of *GFP* driven by the *SAG12* promoter. However, co-expression of *RGL1* or *GAI* with *WRKY75* markedly reduces *GFP* expression in comparison with expression of *WRKY75* or *GUS* alone (Fig. 4B). These results demonstrate that both *GAI* and *RGL1* can repress *WRKY75*-mediated transcriptional activation.

To determine whether the ability of *WRKY75* to bind to target genes is affected by interactions with DELLA proteins, we performed ChIP assays using *Myc-WRKY75/RGL1* plants. When we treated plants with GA, the binding efficiency of *WRKY75* to the *SAG12* promoter increased greatly, implying that *RGL1* may reduce the binding ability of *WRKY75* to its target genes *in vivo*.

3.5. Repression of age-triggered leaf senescence by *GAI* gain-of-function or *RGL1* overexpression

Because several DELLAs physically associate with *WRKY75* and modulate its transcriptional ability, we then wondered whether *GAI* gain-of-function or *RGL1* overexpression could rescue the earlier senescence phenotype caused by *WRKY75* overexpression. Then both *Myc-WRKY75/gai-1* and *Myc-WRKY75/RGL1* plants were used (Zhang et al., 2018). Senescence occurred earlier in *Myc-WRKY75/gai-1* plants than in *gai-1* plants (Fig. 5A). Furthermore, *Myc-WRKY75/gai-1* plants had lower chlorophyll content and higher

levels of *SAG* expression than *gai-1* plants (Fig. 5B–E). *Myc-WRKY75/RGL1* plants showed similar phenotypes (Fig. S4). These findings indicate that *GAI* gain-of-function or *RGL1* overexpression may at least partially delay the early senescence phenotype caused by *WRKY75* overexpression.

3.6. Role of *WRKY75* in GA-mediated leaf senescence

To further determine the role of *WRKY75* in GA-mediated leaf senescence, we compared the GA response in *wrky75-1*, *wrky75-25*, WT, 35S:*WRKY75-L3*, and 35S:*WRKY75-L6* plants. We previously demonstrated that GA induces slight increases in *WRKY75* gene expression (Zhang et al., 2018). Here, we confirm that *WRKY75* expression is promoted in *della* but reduced in *gai1* or *gid1a/gid1b/gid1c* plants (Fig. 6A). Furthermore, when we treated plants with GA, chlorophyll content and *SAG12* expression indicated that GA-mediated leaf senescence was delayed in *wrky75* mutants but accelerated in *WRKY75* overexpressing plants. Combined, these results suggest that *WRKY75* participates in the modulation of leaf senescence through the GA pathway.

3.7. *WRKY75* positively regulates *WRKY45* expression during leaf senescence

Our previous study revealed that *WRKY45* functions as a novel activator of the senescence transcriptional network (Chen et al., 2017). Thus, we wondered whether *WRKY75* would affect the expression of *WRKY45* during leaf senescence. As shown in Fig. 7A, *WRKY45* expression was inhibited in the *wrky75* mutants but was activated in *WRKY75* over-expressing plants (Fig. 7A). To test whether *WRKY45* is a direct target of *WRKY75*, ChIP assays were performed using *WRKY75::YFP-WRKY75::3'-WRKY75* plants. *WRKY75* can directly bind to the *WRKY45* promoter through the W-box cis-element (*pWRKY45-2*) (Fig. 7B and C). These results demonstrate that *WRKY75* directly regulates *WRKY45* expression during leaf senescence, and also imply that there may exist extensive cross-regulation among *WRKY* members during leaf senescence, which contributes to the facilitation of senescence-associated transcriptional reprogramming.

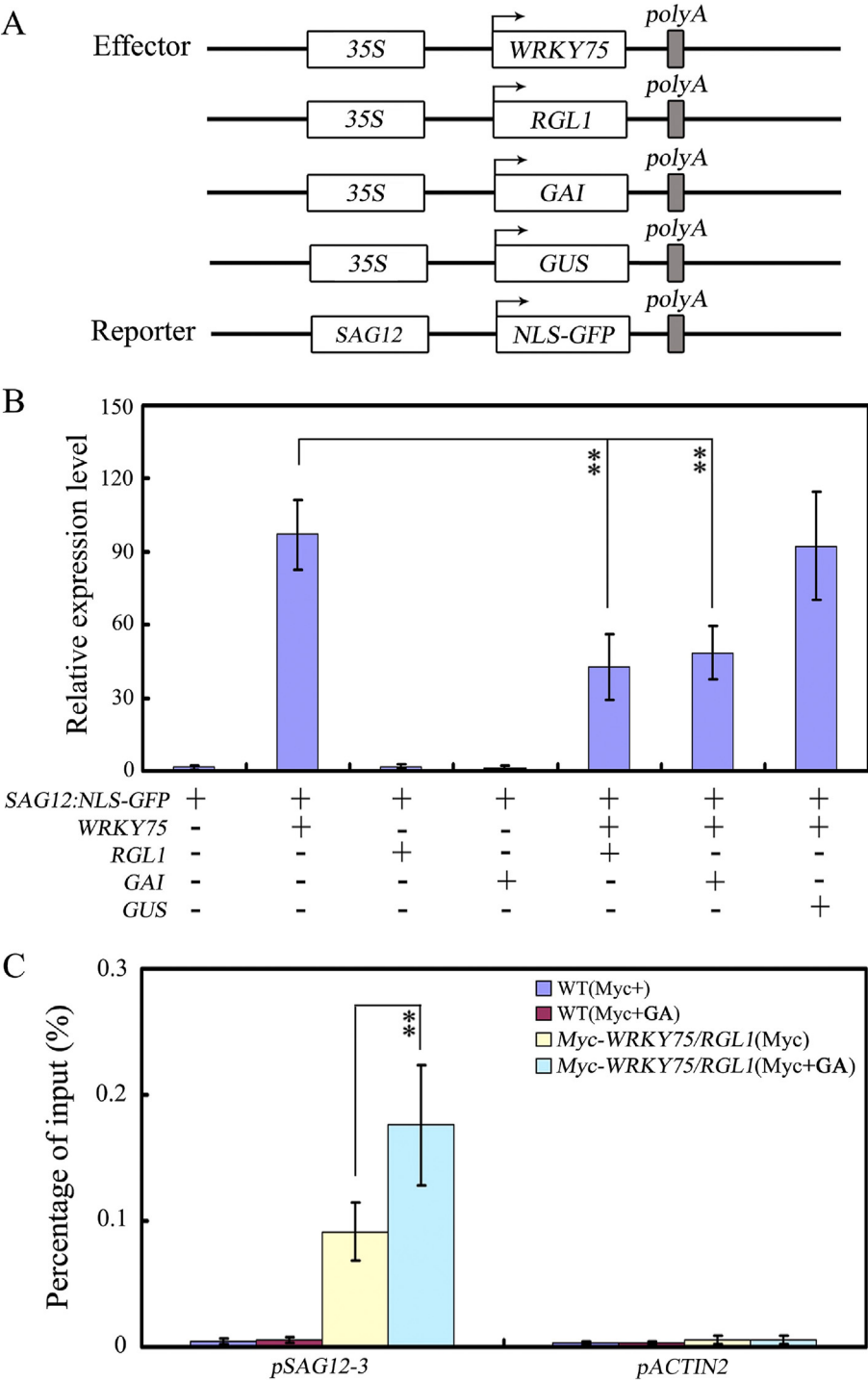


Fig. 4. GAI and RGL1 repress WRKY75 activation ability. (A) Schematic of the *SAG12:NLS-GFP* reporter and *WRKY75*, *RGL1*, *GAI* and *GUS* effectors. (B) Transient expression assays showed that *RGL1* and *GAI* repress transcriptional activation of *WRKY75* determined by qRT-PCR analysis. Values are mean \pm SD of three independent biological replicates. Asterisks indicate Student's t-test significant differences as compared to controls, $**P < 0.01$. (C) *RGL1* interferes the binding of *WRKY75* to its target genes (shown in Fig. 4A). ChIP assays were performed with chromatin prepared from *Myc-WRKY75/RGL1* plants, using an anti-Myc antibody (IP). ChIP results are presented as a percentage of input DNA. Values are mean \pm SD of three independent biological replicates. Asterisks indicate Student's t-test significant differences as compared to controls, $**P < 0.01$. These experiments were performed three times with similar results.

4. Discussion

Plant senescence is tightly controlled by the temporal coordinated expression of numerous SAGs. Over the past decades, our understanding of plant senescence has improved immensely. Microarray expression profiling has identified WRKY factors as the

second largest transcription factor group in the senescent transcriptome (Guo et al., 2004), suggesting that these genes play central roles in modulating transcriptional changes during senescence. However, the biological function of WRKY factors during leaf senescence remains largely unknown. Recently, several *Arabidopsis* WRKY proteins have been shown to play important roles during

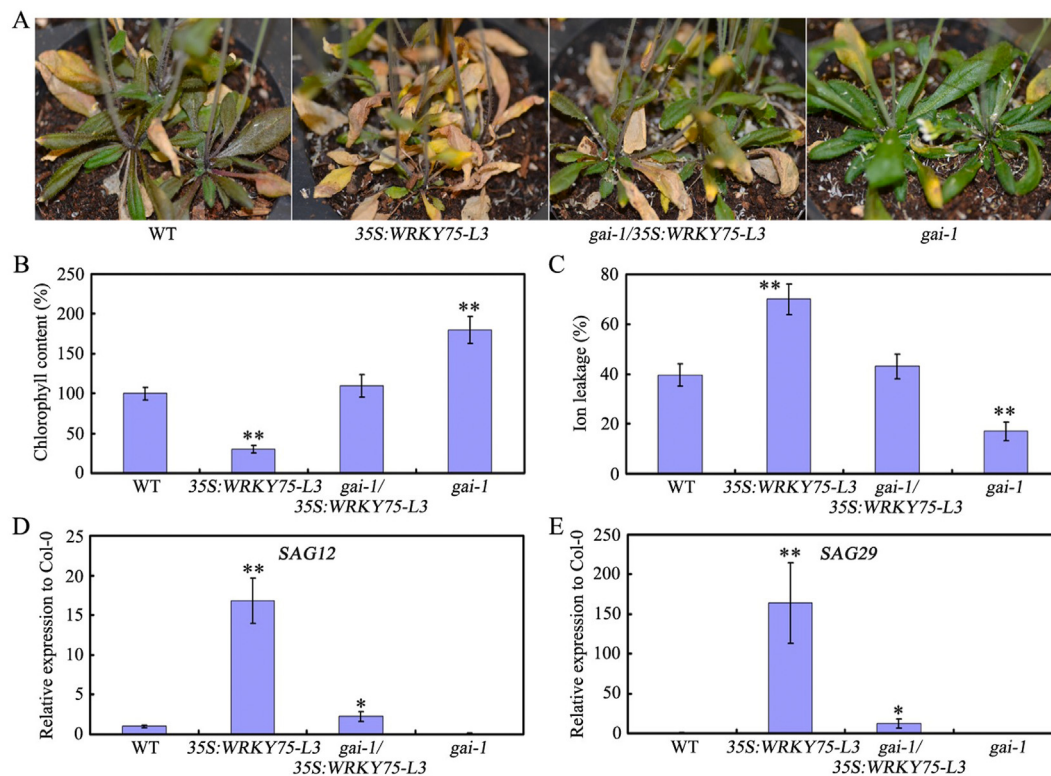


Fig. 5. *GAI* gain-of-function partially rescues the early-senescence phenotype of *WRKY75* overexpressing plants. (A) The senescence phenotypes of the indicated genotypes. (B) Relative chlorophyll content of the indicated genotypes. (C) Membrane ion leakage of the indicated genotypes. (D) and (E) qRT-PCR analysis of transcript levels of senescence marker genes in the indicated genotypes. For B–D, values are means \pm SD of three independent biological replicates. * $P < 0.05$, ** $P < 0.01$, Student's t-test compared with Col-0. These experiments were performed three times with similar results.

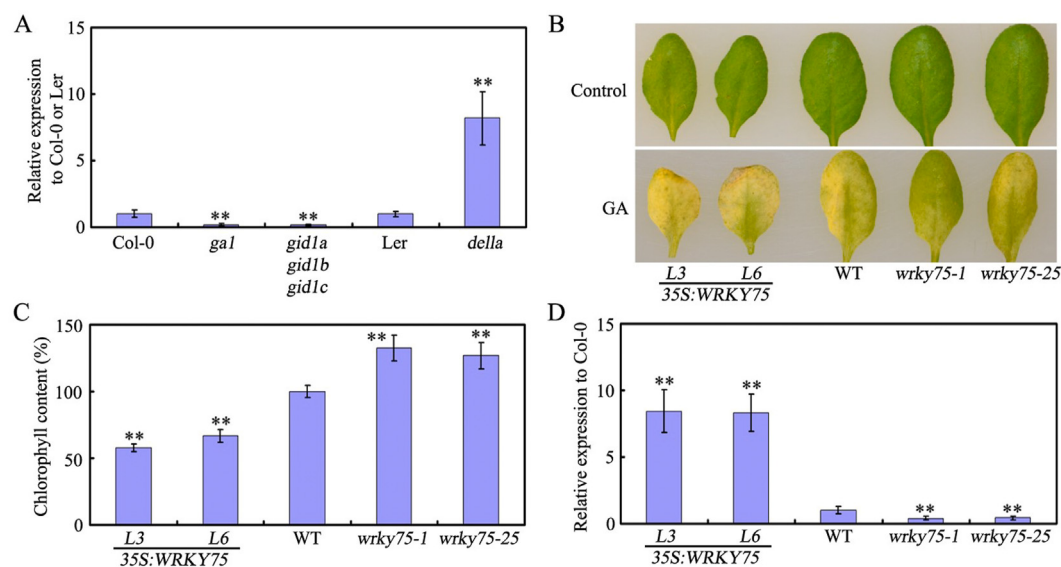


Fig. 6. Effects of GA on *wrky75* and *WRKY75*-overexpressing plants. (A) qRT-PCR analysis of *WRKY75* transcript levels in *gai*, *gid1a*, *gid1b*, *gid1c*, and *della* mutant plants. (B) GA response of the indicated genotypes. (C) Relative chlorophyll content of the indicated genotypes as shown in (B). (D) Relative expression of *SAG12* in the indicated genotypes as treated in (B). For A, C and D, values are means \pm SD of three independent biological replicates. ** $P < 0.01$, Student's t-test compared with Col-0 or Ler. These experiments were performed three times with similar results.

leaf senescence, including *WRKY6*, *WRKY22*, *WRKY45*, *WRKY53*, *WRKY54*, *WRKY57*, and *WRKY70* (Robatzek and Somssich 2002; Zhou et al., 2011; Chen et al., 2017; Miao and Zentgraf, 2007; Jiang et al., 2014; Ulker et al., 2007). Here, we provide further evidence

that *WRKY75* may function as a new component that positively regulates GA-mediated leaf senescence.

We found that *WRKY75* is strongly expressed in senescing leaves at both mRNA and protein levels, when compared with young

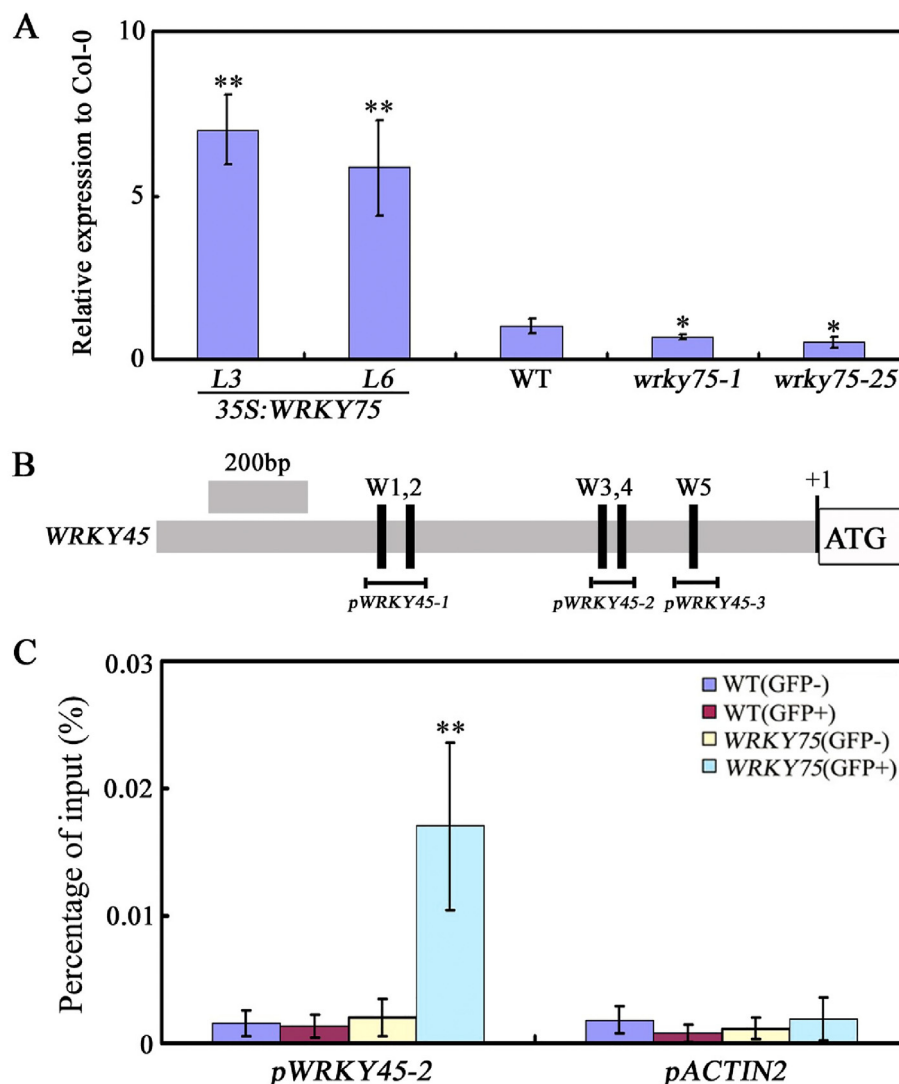


Fig. 7. WRKY75 positively regulates WRKY45 expression during leaf senescence. (A) qRT-PCR analysis of transcript levels of *WRKY45* in the indicated genotypes. Values are means \pm SD of three independent biological replicates. * $P < 0.05$, ** $P < 0.01$, Student's *t*-test compared with Col-0. (B) The promoter structure of *WRKY45* gene and fragment used in the ChIP assay. (C) *WRKY75* directly binds to the promoter of *WRKY45*. ChIP assays were performed with chromatin prepared from *WRKY75::YFP-WRKY75::3'-WRKY75* transgenic plants, using an anti-GFP antibody (IP). ChIP results are presented as a percentage of input DNA. Values are mean \pm SD of three independent biological replicates. Asterisks indicate Student's *t*-test significant differences as compared to controls, ** $P < 0.01$. These experiments were performed three times with similar results.

leaves (Fig. 1), implying that *WRKY75* functions as a SAG to modulate the senescence. Phenotypic analysis using *wrky75* T-DNA mutants and *WRKY75*-overexpressing plants demonstrated that *WRKY75* acts as a positive regulator during age-triggered leaf senescence (Fig. 2 and Fig. S3). Further investigation revealed that *WRKY75* participates in senescence through the direct activation of several SAGs, including *SAG12* and *SAG29* (Fig. 3). A recent study also found that *WRKY75* directly promotes *SA INDUCTION-DEFICIENT 2* (*SID2*) expression, but suppresses *CATALASE 2* (*CAT2*) transcription during leaf senescence (Guo et al., 2017). Therefore, *WRKY75* appears to function as both an activator and repressor to finely modulate leaf senescence. Similarly, our previous study demonstrated that *WRKY8* controls plant defense responses to viral infection by positively regulating *ABI4* while negatively regulating *ACS6* (Chen et al., 2013). Taken together, these findings indicate that *WRKY* factors act as both positive and negative regulators that fine-tune signaling and transcriptional networks involved in mediating plant growth and stress responses.

GA, as an essential hormone, modulates diverse aspects of plant development. Recent studies have demonstrated that GA signaling may have a positive effect on leaf senescence (Chen et al., 2014, 2017; bib_Chen_et_al_2014; bib_Chen_et_al_2017); however, the specific mechanisms by which GA affects this progress have yet to be determined. Numerous studies have suggested that *WRKY* proteins function as key regulators during leaf senescence. We previously found that *WRKY45* regulates age-triggered leaf senescence through a physiological interaction with the DELLA protein RGL1. Other senescence-associated *WRKY* transcription factors may also be involved in GA-mediated age-dependent leaf senescence. Here, we provide evidence that *WRKY75* may positively regulate leaf senescence through the GA pathway.

DELLAs function as crucial transcriptional repressors of the GA pathway, and have been shown to modulate GA-mediated response through interactions with downstream transcription factors. We previously found that GA affects floral initiation via interactions between DELLA and CO or *WRKY* proteins, including *WRKY12*,

WRKY13 and WRKY75 (Li et al., 2016; Wang et al., 2016; Zhang et al., 2018). We also found that GA modulates aged-triggered leaf senescence through a physiological interaction with WRKY45 (Chen et al., 2017). The expression of *WRKY75* is markedly elevated at both mRNA and protein levels in senescing leaves, and the physiological interaction between WRKY75 and DELLA proteins may interfere with WRKY75-mediated transcriptional activation (Fig. 4). Furthermore, *RGL1* can disrupt the association of WRKY75 and its target genes *in vivo* (Fig. 4), and *GAI* gain-of-function and *RGL1* overexpression can partially delay the precocious senescence phenotype caused by *WRKY75* overexpression (Figs. 5 and S4). Together, these data suggest that WRKY75 may function downstream of DELLAs to modulate leaf senescence.

In *Arabidopsis*, leaf senescence is tightly associated with flowering, and a delay in flowering always delays senescence. Previous studies have shown that DELLA proteins retard both flowering and senescence in *Arabidopsis*, and that GA signaling positively regulates flowering and senescence by degrading DELLA proteins (Wilson et al., 1992; Achard et al., 2007; Chen et al., 2017). The elimination of DELLA inhibition is thought to accelerate the onset of the reproductive stage, subsequently promoting leaf senescence. Our findings support these results. Specifically, we found that the life cycle of *della* mutants was shortened, whereas the lifespans of the GA biosynthesis mutant *gai1* and the *gid1a/gid1b/gid1c* triple mutants were prolonged. Combined with our previous findings (Zhang et al., 2018), we have demonstrated that flowering and leaf senescence are delayed in *WRKY75* knockout plants and accelerated in plants overexpressing *WRKY75*. We also found that WRKY75 can regulate flowering and leaf senescence through the GA pathway, indicating that WRKY75 may act as a common component of the GA-mediated regulatory network for both flowering and senescence. Identification of regulatory factors like WRKY75 improves our understanding of plant flowering and senescence processes.

5. Conclusions

In this study, we provide evidence that WRKY75 acts as a positive regulator in age-triggered leaf senescence. Our findings suggest that WRKY75 may modulate the onset and progression of leaf senescence within a senescence-associated transcriptional network by integrating both age and GA signaling. These findings increase our understanding of senescence-associated signaling and transcriptional reprogramming controlled by WRKY proteins.

Author contributions

LGC conceived the project and designed the experiments. HYZ, LPZ, SGW and YLC performed the experiments. LGC wrote the article. All authors interpreted and discussed the data.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pld.2020.10.002>.

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