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Removal of DELLA repression promotes leaf senescence in *Arabidopsis*

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ABSTRACT

Leaf senescence is an integrated response of leaf cells to developmental age and various internal and environmental signals. However, the role of gibberellins (GA) in leaf senescence is not clear. In the current study, we investigated the effect of DELLA on leaf senescence. Compared with the wild type (WT), leaf senescence occurred earlier in the mutant *ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1* (abbreviated as *Q-DELLA/ga1-3*) whose DELLA repression was removed, whereas leaf senescence was retarded in the mutant *ga1-3* whose GA biosynthesis was blocked and whose DELLA proteins accumulated abnormally. During leaf senescence, *SAG12* and *SAG29* were upregulated in *Q-DELLA/ga1-3* and downregulated in *ga1-3* plants. The *Q-DELLA/ga1-3* senescent leaves contained more sugar but less chlorophyll and fatty acids (FAs) than those of *ga1-3* and WT. Both absolute and relative contents of C18:3 in *Q-DELLA/ga1-3* senescent leaves were lower compared with those of the WT and *ga1-3* leaves. The genes regulating FA β-oxidation in *Q-DELLA/ga1-3*, such as *KAT2*, *LACS6*, *LACS7*, *ACX1*, *ACX2* and *MAP2*, were significantly upregulated. The removal of DELLA repression highly upregulated certain genes on various hormone pathways, suggesting that GA signaling acts upstream of the jasmonic acid, salicylic acid, and ethylene pathways in regulating leaf senescence.

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1. Introduction

Leaf senescence, which is a positive and degenerative process, is the final stage of leaf development [1,2]. It can be characterized by cell structure collapse, metabolism, and expression of senescence-associated genes (*SAGs*) [3]. The collapse of the cell structure begins with the breakdown of chloroplast and the catabolism of chlorophyll. Metabolically, carbon assimilation is substituted with catabolism of macromolecules, such as lipids, proteins and nucleic acids. During senescence, the cellular compounds are converted by the catabolic activity into exportable nutrients which are transported to filling seeds or other growing organs. Thus, studies on leaf senescence will provide us better understanding of its fundamental biological process and implications for improvement of agricultural production. To date, hundreds of *SAGs* have been identified in *Arabidopsis* [4–7]. Different *SAGs* exhibit varying temporal expression patterns during the natural leaf senescence process [8]. *SAG12* and *SAG29* are senescence-specific genes in *Arabidopsis* that have been widely used as molecular markers for leaf senescence [9–13].

Leaf senescence is an integrated response of leaf cells to developmental age and various internal signals from various phytohormones [3], as well as to environmental signals that include both biotic and abiotic stresses. Biotic stresses can result from shades of other plants and pathogenic infections, whereas abiotic stresses arise from nutrient limitation, drought, extreme temperatures, oxidative stress by UV-B irradiation, and ozone, among others. Abscisic acid (ABA), jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) promote senescence, whereas auxin and cytokinins retard senescence [8].

GAs are a large group of tetracyclic diterpenoids that are essential for numerous aspects of plant growth and development, such as seed germination and seed fatty acid metabolism, stem elongation, leaf expansion, trichome development, flower and fruit development and seed fatty acid decomposition [14–18]. The GA signal is received and transduced by the GID1 GA receptor/DELLA repressor pathway [19]. In *Arabidopsis*, the DELLA proteins, namely, GA INSENSITIVE (GAI), REPRESSOR OF GA1-3 (RGA), RGA-LIKE1 (RGL1), RGA-LIKE2 (RGL2), and RGA-LIKE3 (RGL3), constitute the nuclear negative regulators of the GA signaling pathway [20–22]. These five DELLA proteins have unique and overlapping functions [23,24]. Genetic studies indicate that GAI and RGA function in stem elongation as GA-sensitive repressors [20,21]. The loss of function of GAI and RGA completely reverses the dwarf phenotype, and the combination of *RGA*, *RGL1*, and *RGL2* loss-of-function mutations lead to recovery of petal, stamen filament, and anther development in *ga1-3* mutants [17,22]. The RGL2 gene encodes the predominant repressor of seed germination in *Arabidopsis*, and its function is enhanced by the other DELLA proteins GAI, RGA, and RGL1 [25,26].

To date, the role of GA signaling in leaf senescence remains unclear. Limited information is available regarding the role of DELLAs, the negative regulators in the GA signaling pathway, on the process of natural leaf senescence. Therefore, in the present study,

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we investigated the process of leaf senescence in plants treated with exogenous GA_3 and in the two mutants *ga1-3*, which has a defective GA biosynthesis, and *Q-DELLA/ga1-3* (*ga1-3 gai-t6 rga-t2 rgl1-1 rgl2-1*), whose DELLA repression has been removed. We also analyzed the molecular and physiological indicators accompanying the senescence process.

2. Materials and methods

2.1. Plant material and growth condition

Arabidopsis thaliana accession 'Landsberg *erecta* (L*er*)' was used as wild-type control (WT), from which GA-deficient mutant *ga1-3* and *Q-DELLA/ga1-3* mutant originated. The seeds of these lines were pre-chilled at 4 °C for 2–4 d and surface sterilized with 6% (w/v) NaClO solution for 6–8 min. Subsequently, the seeds were washed 5–7 times with ddH_2O. WT and *Q-DELLA/ga1-3* mutant were sown onto solid Murashige and Skoog (MS) stock medium, whereas *ga1-3* was placed in an MS stock medium with 200 μM GA_3. After about 2 weeks, the healthy seedlings were transferred into autoclaved soil in a flowerpot 7 cm in diameter. The growing conditions for *Arabidopsis* were based on our previous descriptions [27].

The plants treated with GA_3 solution (500 μM) were of Col-0 ecotype. After the seedlings were transplanted into the soil, eight individual plants in one of three randomly arranged blocks were treated with GA_3 (500 μM) every other day until the formation of the first silique.

The rosette leaves of *Arabidopsis* were numbered from the bottom [28]. The rosette leaves used in this study were harvested and handled as follows [29]. All *Arabidopsis* plants used in a given experiment were taken from a single population growing at the same time and condition. Leaves 5–6 were selected from 12 to 15 plants. Approximately 30 leaves were harvested and pooled. Pooled leaves were dried at 65 °C for 3 d prior to use for leaf weight, total sugar, and FA analysis.

2.2. Chlorophyll determination

The chlorophyll content in the different leaf samples was determined as described [29,30]. Fresh pooled leaves were weighed and ground in liquid nitrogen using mortar and pestle. The pulverized leaf tissue was added to 80% acetone (0.1 mL/mg leaf tissue), vortexed, and incubated for 30 min in the dark at room temperature. The solution was vortexed and centrifuged (3000 × g, 10 min), and the absorbance of the supernatant was measured at 663 and 645 nm to determine the chlorophyll content based on fresh weight.

2.3. Measurement of total soluble sugar

A total of 20 mg of the sample was extracted overnight in 25 mL of 80% ethanol (v/v); the resulting supernatant was analyzed for total soluble sugars as previously described [31,32].

2.4. Fatty acid analysis

About 30 mg of the pooled leaves were used for FA analysis. Extraction and analysis of FA were carried out as previously described [27].

2.5. Real-time quantitative reverse transcription PCR (qRT-PCR)

Leaf number 6 was selected from 10 plants, and about 8 leaves in total were harvested for leaf RNA extraction. The RNA samples were isolated using the Invisorb Spin Plant RNA Mini Kit (Invitek, Berlin, Germany) following the manufacturer's instructions. The RNA samples were treated with RNase-free DNAse I (New England Biolabs, Beijing, China) to remove any trace genomic DNA. For the qRT-PCR templates, first-strand cDNA was synthesized in a 20 μL reaction solution containing approximately 1 μg total RNA using PrimerScript^{TM} reverse transcriptase (Takara, Otsu, Shiga, Japan) and oligo (dT) 12–18 as primer. *ACTIN7* (At5g09810) was used as internal control in the qRT-PCR. All primer pairs used in the qRT-PCR analyses are listed in Table 1. Real-time amplification reactions were performed using the SYBR Green kit (Takara, Japan) and iCycler iQ thermocycler (Bio-Rad, Shanghai, China) according to the manufacturer's instructions. The baseline and threshold cycles (CT value) were automatically determined using Bio-Rad iQ Software (version 3.0, Hercules, CA, USA). The relative amounts of expressed RNA were calculated using a method described previously [33]. The final values were the means of two replicates carried out on cDNA dilutions obtained from two independent RNA extractions.

Table 1
Primers used for qRT-PCR analysis.

Gene symbol (locus of *Arabidopsis* genome)	Primer sequences (5′–3′)	PCR products (bp)
ACTIN7 (*At5g09810*)	GCCCCTGAGGAGCACCCAGTT CCGGTTGTACGACCACTGGCA	161
MFP2 (*AT3G06860*)	GGCGATGGCGTTGCCGTCATA GGCAGCGACAGACGGTTTCCT	282
KAT2 (*AT2G33150*)	AGGGTCAGTCAACCCAGCGGT GCAGCTGCCTTTCTGTGCGA	153
ACX1 (*AT4G16760*)	AGGCCGCTATTGCTCATTGCCA GCGTCCACAAGTGCAACCGC	256
ACX2 (*AT5G65110*)	CCGTCGCCGTCGTTACCGTT GGCGTCTGCAAATCGGGTCG	164
LACS6 (*AT3G05970*)	ACGCCTGGTGGTGGTTGTTGG TGGGTGTCCCAGTTGTTCCGC	188
LACS7 (*AT5G27600*)	GCTTGGTGGACGGGTTCGGT AGGGGAACCGACATGGCCAGA	181
OPR3 (*AT2G06050*)	ACATGACGGCGGCACAAGGG CGCCGCGTTTGGTACTCCGT	137
RNS1 (*AT2G02990*)	TGGAGCTCTAACCAAAGCCGGG ACCGGAACCAGACCGGTCAAC	178
COS1 (*AT2G44050*)	TAGCTTCGCCGCCGTGTCTC CATGGCGAACAGCAGCCGTC	213
EAT1 (*AT1G05010*)	GGAGCTACTGGATCTGCTGTGCG CGTCGGTGTGGGCTCGGAGA	161
EIN3 (*AT3G20770*)	CCTCCGTGGTGGCCTAATGGGA ACCGCAGTCAAAACGCCGACT	131
NIT2 (*AT3G44300*)	AGCACCCGAAGAAACCGGTCA ACATGAACTGACGAATCACAACCGA	89
OSM34 (*AT4G11650*)	CTGGATCCCACCAGCTCCCGA ATGATCACGTCACACGTACGCA	108
EDS1 (*AT3G48090*)	TCAGTCACGCACTTGGGAGAGA CGCCTCTGCGCTTCCAGTCA	263
EDS16 (*AT1G74710*)	TCCTCCGGCGTCGTTCGGTT GGGACGACCAACGTCACTGCG	126
SAG12 (*AT5G45890*)	ACTGGTTTCAAAGGTGTCTCGGCAT ACGCCCAACAACATCCGCAGC	169
SAG29 (*AT5G13170*)	AGGTGTGTCGGAAGTGCACCC TCTTCCTTTGGCCGGCCCTG	246

3. Results

3.1. Comparison of leaf senescence between Q-DELLA/ga1-3, ga1-3 and WT

The leaf senescence processes of *Q-DELLA/ga1-3* and *ga1-3* plants were compared with that of WT (L*er*). The growing

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