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LSD 2.0: an update of the leaf senescence database

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ABSTRACT

This manuscript describes an update of the leaf senescence database (LSD) previously featured in the 2011 NAR Database Issue. LSD provides comprehensive information concerning senescence-associated genes (SAGs) and their corresponding mutants. We have made extensive annotations for these SAGs through both manual and computational approaches. Recently, we updated LSD to a new version LSD 2.0 (http://www.eplantsenescence.org/), which contains 5356 genes and 322 mutants from 44 species, an extension from the previous version containing 1145 genes and 154 mutants from 21 species. In the current version, we also included several new features: (i) Primer sequences retrieved based on experimental evidence or designed for high-throughput analysis were added; (ii) More than 100 images of *Arabidopsis* SAG mutants were added; (iii) *Arabidopsis* seed information obtained from The Arabidopsis Information Resource (TAIR) was integrated; (iv) Subcellular localization information of SAGs in *Arabidopsis* mined from literature or generated from the SUBA3 program was presented; (v) Quantitative Trait Loci information was added with links to the original database and (vi) New options such as primer and miRNA search for database query were implemented. The updated database will be a valuable and informative resource for basic research of leaf senescence and for the manipulation of traits of agronomically important plants.

INTRODUCTION

In plants, senescence occurs at the final stage of leaf development and precedes cell death (1,2). Leaf senescence is a highly coordinated process regulated by a large number of senescence-associated genes (*SAG*s), which are upregulated during senescence (1). Many advances in the understanding of the molecular mechanisms of leaf senescence have been achieved through the identification and characterization of hundreds of *SAG*s and their corresponding mutants (1,3,4). To facilitate systematical research and comparative studies of leaf senescence, we have developed a database of leaf senescence database (LSD) to collect *SAG*s and their mutants and phenotypes, as well as reference citations (4,5). These *SAG*s were retrieved based on genetic, genomic, proteomic or physiological evidence, and were classified into different categories according to their functions in leaf senescence. The first version of LSD was released in August 2010 and publicly accessible for the plant research community (5).

In the past 3 years, many advances had been made in leaf senescence studies. As a result, a large amount of new genes have been newly identified as functional SAGs. For example, SAG113, a gene that encodes protein phosphatase 2C, has been demonstrated to be a positive regulator of *Arabidopsis* leaf senescence (6). Transgenic plants overexpressing *SAG113* exhibited an early senescence phenotype, whereas mutation in *SAG113* delayed developmental senescence process (6). UGT76B1, a UDP-glycosyltransferase encoding gene, was reported to be a negative regulator of leaf senescence in *Arabidopsis* (7). Overexpression of *UGT76B1* delayed leaf senescence, while *ugt76b1* knockout lines showed a clearly early

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senescence phenotype (7). Moreover, genome-scale analyses have been widely used in leaf senescence studies, and thousands of *SAG*s have been identified and categorized by using the ATH1 Arabidopsis GeneChip microarray (8), providing a systematic view of transcriptional regulation in leaf senescence (9).

To cover the above-mentioned advances and extending the functionality of the previous LSD (5), we updated our database to a new version LSD 2.0. In this updated version, we made tremendous modifications and improvements to the original database and added several new features. First, we manually updated our collection of SAG genes and senescence-associated mutants based on recent papers published in the latest 3 years. The updated version contains 5356 genes and 322 mutants, increased by ~4.5-fold and ~2.8-fold, respectively (Table 1). Second, new features were included in the current version, including primer sequences, subcellular localization information, Arabidopsis seed information, images of *Arabidopsis* mutants and Quantitative Trait Loci (QTL) information.

Table 1. Comparisons of gene number between the two versions of the database

Species	Common name	LSD 1.0	LSD 2.0
Arabidopsis thaliana	Thale Cress	949	3744
Oryza sativa	Rice	104	132
Medicago truncatula	Barrel Clover	31	31
Brassica napus	Rapeseed	15	8
Lycopersicon esculentum	Tomato	8	23
Nicotiana tabacum	Tobacco	5	9
Brassica oleracea	Broccoli	4	9
Glycine max	Soybean	4	12
Pisum sativum	Pea	4	6
Sorghum bicolor	Sorghum	4	26
Hordeum vulgare	Barley	3	14
Solanum tuberosum	Potato	3	3
Zea mays	Maize	3	94
Astragalus sinicus	Chinese Milk Vetch	1	1
Chenopodium rubrum	Red goosefoot	1	1
Festuca pratensis Huds.	Fescue	1	1
Ipomoea nil	Japanese morning glory	1	1
Medicago sativa	Alfalfa	1	2
Rosa hybrida	Rose	1	1
Triticum aestivum	Wheat	1	256
Triticum turgidum	Wheat	1	65
Amaranthus hypochondriacus	Grain Amaranths	0	1
Arabidopsis lyrata	Thale Cress	0	2
Brassica campestris	Chinese cabbage	0	2
Brassica rapa subsp. Rapa	Turnip	0	1
Brassica rapa var.parachinensis	Choy sum	0	5
Camellia sinensis	Tea	0	1
Capsicum annuum	Pepper	0	1
Crocus sativus	Saffron	0	1
Cucumis melo	Muskmelon	0	1
Daucus carota	Carrot	0	1
Dianthus caryophyllus	Carnation	0	1
Festuca arundinacea	Tall fescue	0	1
Fragaria x ananassa	Strawberry	0	1
Ipomoea batatas	Sweet potato	0	4
Lolium perenne	perennial ryegrass	0	4
Mangifera indica	Mango	0	1
Musa acuminata	Banana	0	882
Neosinocalamus affinis	Rendle	0	1
Nicotiana attenuata	Solanaceae	0	1
Petunia hybrida	Petunia	0	1
Platycodon grandiflorum	Balloon flower	0	1
Spinacia oleracea	Spinach	0	2
Vigna unguiculata	cowpea	0	1
Total	44	1145	5356

NEW FEATURES

Browsing and searching

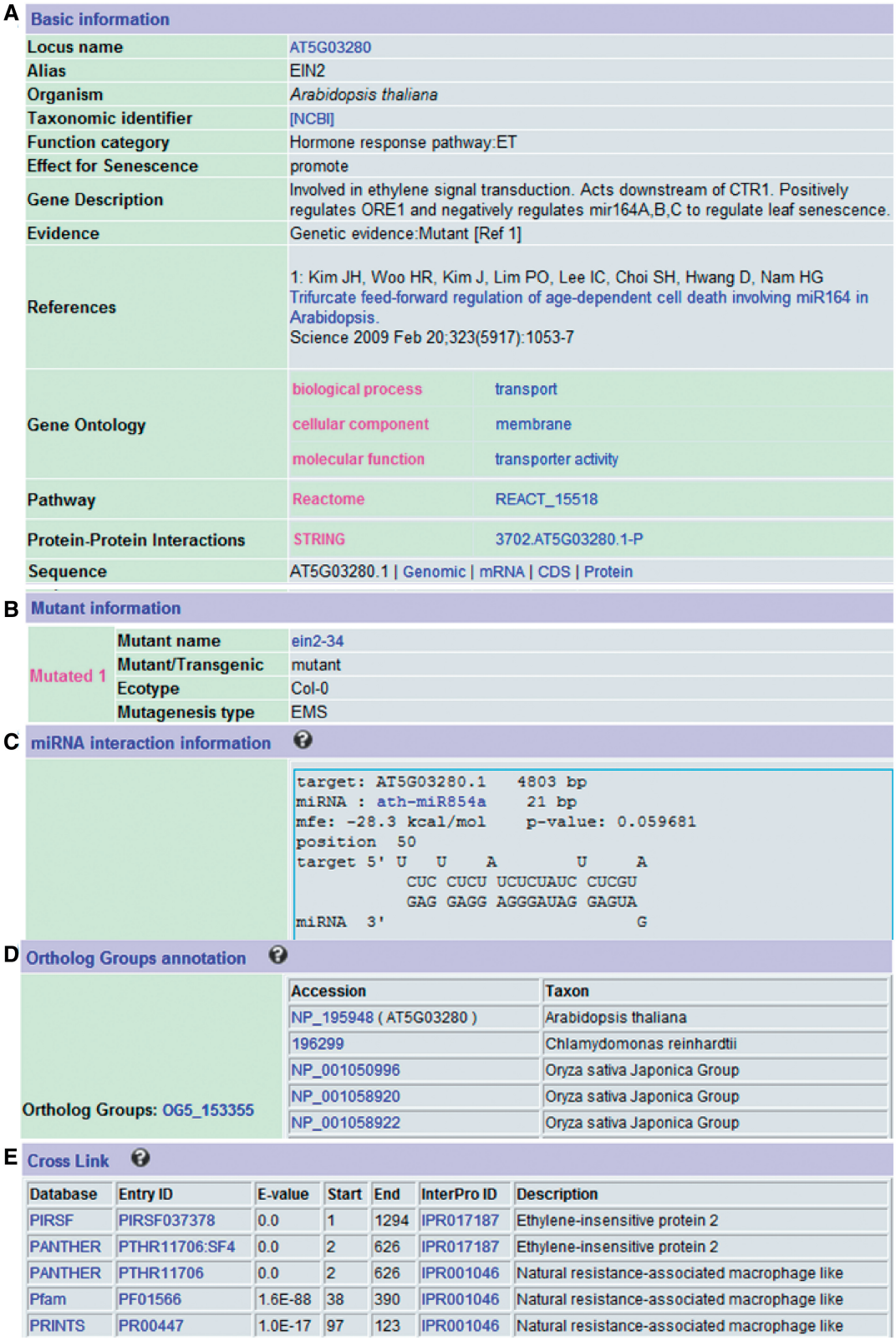
LSD 2.0 enables users to retrieve and analyze *SAG*s through the Browse or Search page. Users may browse the entries by clicking the buttons of Species, Mutants, Phenotypes, QTLs and Arabidopsis Seed at the main page. A tree-like structure was designed for both species and phenotypes, and tables were also created for species, mutants, QTL and Arabidopsis Seed. In the updated version, new options such as primer and miRNA search for database query were added. Currently, the text search interface allows users to make queries with five types of data: (i) locus name, GenBank ID, alias, species and description of genes; (ii) name, type and ecotype of mutants; (iii) title, author, journal and date of literature papers; (iv) locus name, alias and keywords and (v) miRNA name.

Example of annotation

Figure 1 shows the annotation for a typical LSD entry EIN2 (AT5G03280), an essential positive regulator of ethylene signaling (10), which accelerates *Arabidopsis* leaf senescence through suppressing *miR164* expression (11). We made detailed annotation including general information (gene ontology, sequences, protein–protein interactions and references) (Figure 1A), mutant information (Figure 1B), miRNA interaction information (Figure 1C), ortholog groups (Figure 1D), as well as cross links to several protein domain and family databases such as Pfam and Prosite (Figure 1E).

Phenotype information

In order to determine whether *SAG*s collected in LSD 2.0 really affect leaf senescence process, T-DNA insertion lines were selected using the SIGnaL database (http://signal.salk.edu/) and ordered from ABRC (4). If multiple insertions were available in the same genes, the selection was based on the position of the insertions that disrupt the gene function as much as possible, such as those insertions located within exon (4). Some RNAi lines were generated by ourselves or obtained from other laboratories for further study if there was no suitable insertion line available from the Salk collections. In the previous version, phenotypes were described by text only (5). In the updated version, more than 100 pieces of phenotypic information of *Arabidopsis* mutants were added. As shown in Figure 2A, transgenic plants overexpressing *EIN2* (12), a senescence-associated gene, exhibited early flowering and early senescence phenotype compared with *ein2-5* mutant. Previous studies demonstrate that loss-of-function of EIN2 delay leaf senescence process (13), suggesting that EIN2 is a positive regulator of senescence (11), which is further supported by our results (Figure 2A).



A Basic information

Locus name	AT5G03280	
Alias	EIN2	
Organism	*Arabidopsis thaliana*	
Taxonomic identifier	[NCBI]	
Function category	Hormone response pathway:ET	
Effect for Senescence	promote	
Gene Description	Involved in ethylene signal transduction. Acts downstream of CTR1. Positively regulates ORE1 and negatively regulates mir164A,B,C to regulate leaf senescence.	
Evidence	Genetic evidence:Mutant [Ref 1]	
References	1: Kim JH, Woo HR, Kim J, Lim PO, Lee IC, Choi SH, Hwang D, Nam HG Trifurcate feed-forward regulation of age-dependent cell death involving miR164 in Arabidopsis. Science 2009 Feb 20;323(5917):1053-7	
Gene Ontology	biological process	transport
	cellular component	membrane
	molecular function	transporter activity
Pathway	Reactome	REACT_15518
Protein-Protein Interactions	STRING	3702.AT5G03280.1-P
Sequence	AT5G03280.1 | Genomic | mRNA | CDS | Protein	

B Mutant information

Mutated 1	Mutant name	ein2-34
	Mutant/Transgenic	mutant
	Ecotype	Col-0
	Mutagenesis type	EMS

C miRNA interaction information

```
target: AT5G03280.1   4803 bp
miRNA : ath-miR854a    21 bp
mfe: -28.3 kcal/mol    p-value: 0.059681
position  50
target 5' U   U    A        U     A
           CUC CUCU UCUCUAUC CUCGU
           GAG GAGG AGGGAUAG GAGUA
miRNA  3'                       G
```

D Ortholog Groups annotation

Ortholog Groups: OG5_153355

Accession	Taxon
NP_195948 (AT5G03280)	Arabidopsis thaliana
196299	Chlamydomonas reinhardtii
NP_001050996	Oryza sativa Japonica Group
NP_001058920	Oryza sativa Japonica Group
NP_001058922	Oryza sativa Japonica Group

E Cross Link

Database	Entry ID	E-value	Start	End	InterPro ID	Description
PIRSF	PIRSF037378	0.0	1	1294	IPR017187	Ethylene-insensitive protein 2
PANTHER	PTHR11706:SF4	0.0	2	626	IPR017187	Ethylene-insensitive protein 2
PANTHER	PTHR11706	0.0	2	626	IPR001046	Natural resistance-associated macrophage like
Pfam	PF01566	1.6E-88	38	390	IPR001046	Natural resistance-associated macrophage like
PRINTS	PR00447	1.0E-17	97	123	IPR001046	Natural resistance-associated macrophage like

Figure 1. A typical entry for the Arabidopsis EIN2 gene (AT5G03280) in LSD. (**A**) Basic information, (**B**) Mutant information, (**C**) miRNA targets, (**D**) Ortholog Groups and (**E**) Cross Links to other databases.

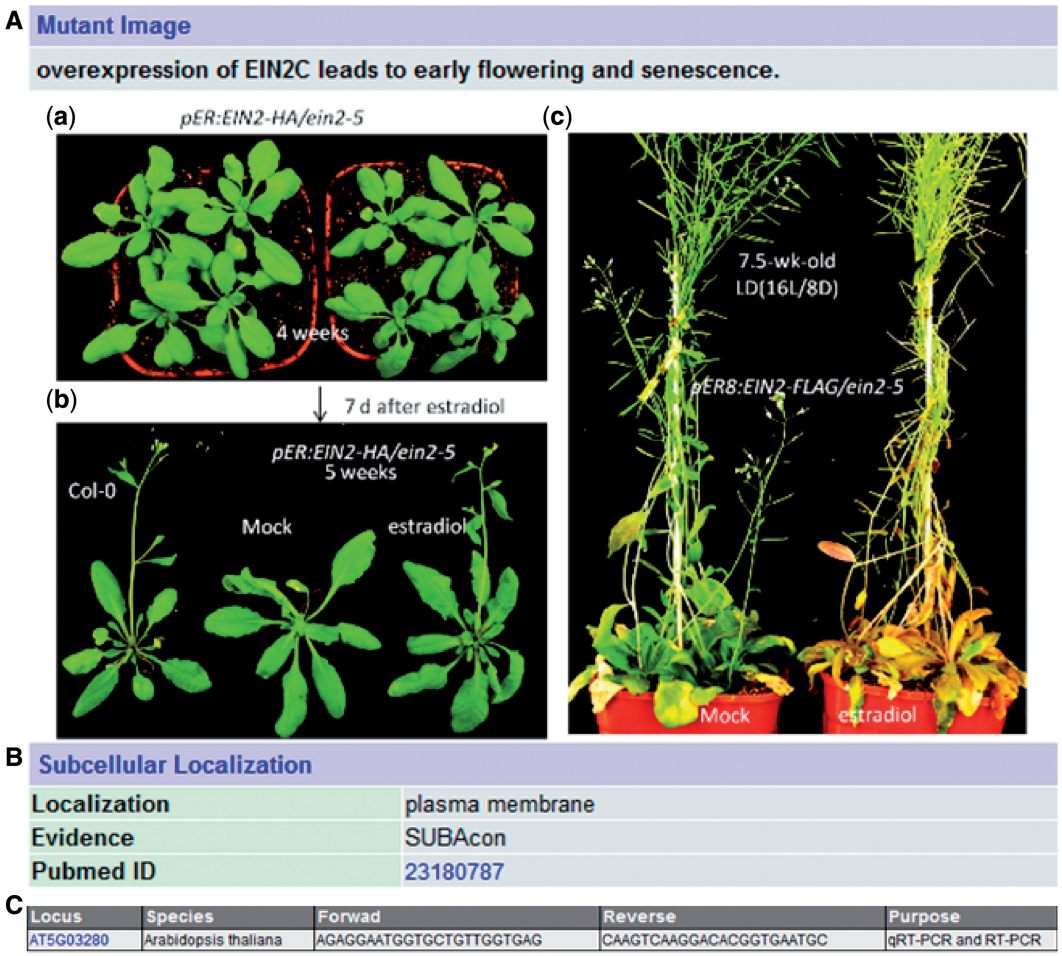


Figure 2. Added information for the Arabidopsis *EIN2* (AT5G03280) gene in LSD 2.0. (**A**) Mutant image, (**B**) Subcellular localization and (**C**) primer sequences.

Newly added annotations

To help researchers easily and high-throughput study the function of *SAG*s, Arabidopsis seed information obtained from The Arabidopsis Information Resource (http://www.arabidopsis.org/) was integrated into LSD. In addition, subcellular localization information of *SAG*s in *Arabidopsis* mined from literature or generated from the SUBA3 program (http://suba.plantenergy.uwa.edu.au/) (14) was added (Figure 2B). Leaf senescence is an essential developmental process that dramatically impacts on crop yields and qualities. So, in the updated version, QTL information, which was linked to the original database, was added to help scientists to study leaf senescence, one of agronomic traits, in crop, such as rice, maize and sorghum.

Design and experimental evaluation of the primers

To help researchers with high-throughput study of the expression level of *SAG* genes, primer sequences retrieved based on experimental evidence or designed by array designer 4 were presented (Figure 2C). To facilitate the conduction of multiple PCRs, all primers are designed to have similar properties. All primers are 19–23 nt long, with a preferred length of 21 residues. This is long enough to permit generation of gene-specific primers, while reducing the potential for cross-reactivity and allowing cost-effective generation of large primer sets. The GC contents are also similar (35–65%) to ensure uniform priming. Since PCR efficiency is decreased for very long amplicons, only short amplicons of 150–450 bp are considered during primer selection.

To evaluate the quality of the primers designed, 15 primer pairs (nine for Arabidopsis and six for rice) were tested in conventional RT–PCR experiments (Figure 3). The genes were chosen because they had been shown to be expressed specifically (*SAG12*) or preferentially (e.g. *SAG13*, *WRKY75* and *WRKY53*) in senescent leaves as reported previously (4,15). All 15 PCRs resulted in single specific amplicons, determined by gel electrophoresis (Figure 3).

DICUSSION AND FUTURE PLANS

LSD is a useful resource for leaf senescence study

In recent years, genetic and genome-wide studies of leaf senescence have generated a wealth of information and led to the identification of *SAG*s (16). Development of the LSD with wide-spread collection and systematic

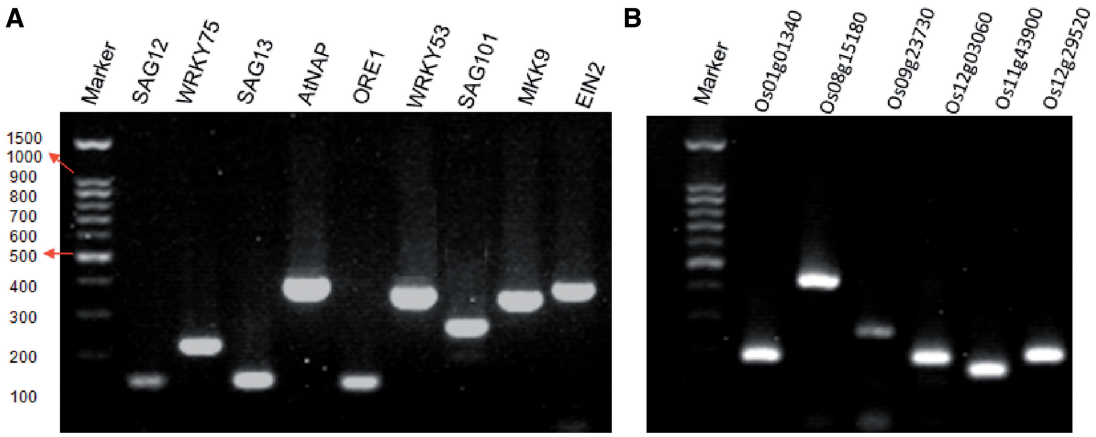


Figure 3. Gel electrophoresis of PCR products. (**A**) PCR amplifications of nine *SAG*s in *Arabidopsis thaliana*. (**B**) PCR amplifications of six *SAG*s in rice. Marker, 100-bp DNA ladder.

annotation of *SAG*s provides a useful resource for the investigation of the molecular aspects of leaf senescence (4,5). After the first release in 2010, LSD has been used for comparative studies in several important crops, such as maize and wheat. For example, Sekhon *et al.* have annotated the maize orthologs and paralogs of the *SAG*s and identified 1618 genes belonging to 615 orthologous groups (17). Comparison of 815 differentially regulated genes in wheat during senescence with *SAG*s in LSD showed significant similarities for 181 genes (18). Functional study of some of *SAG*s in LSD found that transcription factors, *WRKY75* and *AZF2*, are positive regulator of leaf senescence, while *AtMKP2* delays senescence process in *Arabidopsis* (4).

Update and improvement of LSD in the future

In the updated version of LSD, thousands of *SAG*s and hundreds of mutants were added. New features including primer sequences, subcellular localization information, Arabidopsis seed information, images of *Arabidopsis* mutants and QTL information were also included in the current version. We will continue on this project to make further updates and improvements of LSD in the future. (i) More phenotypic information such as images and descriptions will be added. Currently, more than 2000 T-DNA homology lines of *SAG*s in *Arabidopsis* are available in our institute, and senescence phenotypic information will be collected and added into the database in the near future. In addition, we are constructing transgenic lines overexpressing *SAG*s in the model plant *Arabidopsis thaliana* and will provide phenotype information in LSD. For example, overexpression of *WRKY75*, a positive regulator of senescence (4), accelerates leaf senescence in *Arabidopsis* (Figure 4); (ii) Subcellular localization information and primer sequences of *SAG*s in all species will be provided; (iii) new *SAG*s and mutants will be added once they are identified and available and (iv) we will improve the user interface according to the suggestions and comments from the user community. We hope that LSD can be a useful platform for the research community of leaf senescence worldwide.

Figure 4. Overexpression of *WRKY75* accelerates *Arabidopsis* leaf senescence. *Arabidopsis thaliana* wild-type (WT) and *WRKY75ox* transgenic lines (#1 and #5) grown in soil under long-day growth conditions (16 h-light/8 h-dark) at 22°C for up to 40 days were photographed.

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