Early senescence in the ubiquitin ligase mutant *saul1* involves salicylic acid signaling

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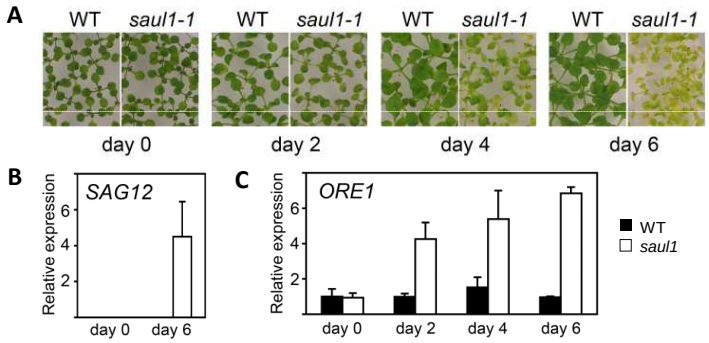
Introduction

Regulation and onset of age-dependent leaf senescence in plants is a well-defined process, which enables the recycling of resources. In *Arabidopsis thaliana,* the PUB-ARM E3 ubiquitin ligase SAUL1 was shown to suppress premature senescence in young seedlings. When growing under low light conditions, *saul1* mutant seedlings start senescing prematurely as indicated by yellowing of leaves and confirmed by physiological and molecular markers (Raab *et al*., 2009, *Plant J*, 59). Age-dependent leaf senescence in Arabidopsis has been shown to be controlled by the transcription factor ORE1 being required for a trifurcate regulatory switch that turns on the senescence program (Kim *et al*., 2009, *Science* 323).

Aim

In this work, the function of SAUL1 should be further defined by analyzing the role of ORE1 during the onset of senescence in *saul1* mutant seedlings. Furthermore, early molecular events that determine *saul1* senescence should be identified by microarray analyses and characterized.



1. The molecular switch for age-dependent cell death during senescence is turned on in low light-treated *saul1* mutants

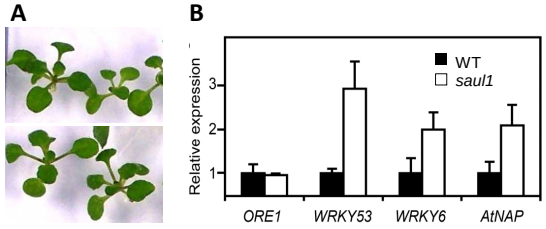
For qPCR analysis of senescence marker and key regulatory genes, wild-type and *saul1* seedlings were grown on permissive light conditions for 12 days and transferred to low light for additional 6 days. Yellowing of leaves and growth arrest were observed as visible phenotypes in *saul1* mutants (A). After 6 days under low light conditions, expression of the senescence marker gene *SAG12* was detectable in *saul1* mutants, while it was completely absent in wild-type seedlings (B). The key regulator for age-dependent cell death during senescence *ORE1* started to accumulate in *saul1* seedlings after 2 days in low light (C).

2. Absence of *ORE1* is not sufficient to suppress *saul1* phenotypes

To test for involvement of the senescence regulatory gene *ORE1* in *saul1* cell death and senescence, we generated *saul1/ore1* double mutants. Under growth conditions that trigger senescence and growth arrest in *saul1*, these double mutants showed identical visible symptoms. Thus, the additional knock-out of *ORE1* was not sufficient to suppress the *saul1* phenotypes.

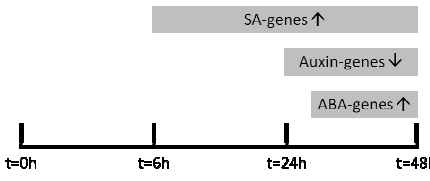
3. Regulatory events in *saul1* precede occurrence of visible phenotypes and induction of *ORE1*

Because the senescence marker genes *SAG12* and *ORE1* were expressed in late stages during senescence in *saul1* seedlings, we investigated earlier transcriptional events in the absence of visible symptoms (A). After 1 day under low light conditions, transcript levels of *ORE1* were not increased. Transcript levels of the senescence regulatory genes *WRKY6*, *WRKY53* and *AtNAP*, however, were already upregulated at this early point in time (B). In wild-type plants, SAUL1 functions to prevent these early changes in gene expression.

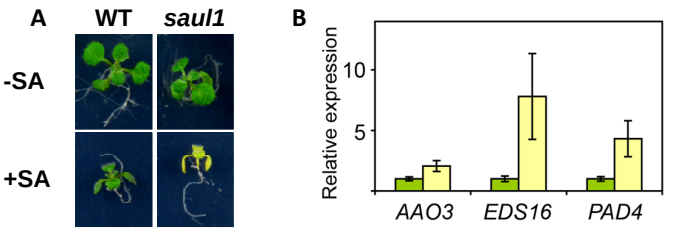


4. Induction of SA genes is the earliest change in gene expression in *saul1*

To resolve changes in gene expression leading to onset of senescence in *saul1*, microarray experiments were performed. Wild-type and *saul1* seedlings were grown under permissive light conditions and then transferred to low light for 6, 12 or 24 hours. The earliest changes in gene expression were related to genes that are known to be regulated by salicylic acid (SA) and/or pathogens such as powdery mildew. Repression of auxin and induction of ABA genes started at later points in time. These data suggested a participation of SA signaling in the early events during premature senescence in *saul1* seedlings.



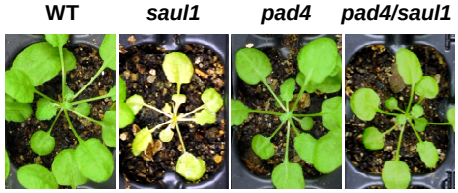
5. Salicylic acid is important for initiation of senescence in *saul1* mutant seedlings

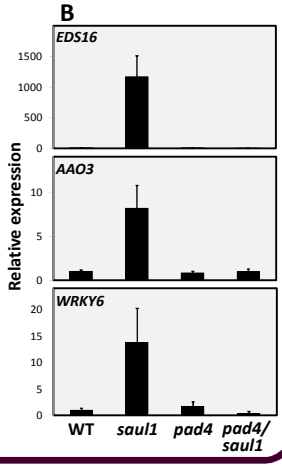


To further investigate the role of SA in *saul1*, we tested for SA-dependent growth in permissive light conditions. Indeed *saul1* mutants were hypersensitive to SA (A). Additionally, the expression of SA-responsive genes, analyzed by qPCR, was increased in *saul1* mutants (B). SA signalig seems to play an important role during the onset of senescence in *saul1* mutants.

6. Disruption of PAD4-mediated SA signaling is sufficient to rescue *saul1* phenotypes

To prove the importance of SA signaling in *saul1* mutants, *saul1* plants were crossed with *pad4*. In growth conditions that led to senescence in *saul1* plants, *saul1/pad4* double mutants showed WT-like growth (A). This was also confirmed on the molecular level by qPCR analysis of marker gene expression (B). Impairment of the PAD4-dependent SA-pathway in *saul1/pad4* resulted in complementation of *saul1* phenotypes.





Conclusion

- The ORE1-dependent regulatory switch leading to age-dependent senescence is turned on in *saul1* mutants. However, disruption of this pathway does not lead to complementation of *saul1* phenotypes.
- Early senescence in *saul1* seedlings involves PAD4-dependent salicylic acid signaling.