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**Stress adaptation and ageing is controlled by senescence-inducing age-related changes in *Arabidopsis thaliana***

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## Abstract

Senescence is the final stage of leaf development and leads to the death of a leaf. In leaves, chloroplasts are the major source of nitrogen (75%-80%), which is found mainly in proteins. The disassembly of chloroplasts during the senescence process releases a considerable amount of nitrogen, which is then remobilized to other growing parts of the plant. Thus, nutrients from dying parts of the plants are crucial for the initial development of seeds and new plant organs. Therefore, while leaf senescence is a destructive process, efficient senescence also increases viability of the whole plant and its survival to the next season or generation. However, senescence can also be induced prematurely by abiotic stress. Early senescence caused by environmental stress can be undesirable as it may affect the growth and yield of a plant. Plants grown under abiotic stress conditions such as high salinity, drought, cold or heat, display a variety of molecular, biochemical and physiological changes. Plants under environmental stress conditions activate several signalling pathways which, in coordination with hormones such as ethylene and abscisic acid, allow for an adaptive response to stress, resulting in adjustments of plant growth and development, in an attempt to maximise survival chances. Early senescence of old leaves is one of the important strategies adapted by plants for the survival of young growing tissues. The remobilisation of nutrients from old leaves to young tissues allows survival of the whole plant under stressed conditions. However, the outcome of the stress, i.e. survival or death, depends on the strength and duration of the stress in combination with the stress response.

A plant's response to stressed conditions also depends on the age of the plant. It has been reported by multiple studies that the tolerance to stress decreases with age, however, the underlying molecular mechanisms are not well understood. In chapter 1, it is reviewed and proposed that plants of different age show distinct responses to environmental stress because of senescence-inducing age-related changes (ARCs). Research work in chapter 3 sought to understand the synchrony between ageing and reduction of plant stress tolerance, using *Arabidopsis thaliana* as a model plant. Transcriptomic studies were carried out to examine the occurrence of senescence-inducing ARCs in *Arabidopsis* first rosette early expanding leaves (EEL), mid expanding leaves (MEL) and fully expanded leaves (FEL). The transcriptomic dataset showed that, as the leaf grows, genes associated to DNA repair mechanisms are downregulated and genes linked to stress hormone biosynthesis, oxidative stress, senescence and other stress responses, are upregulated. This research confirmed that *Arabidopsis* young, mature and adult plants, when treated with drought, salt, and dark stresses, had greater stress sensitivity with increased age, consistent with the role of senescence-inducing ARCs in stress resistance. This study suggests that young plants are more tolerant to stress because

of negligible senescence-inducing ARCs in young leaves, whereas the gradual accumulation of ARCs in mature leaves, and rapid accumulation in old leaves, results in decreased resistance to stress.

Next, to characterise mutants that modulate senescence-induced ARCs, I used stress-sensitive *onset of leaf death* (*old*) mutants of *Arabidopsis thaliana*. The mutants were characterised based on stress responses observed in *old13* and *old14* mutant plants compared to the wild type (WT) (Chapter 4). The *old13* mutant was selected as an appropriate mutant to study the regulatory pathway of senescence-inducing ARCs as I found that the *old13* mutant plants are susceptible to stress in an age-dependent manner (Chapter 5). The transcriptomes of *old13* leaves compared with the WT samples illustrate that stress susceptibility in the *old13* mutant is because of early acquisition of senescence-inducing ARCs. Compared to the WT leaves, *old13* showed significant downregulation of genes involved in antioxidant activity, stress tolerance, and cell-wall morphology, while genes involved in oxidative stress, senescence and stress responses were upregulated. Furthermore, transcriptional and metabolomic data illustrated that an unbalanced sugar level in *old13* leaves is one of the important senescence-inducing ARCs involved in ageing and stress responses. Chapter 6 includes an attempt to identify the mutated gene in *old13* using high throughput next generation sequencing. Further study on *old13* gene recognition will offer an exciting opportunity to gain an in-depth knowledge of the coupling between ageing and stress responses in plants. Together, this study suggests that the occurrence of senescence-inducing ARCs is an intrinsic process integrated into the stress response and ensures certain death in plants.

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## Abbreviations

<i>AAF</i>	<i>ARABIDOPSIS A-FIFTEEN</i>
<i>AAO3</i>	<i>ABSCISIC ALDEHYDE OXIDASE 3</i>
AA	Ascorbic acid
ABA	Abscisic acid
ABP1	Auxin-Binding Protein 1
ACC	1-aminocyclopropane-1-carboxylic acid
ACS	1-aminocyclopropane-1-carboxylic acid synthase
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
<i>ACT2</i>	<i>ACTIN 2</i>
<i>AOS</i>	Allene Oxide Synthase
<i>AOC</i>	Allene Oxide Cyclase
ARCs	Age-related changes
ATP	Adenosine Triphosphate
APX	Ascorbate peroxidase
BAM	Binary Alignment Map
°C	Degrees celcius
CAT	Catalases
cDNA	Complementary DNA
Chr.	Chromosome
CTAB	Cetrimonium bromide
<i>COII</i>	<i>CORONATINE INSENSITIVE 1</i>

Col-0	Columbia
CW	Cell-wall
DAG	Days after germination
DAB	3,3-diaminobenzidine
<i>DDB2</i>	<i>DAMAGED DNA-BINDING PROTEIN 2</i>
DHA	Dehydroascorbic acid
DHAR	Dehydroascorbate reductase
DMF	N, N'-dimethylformamide
DNA	Deoxyribose nucleic acid
DNase	Deoxyribonuclease
<i>EDS1</i>	<i>ENHANCED DISEASE SUSCEPTIBILITY 1</i>
EDTA	Ethylene diamine tetra acetic acid
EEL	Early expanding leaves
<i>EIN</i>	Ethylene Insensitive
EMS	Ethyl methanesulfonate
ETCs	Electron transport complexes
ERFs	Ethylene response factors
FC	Field capacity
FEL	Fully expanded leaves
<i>FSD3</i>	<i>Fe SUPEROXIDE DISMUTASE</i>
FW	Fresh weight
GA	Gibberellic acid
<i>GAST1</i>	<i>GA-STIMULATED TRANSCRIPT 1</i>

gDNA	Genomic DNA
GGC-MS	Gas Chromatography-Mass Spectrometry
Gi	Gigantea
GO	Gene Ontology
GSH	Glutathione
GR	Glutathione reductase
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
•OH	Hydroxyl radical
<i>ICS</i>	<i>ISOCHORISMATE SYNTHASE</i>
IGV	Integrative genomics viewer
IKI	Lugol's Iodine
JA	Jasmonic acid
Kb	Kilobase Pair
<i>Ler-0</i>	Landsberg <i>erecta</i>
LD	Long-day
LOX	Lipoxygenase
MAPKs	Mitogen-activated protein kinases
MDHAR	Monodehydroascorbate reductase
MDA	Monodehydroascorbate
MEL	Mid expanding leaves
MGS	Massey Genome Service
Mb	Megabase Pair
µg	Micro-gram

$\mu\text{L}$	Micro-litre
mM	Milli-molar
mg	Milli-gram
mL	Milli-litre
mm	Milli-metre
min	Minute(s)
mRNA	Messenger ribonucleic acid
MPI	Max Planck Institute
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
<i>NCED3</i>	<i>NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3</i>
NIKS	Needle in a K-stack
ng	Nano-gram
NGS	Next generation sequencing
OD	Optical density
<i>old</i>	<i>onset of leaf death</i>
O <sub>2</sub>	Oxygen
<sup>1</sup> O <sub>2</sub>	Singlet oxygen
O <sub>2</sub> <sup>-•</sup>	Superoxide
OGBF	Otago Genomics and Bioinformatics Facility
<i>ORE</i>	<i>ORESARA</i>
<i>PAD4</i>	Phytoalexin Deficient 4

PCD	Programmed cell death
PCR	Polymerase chain reaction
<i>PEN2</i>	<i>PENETRATION2</i>
PE	Paired end
<i>phs</i>	<i>pre-harvest sprouting</i>
pmol	Picomoles
PS I	Photosystem I
PS II	photosystem II
<i>PYL</i>	<i>PYRABACTIN RESISTANCE 1-LIKE</i>
qRT-PCR	quantitative Real-Time polymerase chain reaction
<i>RBOHD</i>	<i>RESPIRATORY BURST OXIDASE HOMOLOG D</i>
ROS	Reactive Oxygen Species
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RNase	Ribonuclease
<i>RPS18</i>	<i>RIBOSOMAL PROTEIN S18</i>
RuBisCO	Ribulose-1,5-bisphosphate carboxylase oxygenase
RWC	Relative water content
SA	Salicylic acid
SAM	S-adenosyl-L-methionine
SAG	Senescence-associated gene
SD	Short-day
SDS	Sodium dodecyl sulfate

sec	Seconds
SFC	Soil field capacity
SNPs	Single nucleotide polymorphisms
SOD	Superoxide dismutase
TET	Tetraspanin
TFs	Transcription factors
TPM	Transcripts per million
<i>TUB 2</i>	<i>TUBULIN BETA-2</i>
<i>UPL 7</i>	<i>UBIQUITIN-PROTEIN LIGASE 7</i>
UV	Ultraviolet
WT	Wild type
<i>ZEP</i>	<i>ZEAXANTHIN EPOXIDASE</i>