Identification of genetic regulators of longevity in dark-held detached Arabidopsis inflorescences

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Abstract

Harvested green plant tissues experience a number of stresses including energy deprivation, water disruption, and changes in hormone levels. These stresses accelerate the senescence of the tissues, which causes their deterioration. A comprehensive understanding of how these stresses cause senescence is essential if this unwanted deterioration is to be minimised. In this thesis, I used detached dark-held immature inflorescences of *Arabidopsis thaliana* (Arabidopsis) to investigate the regulatory programme responsible for the senescence of harvested energy-deprived tissue. Detached dark-held Arabidopsis inflorescences completely degreened at day 5 when held in the dark at 21°C. The degreening was accelerated by exogenously applying ACC, ethrel, MeJA, and ABA that have previously been shown to accelerate senescence in detached dark-held leaves. Higher MeJA concentrations unexpectedly delayed rather than accelerated degreening of the detached dark-held inflorescences and this was associated with reductions in transcripts for the senescence-associated genes *SEN4*, *ANAC029*, *NAC3,* and *SAG12*. To identify key genetic regulators of inflorescence senescence an untargeted forward genetics approach was utilized. This involved detaching the immature inflorescences grown from ~20,000 ethyl methane-sulfonate-treated (EMS-treated) Arabidopsis (Landsberg *erecta*) seeds, holding them in the dark at 21°C and visually identifying those that showed a different timing of degreening to wild type. This approach successfully identified inflorescences that were completely degreened at day 3 of dark incubation (two days earlier than wild type) that were designated *accelerated inflorescence senescence* (*ais*) and inflorescences that were more green than wild type at day 5 that were designated *delayed inflorescences senescence* (*dis*). A total of 10 *ais* and 20 *dis* mutants were identified. Interestingly, most of the *dis* mutants were specific for inflorescence senescence as they did not show delayed senescence in detached dark-held leaves. By utilizing a traditional map-based cloning approach, five *dis* mutants were mapped to particular chromosomal regions. *dis9* was mapped to the top arm of chromosome 3, *dis15* was to the bottom of chromosome 2, and *dis1*, *dis34*, and *dis58* were mapped to chromosome 4. Whole genome sequencing of *dis15* and *58* identified the EMS-induced lesions as G to A transitions in the eukaryotic *ASPARTYL PROTEASE* (AT2G28030) and *NON-CODING RNA* (AT4G13495), respectively. Transformation of the AT4G13495 DNA fragment into *dis58* reverted the *dis58* phenotype to wild-type confirming that the non-coding RNA is involved in

regulating inflorescence senescence. In addition to these fertile mutants, a sterile *agamous-like* mutant that had a sepal-petal-petal phenotype was identified. The mutant showed delayed degreening of detached dark-held inflorescences. This prompted me to investigate the mechanism behind the delayed senescence of the sterile homeotic *ag-1* mutant. The sepals of the *ag-1* inflorescences were found to have both delayed *in planta* and detached dark-induced senescence. They were also found to be devoid of JA and like wild-type senesced when treated with MeJA. The delayed *in planta* sepal senescence appeared to be due to the lack of produced JA as the *dde2* mutant (defective in JA biosynthesis and devoid of JA) also showed delayed *in planta* sepal senescence. However, the *dde2* mutant did not show delayed dark-induced senescence suggesting that the delayed dark-induced senescence of *ag-1* may be through a mechanism that is unrelated to the JA hormone. Taken together, in addition to identifying common regulators of inflorescence and leaf senescence, this screen has also identified novel regulators specific to inflorescence senescence that traditional screens based on leaf senescence would have missed. This suggests that there are both similarities and differences in the genetic pathways regulating leaf and inflorescence senescence. The identification of a range of mutants, some of which appear to be novel, also indicates that the immature detached Arabidopsis inflorescences are a useful system for studying energy-deprivation driven senescence. Understanding the role of the *dis58* non coding RNA and the other regulators in the mutant collection offers a new and exciting opportunity for ascertaining the regulatory genetic network initiated in energy-deprived tissues that control the deterioration of harvested produce.

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Abbreviations

AAF	ARABIDOPSIS A FIFTEEN
ABA	abscisic acid
ABI3	ABSCISIC ACID INSENSITIVE 3
ACC	1 aminocyclopropane 1 carboxylic acid
ACO	1 AMINOCYCLOPROPANE 1 CARBOXYLATE OXIDASE
ACS	1 AMINO CYCLOPROPANE 1 CARBOXYLATE SYNTHASE
AHK1	ARABIDOPSIS HISTIDINE KINASE 1
ag-1	*agamous-1*
agl	*agamous-like*
agl15	*agamous like 15*
ais	*accelerated inflorescence senescence*
AMP	adenosine monophosphate
AMPK	ADENOSINE MONOPHOSPHATE ACTIVATED PROTEIN KINASE
ANAC	ABSCISIC-ACID-RESPONSIVE NO APICAL MERISTEM *ARABIDOPSIS THALIANA* ACTIVATING FACTOR1/2 CUP-SHAPED COTYLEDONS 2
AP	APETALA
APG	autophagy
ARR2	ARABIDOPSIS RESPONSE REGULATOR 2
AS	ANTHRANILATE SYNTHASE
NAP	NAC-LIKE ACTIVATED BY AP3/PI
ant5	*arabidopsis nac domain containing protein 5*
aos	*allene oxide synthase*
AOS	ALLENE OXIDE SYNTHASE
ARCs	age-related changes
ASK1	ARABIDOPSIS SERINE/THREONINE KINASE 1
Asn	asparagine
ATP	adenosine triphosphate
AZF2	A Cys2/His2 TYPE ZINC FINGER 2
6-BAP	6-benzylaminopurine

BoCLH	*Brassica oleracea* CHLOROPHYLLASE
BoPaO	*Brassica oleracea* PHEOPHORBIDE A OXYGENASE
BOT1	BOTERO1
BoCP1	*Brassica oleracea* CYSTEINE PROTEASE
BR	Brassinosteroids
bri1	*brassinosteroids insensitive 1*
bp	base pair
BZIP1	BASIC LEUCINE-ZIPPER 1
CAB	CHLOROPHYLL A/B BINDING PROTEIN
CBF2	C-REPEAT/DRE BINDING FACTOR 2
CKs	cytokinins
CKX6	CYTOKININ OXIDASE/DEHYDROGENASE 6
cM	centimorgan
CND41	CHLOROPLAST NUCLEOID DNA-BINDING 41
Col-0	Columbia-0
coi1	*coronatine insensitive 1*
COS1	CORONATINE INSENSITIVE 1 SUPPRESSOR 1
CPR5	CONSTITUTIVE EXPRESSION OF PR GENES 5
cuc1	*cup shaped cotyledon 1*
cuc2	*cup shaped cotyledon 2*
DAD1	DEFECTIVE IN ANTHER DEHISCENCE 1
DDE2	DELAYED DEHISCENCE 2
DET	detached dark-held leaves
dis	*delayed inflorescence senescence*
DIS	attached shaded leaves
D-2HG	d-2-hydroxyglutarate
d-2HGDH	d-2-HYDROXYGLUTARATE DEHYDROGENASE
dls1	*delayed senescence 1*
DNA	deoxyribonucleic acid
AtαDOX1	ARABIDOPSIS ALPHA-DIOXYGENASE 1
EBF1	ETHYLENE-INSENSITIVE 3-BINDING F BOX PROTEIN 1
EBF2	ETHYLENE-INSENSITIVE 3-BINDING F BOX PROTEIN 2
EEP	EARLY EXTRA PETALS
EIN2	ETHYLENE INSENSITIVE 2

EIN3	ETHYLENE INSENSITIVE 3
EIL3	ETHYLENE-INSENSITIVE 3 LIKE
EMS	ethyl methane-sulphonate
ER	ethylene receptor
EREBP	ETHYLENE RESPONSE ELEMENT BINDING PROTEIN
ERF	ETHYLENE RESPONSE FACTOR
ESTs	expressed sequence tags
ESR	EPITHIOSPECIFYING SENESCENCE REGULATOR
ETD	ethylene detector
ETP1	ETHYLENE INSENSITIVE 2 TARGETING PROTEIN 1
ETR1	ETHYLENE RESPONSE 1
ETR2	ETHYLENE RESPONSE 2
F1	*filial 1*
F2	*filial 2*
FTS	FILAMENTING TEMPERATURE-SENSITIVE
FTSH5	FILAMENTOUS TEMPERATURE SENSITIVE H PROTEIN 5
g	gravity or g-force
GA	gibberellic acid
GABA	gamma-aminobutyric acid
GDH	GLUTAMATE DEHYDROGENASE
GIN2	GLUCOSE INSENSITIVE 2
GFP	green fluorescent protein
Gln	glutamine
GMO	genetically modified organism
GmSARK	GLYCINE MAX SENESCENCE-ASSOCIATED RECEPTOR-LIKE KINASE
GS	GLUTAMINE SYNTHETASE
GUS	β-glucuronidase
HIF	heterogeneous inbred family
HRM	high-resolution melting
HXK	HEXOKINASE
IDT	integrated DNA technologies
IPT	ISOPENTENYL TRANSFERASE
JA	jasmonic acid

JAZ10	JASMONATE-ZIM-DOMAIN PROTEIN 10
KIN10	KINASE 10
LP	left primer
LBP	left border primer
L*er*-0	Landsberg *erecta*-0
LC-MS	liquid chromatography-mass spectrometry
lfy-4	*leafy-4*
lfy-5	*leafy-5*
LOX	LIPOXYGENASE
LRR	leucine-rich repeat
LHCII	LIGHT HARVESTING COMPLEX II
M	molar
MAPK	MITOGEN-ACTIVATED PROTEIN KINASES
MAP65	MICROTUBULES ASSOCIATED PROTEIN 65
MAP70-1	MICROTUBULES ASSOCIATED PROTEINS 70-1
MeJA	methyl jasmonate
Mg^{2+}	magnesium
µg	microgram
µL	microlitre
µM	micromolar
min	minutes
mg	milligram
mL	milliliter
mM	millimolar
MKK	MITOGEN-ACTIVATED PROTEIN KINASE KINASE
MMP	MATRIX METALLOPROTEINASE
MT	microtubules
ms5	*male sterile 5*
MYB	myeloblastosis
MYBL	MYELOBLASTOSIS-LIKE PROTEIN
MYC2	MYELOCYTOMATOSIS VIRAL ONCOGENE 2
NAC	NAM (NO APICAL MERISTEM), ATAF1,2 (Arabidopsis thaliana NAC transcription factor1,2) and CUC2 (CUP SHAPED COTYLEDON 2)

NCCs	non-fluorescent chlorophyll catabolites
ncRNA	non coding RNA
NIKS	needle in *K*-stack
NGS	next generation sequencing
NO	nitric oxide
NOL	NYC-ONE LIKE
NR	NITRATE REDUCTASE
NS	natural leaf senescence
NOS1	NITRIC OXIDE SYNTHASE 1
NTAG1	CHINESE NARCISSUS AGAMOUS HOMOLOGUE 1
NYC1	NON-YELLOW COLORING 1
$^1O_2$	singlet oxygen
O_2	oxygen
2OG	2-oxoglutarate
ORA59	OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59
OLD5	ONSET OF LEAF DEATH 5
OPR3	OXOPHYTODIENOATE REDUCTASE 3
ore	*oresara*
ORF	open reading frame
PAO	PHEOPHORBIDE A OXYGENASE
PCD	programmed cell death
PCR	polymerase chain reaction
PDF1.2	PLANT DEFENSE 1.2
pH	potential of Hydrogen
PI	PISTILLATA
Pi	inorganic phosphate
PHEIDE A	PHEOPHORBIDE A
PLD-α	PHOSPHOLIPASE D-A
PPH	PHEOPHYTIN PHEOPHORBIDE HYDROLASE
PPDK	PYRUVATE ORTHOPHOSPHATE DIKINASE
PT2	PHOSPHOROUS TRANSPORT 2
qRT-PCR	quantitative real-time polymerase chain reaction
RAP2.4	RELATED TO AP 2.4
RAV1	RELATED TO ABI3/VP1

RCBs	rubisco-containing bodies
RCC	red chlorophyll catabolite
RCCR	RED CHLOROPHYLL CATABOLITE REDUCTASE
reb1-1	*rabbit ears 1-1*
DsRed	red fluorescent proteins
RLK	receptor like kinases
ROS	reactive oxygen species
RNA	ribonucleic acid
RNAi	RNA interference
RNS2	RIBONUCLEASE 2
RP	right primer
RPK1	RECEPTOR-LIKE PROTEIN KINASE 1
RTPCR	reverse transcriptase PCR
AtS40-3	ARABIDOPSIS SENESCENCE 40-3
SA	salicylic acid
SAGs	senescence associated genes
SAVs	senescence-associated vacuoles
SAUL1	SENESCENCE-ASSOCIATED E3 UBIQUITIN LIGASE 1
SEN1	SENESCENCE 1
SEN4	SENESCENCE 4
sep1/sep2/sep3	*sepallata 1/ sepallata 2 /sepallata 3*
SGR	STAY-GREEN
sid2	*salicylic acid induction deficient 2*
SIRK	SENESCENCE-INDUCED RECEPTOR LIKE KINASE
SnRK	SUCROSE NON-FERMENTING RELATED PROTEIN KINASE
Snf1	SUCROSE NON-FERMENTING
SNP	single nucleotide polymorphism
TAIR	The Arabidopsis Information Resource
TCA	tricarboxylic acid
T6P	trehalose-6-phosphate
VIN2	VACUOLAR INVERTASE ENZYME 2
VP1	VIVIPAROUS-1
VSP1	VEGETATIVE STORAGE PROTEIN 1
VTC1	VITAMIN C DEFECTIVE 1

WGS	whole genome sequencing
wt	wild-type
XDH	XANTHINE DEHYDROGENASE

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