

Molecular mechanisms of abiotic stress-induced senescence in plants (AbioSen)

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ERA-NET for Coordinating
Action in Plant Sciences



Objective

The main objective of AbioSen is to chart and study the networks that integrate abiotic stress-derived signals into the senescence pathway.

Project has 4 WPs

Senescence – an integrated response to age and environment

Environment: salt, drought, temperature,
ROS (H_2O_2)

TFs?

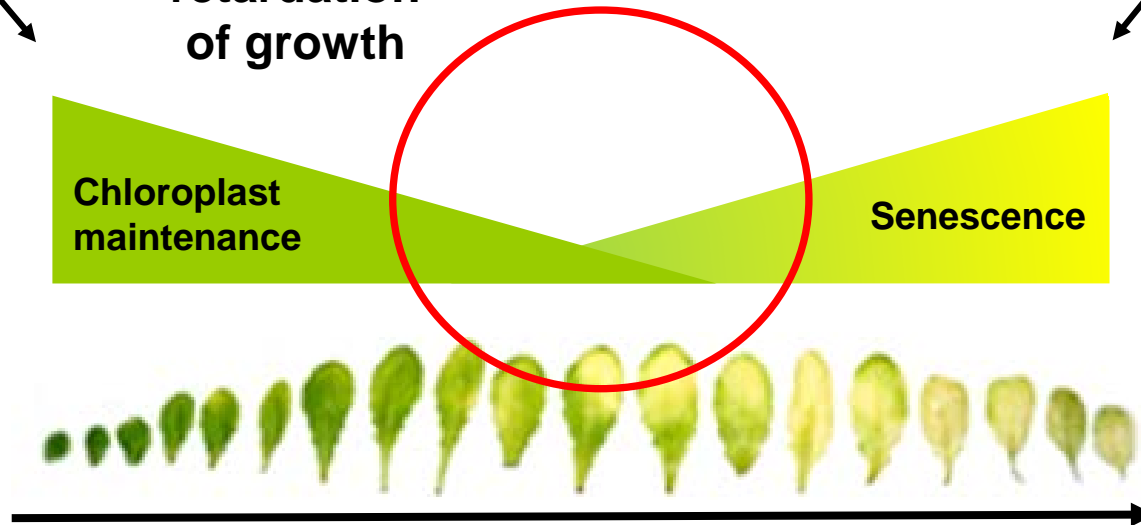
juvenile, never
senescence

retardation
of growth

always
senescence

Chloroplast
maintenance

Senescence

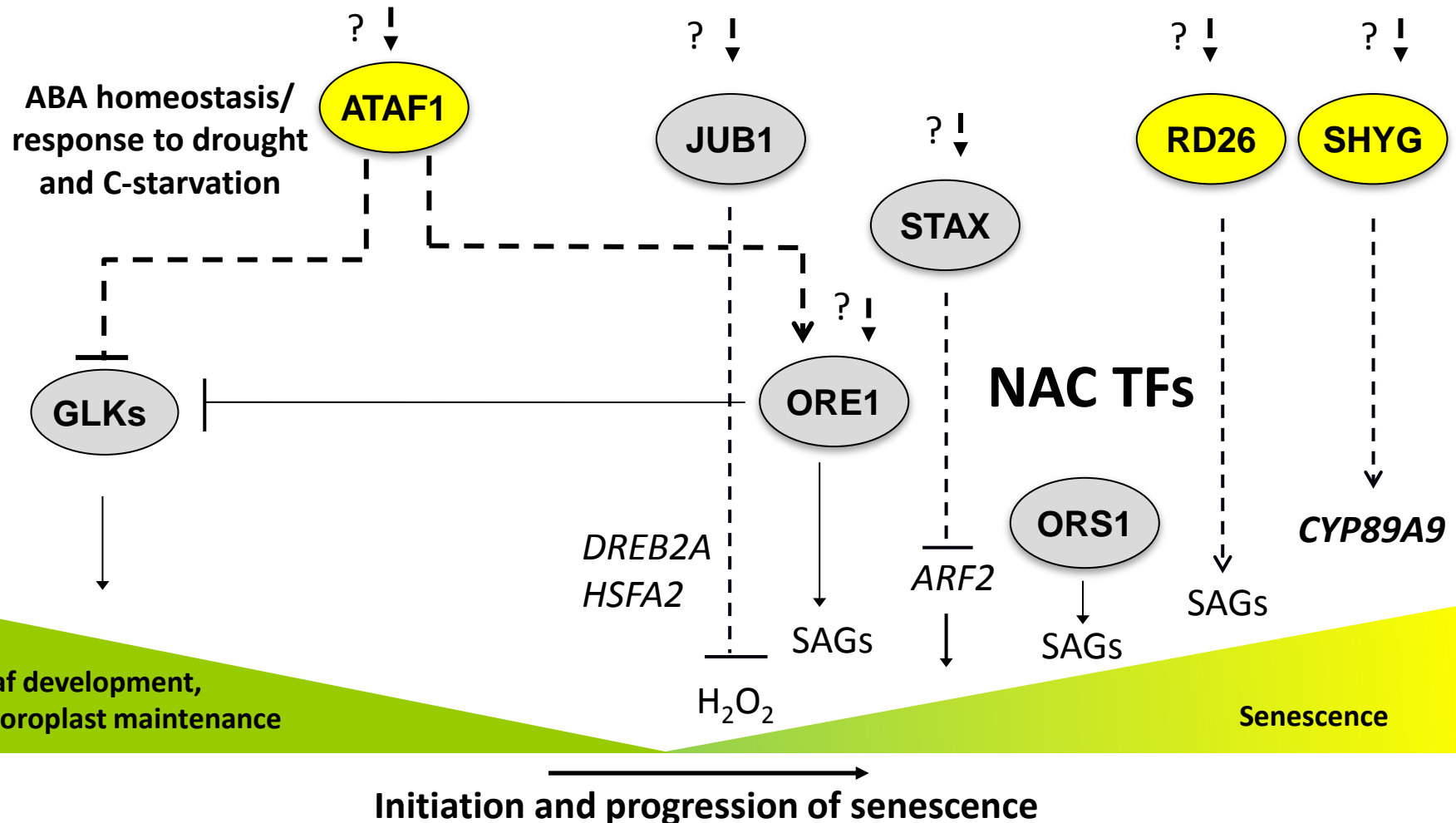


Leaf development

Remobilisation of
N, C,

WP1 Unravelling the GRNs of TFs that modulate oxidative- and abiotic stress-induced senescence

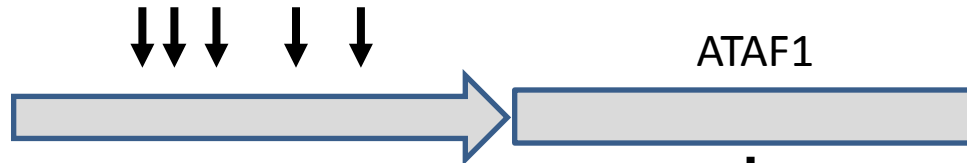
age / development, stress, nutrient status, H_2O_2



WP1 Unravelling the GRNs of TFs that modulate oxidative- and abiotic stress-induced senescence

Task 1.1. Upstream TFs and regulators of *ATAF1*

- Y1H, EMSA, ChIP, TA, mutants
- LUC screen (EMS)

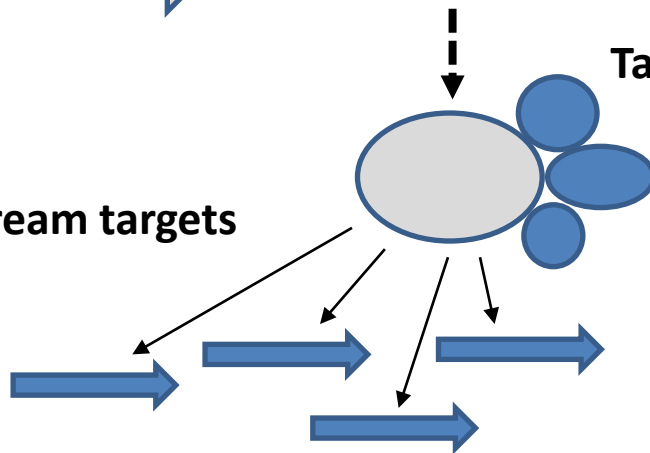


Task 1.3. *ATAF1* interacting proteins

- Co-IP
- Functional confirmation

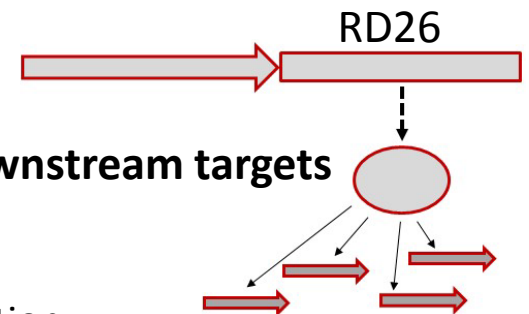
Task 1.2. Downstream targets

- ChIP-seq
- RNA-seq
- confirmation



Task 1.4. Downstream targets

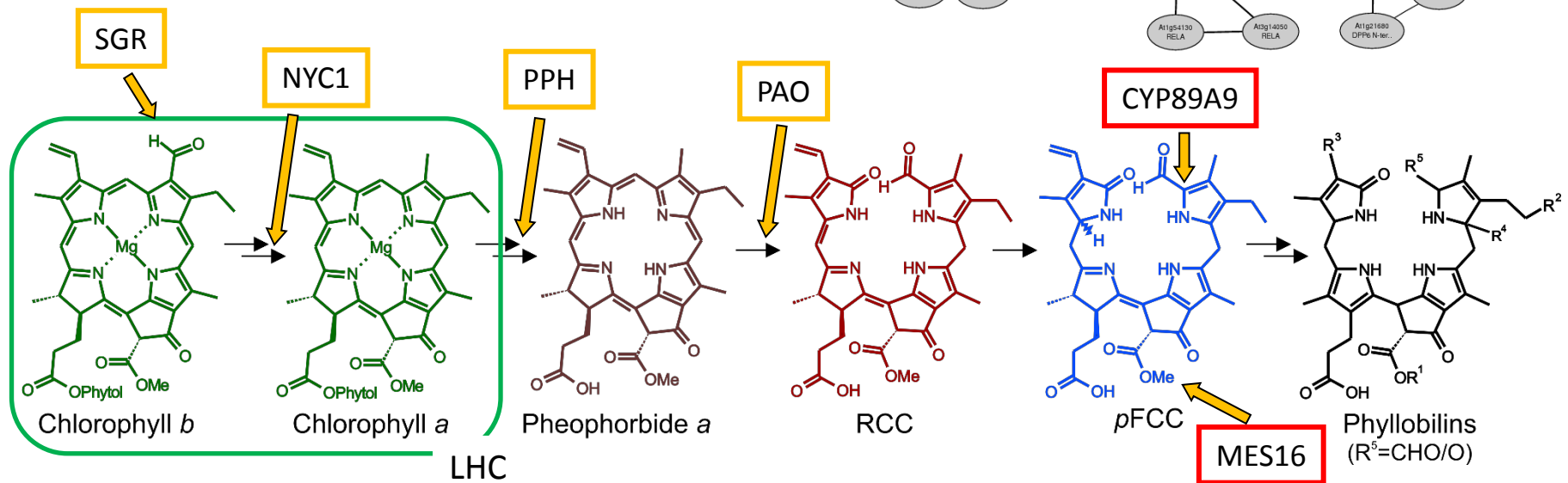
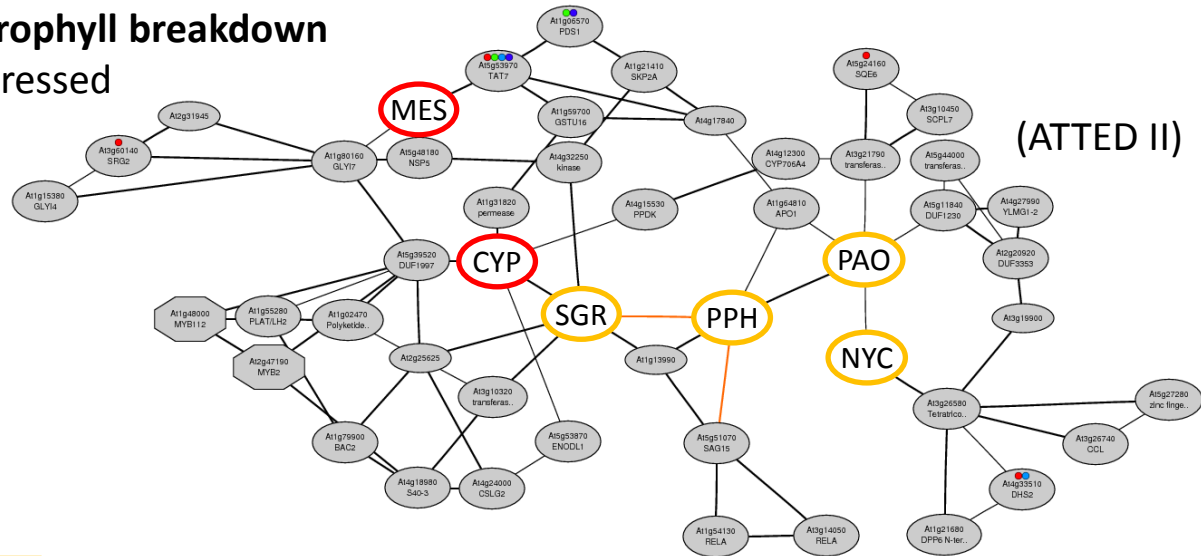
- ChIP-seq
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- confirmation



WP2 Investigating the GRN of chlorophyll breakdown during developmental and stress-induced senescence in *A. thaliana*

Task 1: Gene regulatory aspects of chlorophyll breakdown

- Chlorophyll catabolic genes are co-expressed
- Common regulation?



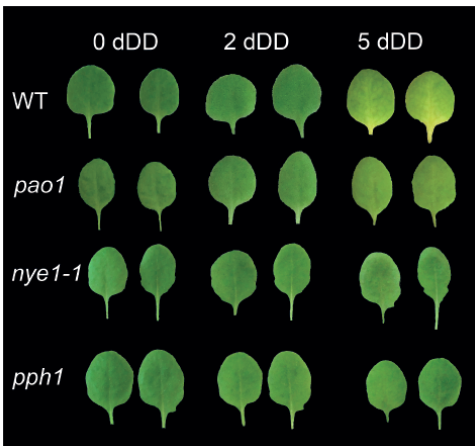
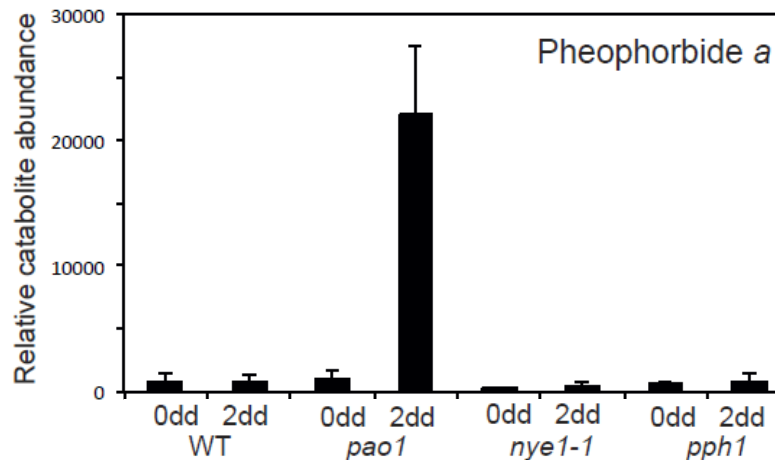
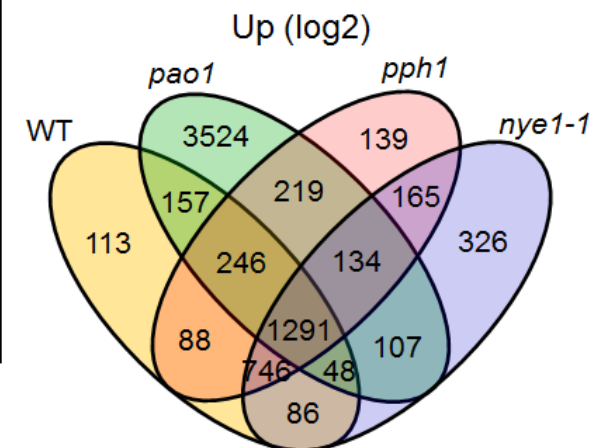
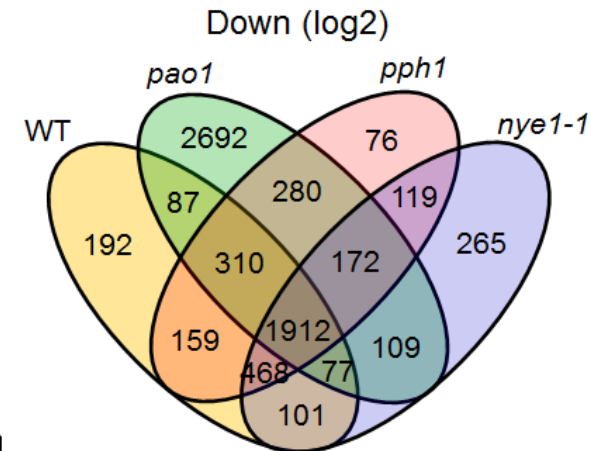
WP2 Investigating the GRN of chlorophyll breakdown during developmental and stress-induced senescence in *A. thaliana*

Task 1: Gene regulatory aspects of chlorophyll breakdown

- Chlorophyll catabolic genes are co-expressed
- Common regulation?

RNAseq:

- senescence induces dramatic changes in gene expression (RNAseq)
- in *pao1*: specific alteration of 1000s of genes
- caused by pheophorbide *a* accumulation?



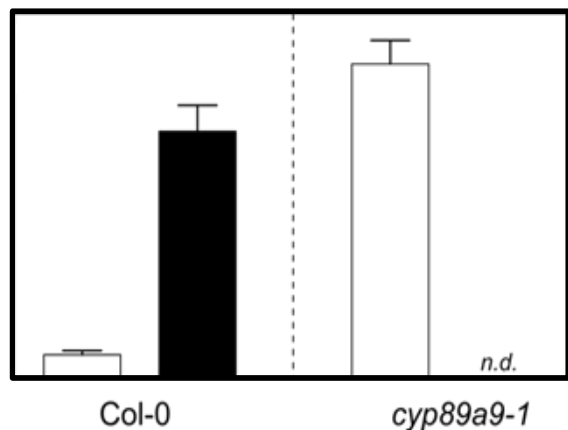
- TFs in the data sets?
- Search for *cis*-elements in CCEs: phylogenetic footprinting, promoter deletion studies, Y1H

WP2 Investigating the GRN of chlorophyll breakdown during developmental and stress-induced senescence in *A. thaliana*

Task 2: Role of SHYG in chlorophyll breakdown and senescence regulation

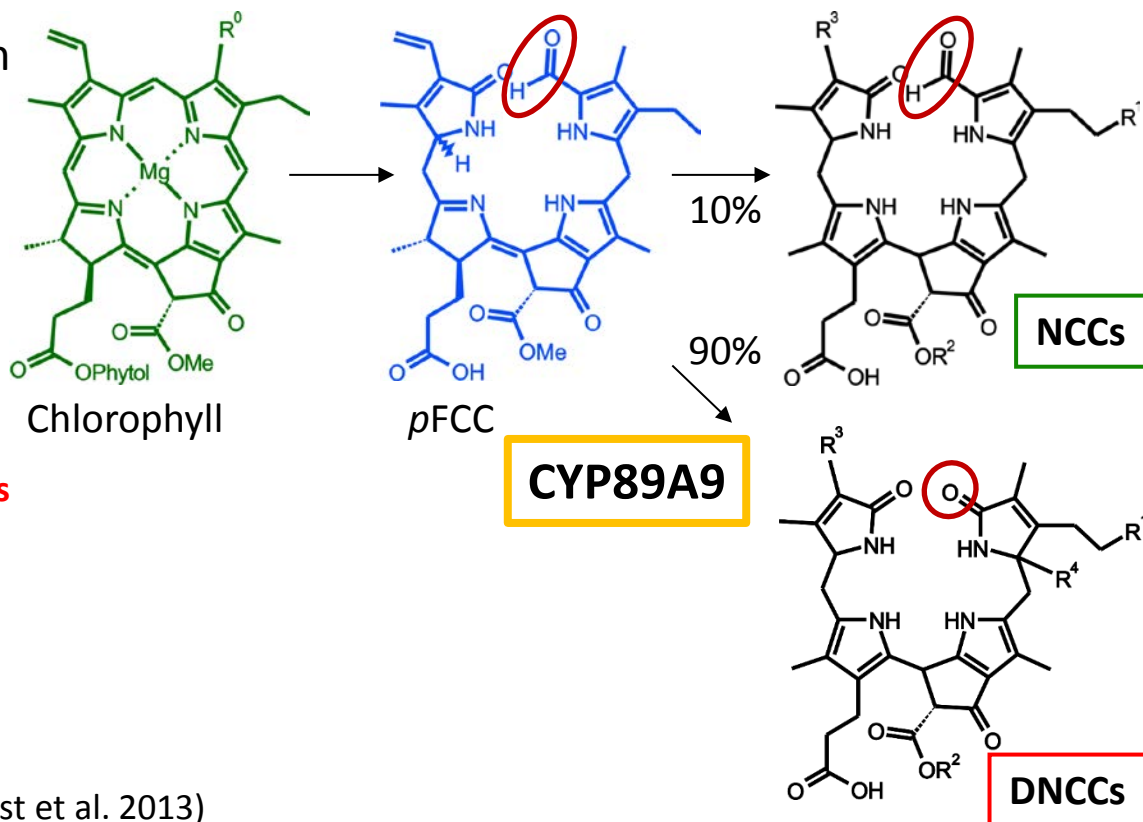
- two types of phyllobilins as end products of chlorophyll breakdown: DNCCs and NCCs
- CYP89A9 forms DNCCs (90% of all phyllobilins) in Arabidopsis
- *cyp89a9* mutants are deficient in DNCCs
- SHYG activates *CYP89A9* expression

➤ Test for direct control of *CYP89A9* by SHYG



□ NCCs
■ DNCCs

(Christ et al. 2013)



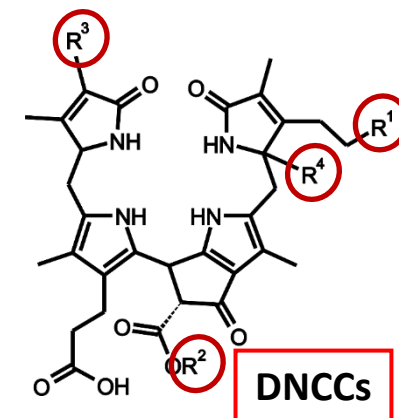
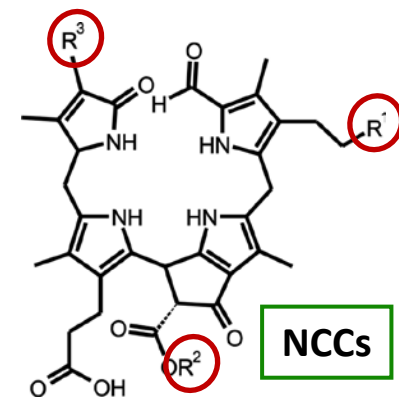
WP2 Investigating the GRN of chlorophyll breakdown during developmental and stress-induced senescence in *A. thaliana*

Task 3: Establishment of an LC-MS system for chlorophyll catabolites as diagnostic tool to monitor senescence

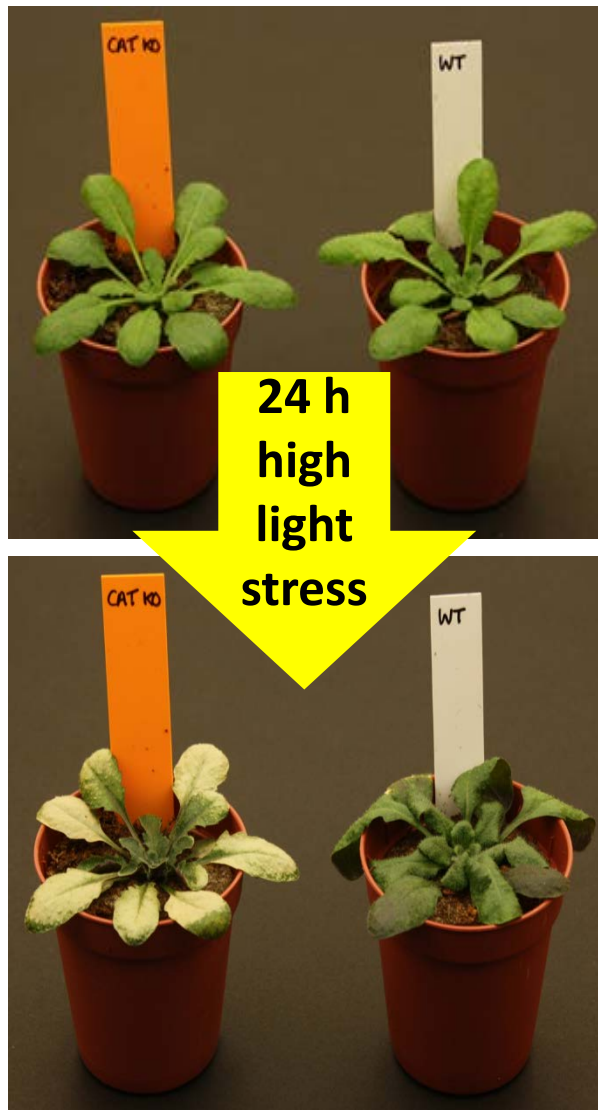
- complex pattern of phyllobilins in a given species
- in Arabidopsis: at least 10 different phyllobilins
- requirement of an LC-MS platform for sensitive detection/quantification

	maple	Arabidopsis	canola	quince	katsura tree	barley	sweetgum	apple	tobacco	pear	spinach	peace lily	basswood	maize
R ¹	D	N	N	N	N	N	D	D	D	D	N	N	N	N
R ²														
R ³														
R ⁴														

Name	R ¹	R ²	R ⁴	m/z [M+H] ⁺
At-NCC-1	O-glucosyl	H	H	793.3296
At-NCC-2	OH	H	H	631.2768
At-NCC-3	OH	H	H	631.2768
At-NCC-4	O-glucosyl	CH ₃	H	807.3453
At-NCC-5	H	H	H	615.2741
At-DNCC-1	OH	H	H	619.2770
At-DNCC-2	O-glucosyl	H	H	781.3296
At-DNCC-3	H	H	CH ₂ OH	633.2924
At-DNCC-4	H	H	H	603.2819
At-DNCC-5	H	CH ₃	CH ₂ OH	647.2717

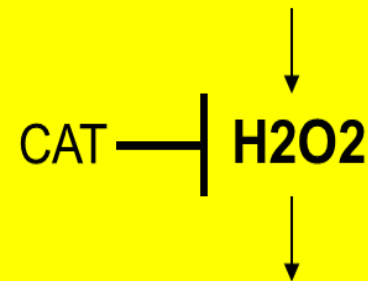


WP3 Framing a genetic network of H_2O_2 -induced chlorosis/cell death/senescence



***Catalase2*-deficient Arabidopsis:
tool for non-invasive H_2O_2 modulation**

Photorespiration (HL, low CO_2)



defense response
cell death

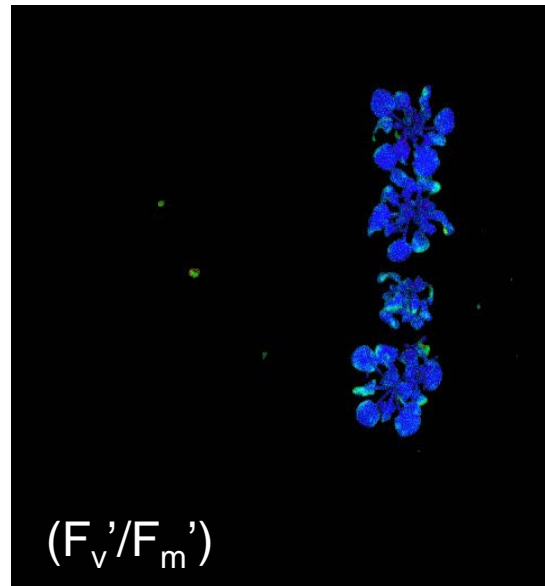
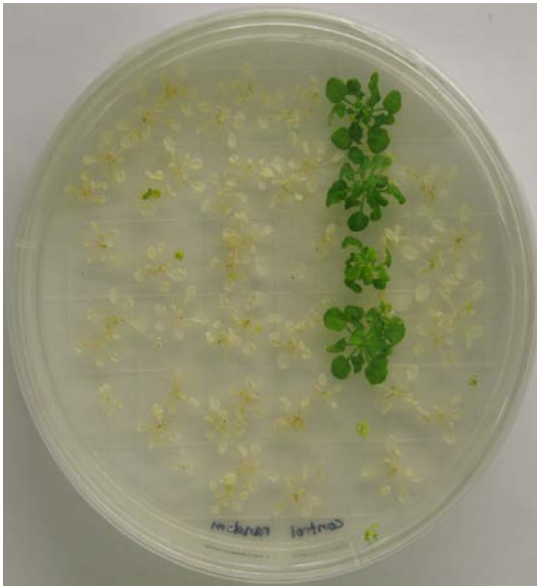
Vandenabeele et al. (2003; 2004)

Vanderauwera et al. (2005)

Queval et al. (2007)

WP3 Framing a genetic network of H_2O_2 -induced chlorosis/cell death/senescence

A mutant screen for revertants of H_2O_2 -induced cell death in *Arabidopsis thaliana*

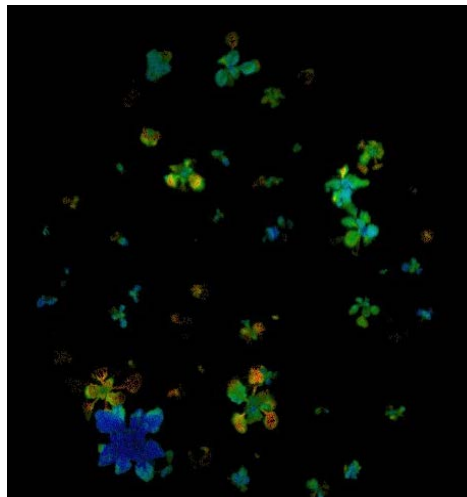
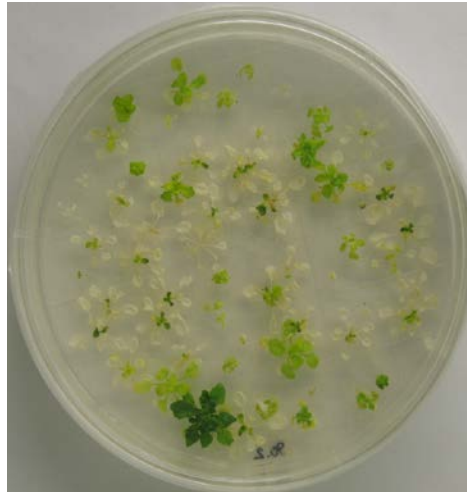


Photorespiratory conditions induce cell death in *CAT2* knock-out plants

Catalase-deficient cell death phenotype within an in vitro plate assay (4 survivors are wild-type plants)

WP3 Framing a genetic network of H₂O₂-induced chlorosis/cell death/senescence

Genetic screen for second-site mutations (EMS) reversing *cat2*^{KO} phenotypes



11.000 second site
mutated *cat2-2* M1 plants



screened 113.000 M2 plants

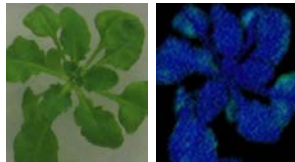


> 30 revertants

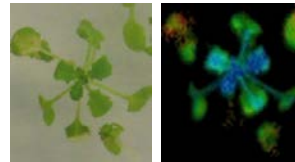
- Reduced photorespiration ?
- Increased anti-oxidant capacities ?
- Impaired cell death pathways ?
- Induced anti-cell death pathways ?
- ...

WP3 Framing a genetic network of H₂O₂-induced chlorosis/cell death/senescence

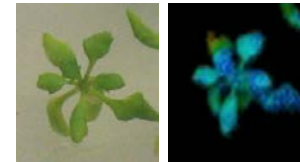
WT



48-1



211-4



Tasks

Task 3.1. Physiological, biochemical and molecular phenotyping of revertants.

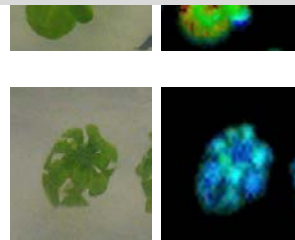
Task 3.2. Identification of causative mutations by deep-sequencing.

Task 3.3. Detailed functional analysis of causative mutations.

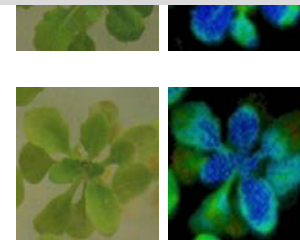
40-2



182-1



378-3



13 mutants currently under analysis

WP4 Molecular mechanisms of abiotic stress-induced senescence in *H. rhodopensis*

Background:

- *Haberlea rhodopensis* is a desiccation-tolerant species; survives months without water and then rapidly regains normal physiology after re-watering. Genome not yet sequenced but transcriptomes and metabolomes recently studied (Gechev et al. 2013).
- *H. rhodopensis* is tolerant to high oxidative stress (that would kill other species like *A. thaliana*).
- The abiotic stress-induced senescence in *H. rhodopensis* is rather different from the stress-induced senescence in *A. thaliana* and many other species. Drought and low temperatures do not induce senescence; darkness induces senescence only after 5-6 months (Denev et al. 2012).



well-watered



20 days desiccation



2 days rehydration

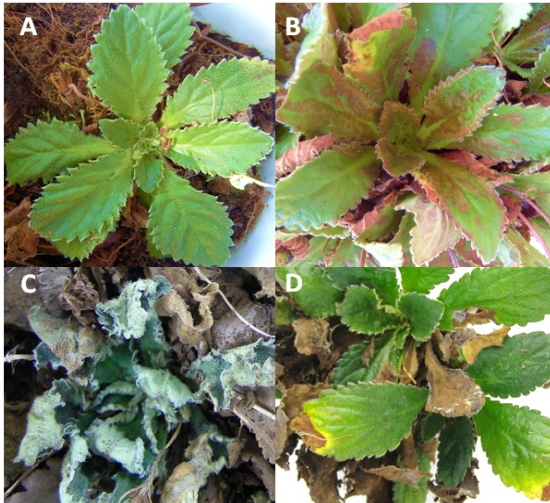
WP4 Molecular mechanisms of abiotic stress-induced senescence in *H. rhodopensis*

Preliminary results:

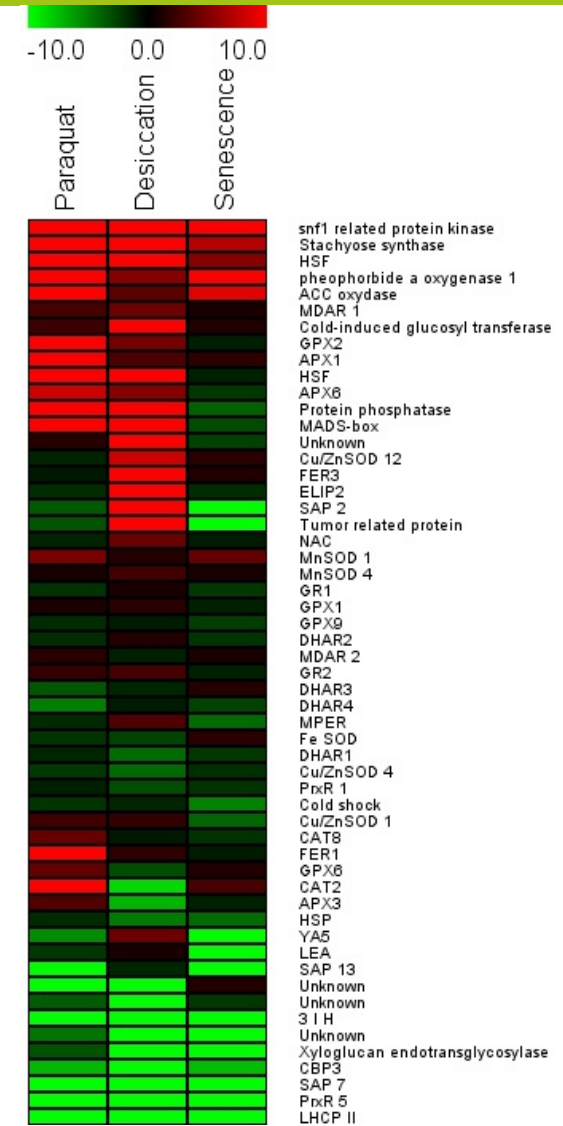
- RNA-seq identified highly regulated TFs and senescence-related genes during dehydration and rehydration of *H. rhodopensis*.
- Homologues of Arabidopsis *STAY-GREEN 1 (SGR1)*, *PHEOPHORBIDE a OXYGENASE (PAO)*, etc. identified.
- A qRT-PCR platform for senescence-, oxidative-, and abiotic stress-related genes established.

WP4 Molecular mechanisms of abiotic stress-induced senescence in *H. rhodopensis*

qRT-PCR platform with senescence-, oxidative-, and abiotic stress-related genes



Commonly regulated genes identified



- A, unstressed controls (RWC 85%)
- B, oxidative stress (4 mM paraquat)
- C, desiccated plants (RWC 5%)
- D, senescence

WP4 Molecular mechanisms of abiotic stress-induced senescence in *H. rhodopensis*

Tasks:

Task 4.1: Transcriptomics and metabolomics of senescence in *H. rhodopensis*.

- High-resolution temporal transcriptome (qRT-PCR, RNA-seq) and metabolome (GC/LC-MS) profiling of dark-induced senescence.
- Detailed inventory of SAGs and expression analysis during senescence.
- Identification and temporal profile of chl catabolites (with **P2**) during abiotic stresses.

Task 4.2. Functional analysis of *H. rhodopensis* senescence-related genes.

- Functional studies of SAGs (*SGR*, *PAO*, and a few more) from *H. rhodopensis* (with P1 and P2); expression in *A. thaliana sgr* and *pao* mutants to test for functional complementation.

Thanks!



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