



Redox Biology Congress
2022 Annual Meeting of the Society of Free Radical Research - Europe
& Biannual Meeting of the Plant Oxygen Group

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www.SFRR-Europe.org | www.PlantOxygenGroup.eu



Ghent, Belgium
Oude Vismijn
24-26 Aug, 2022

Redox Biology Congress
2022 Annual Meeting of the Society of Free Radical Research - Europe
& Biannual Meeting of the Plant Oxygen Group

**Oxidative stress, redox biology and
antioxidants from plants to humans**

This congress is a joint event organized by the Society for Free Radical Research - Europe (SFRR-E) and the Plant Oxygen Group (POG)

New Abstract submission deadline: April 28th

Late abstract submission: May 20th

Outline today's talk.

Oxidative stress (signaling) in plants.

ROS production, scavenging, transport

Oxidative Stress Resilience

Acclimatory responses: towards stress resilient crops?

H₂O₂ as a signaling molecule.

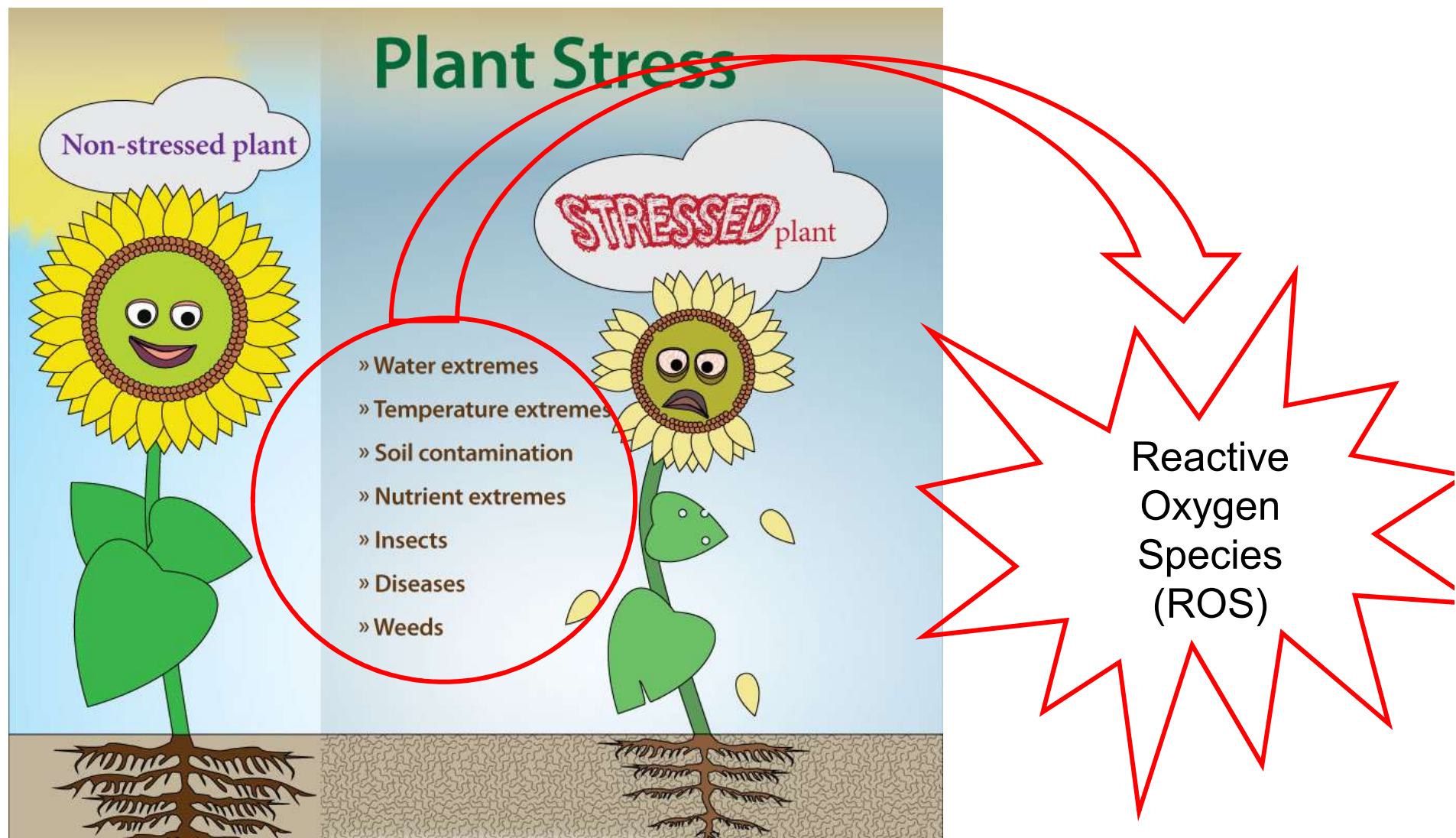
PROJECTS

Forward Chemical and Genetic screens

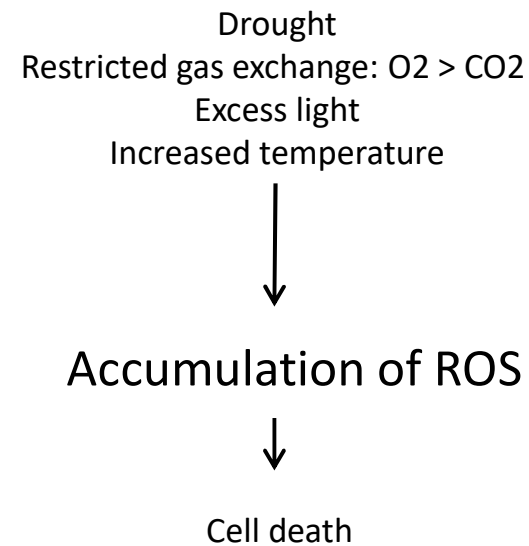
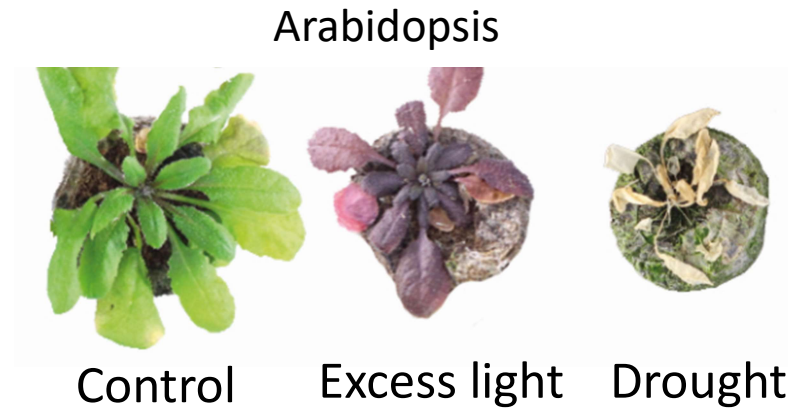
Redox proteomics. Sensitive identification of oxidative protein modifications

Chemical and Genetic Oxi-TRAPs

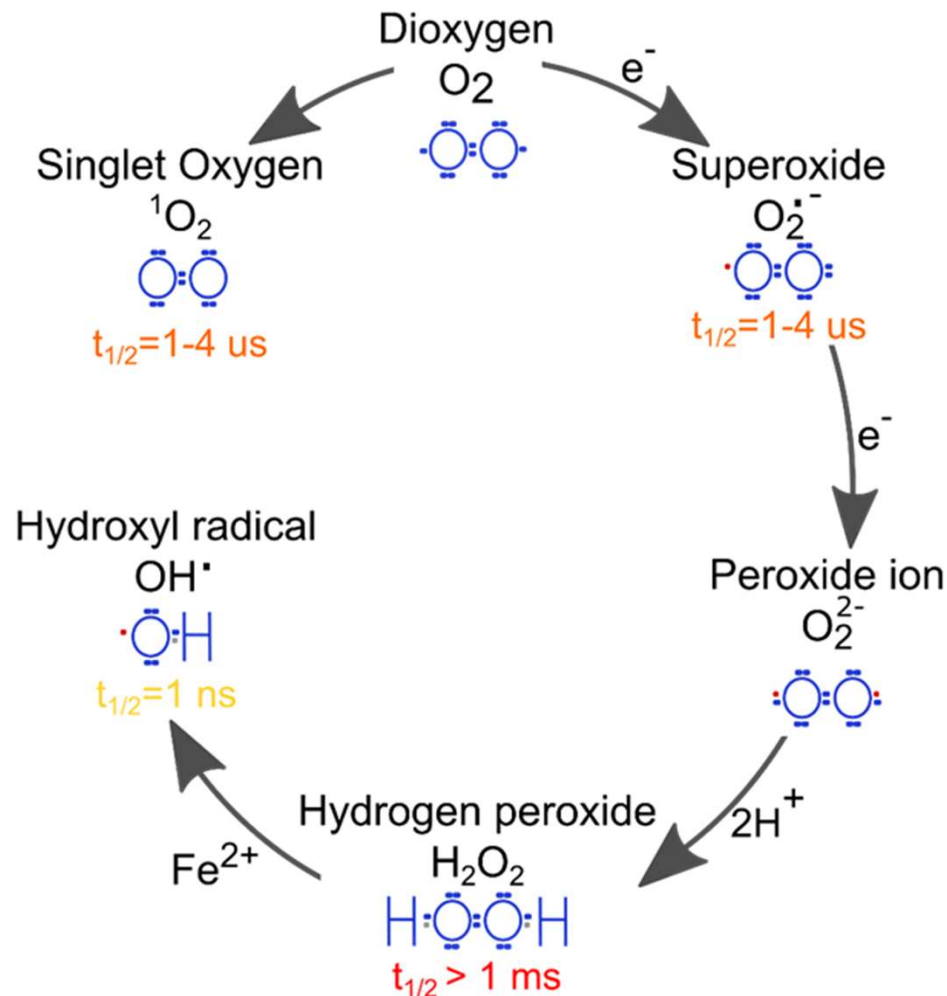
Subcellular sulfenomes



Abiotic stresses are accompanied by increased ROS production

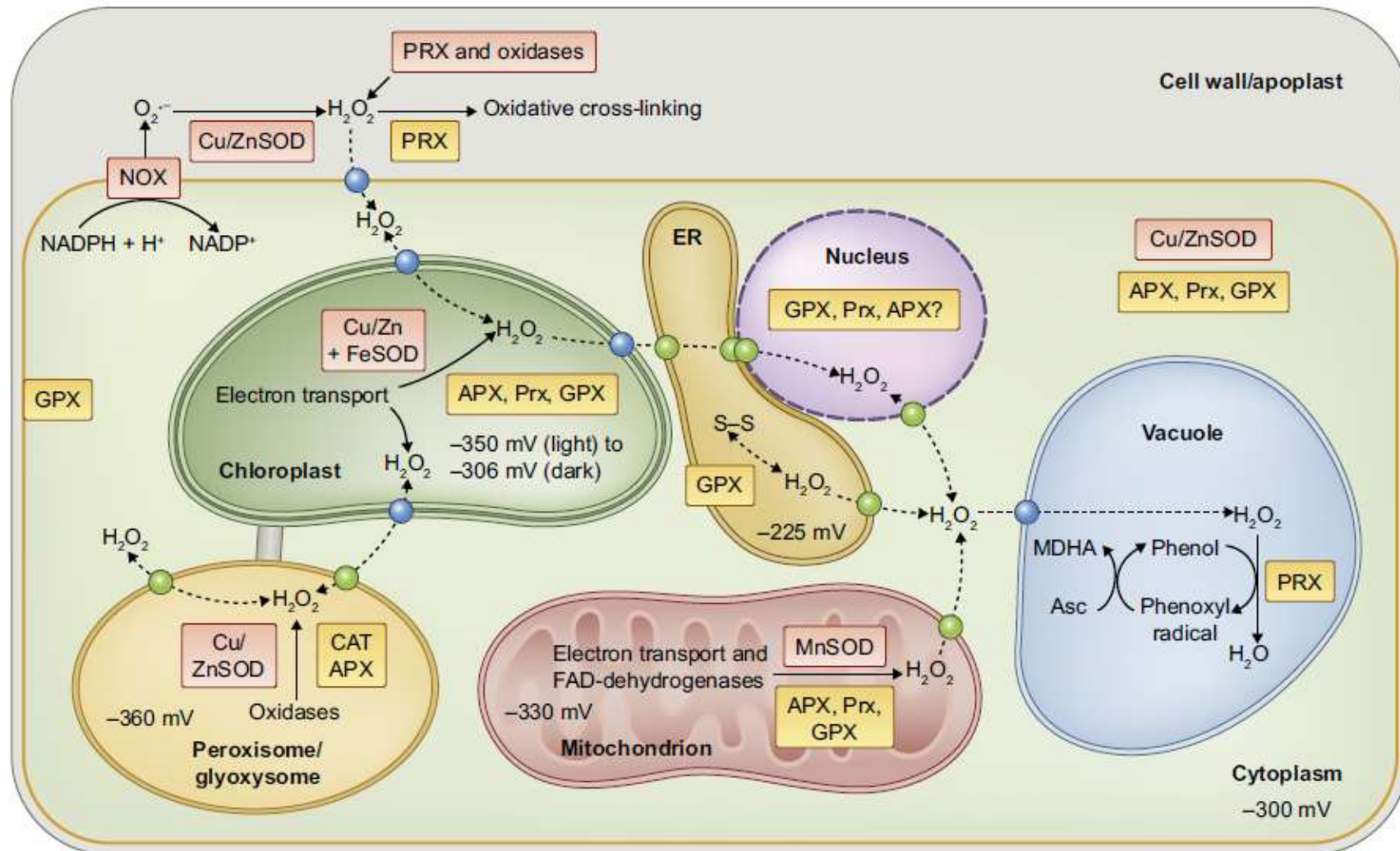


The high reactivity of ROS is due to their altered chemistry, compared to O₂, that allows them to donate an electron or transfer an excited energy state to an acceptor molecule.

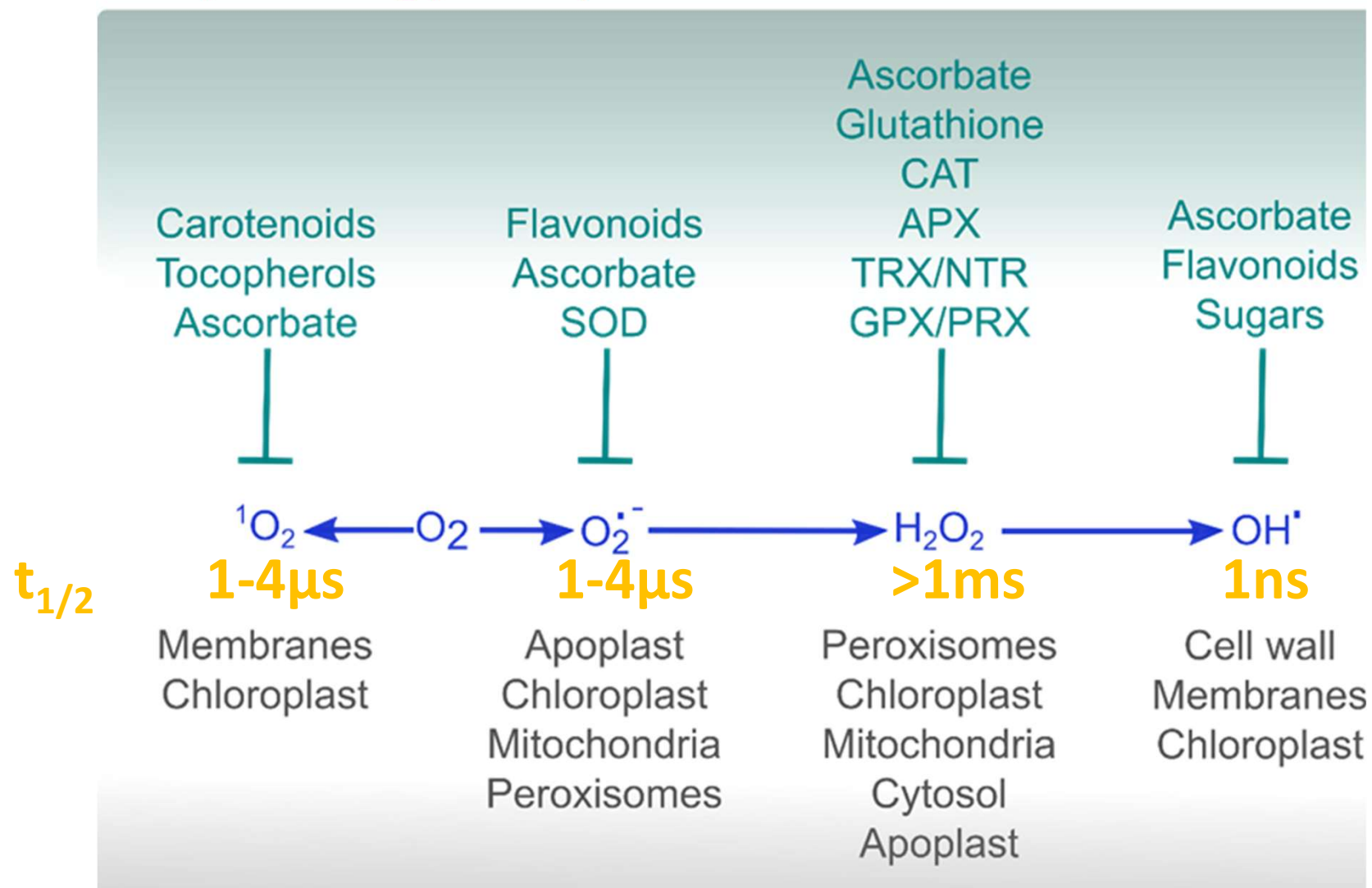


Mhamdi and Van Breusegem, 2018

Subcellular sites of hydrogen peroxide production, scavenging & transport



ROS processing pathways

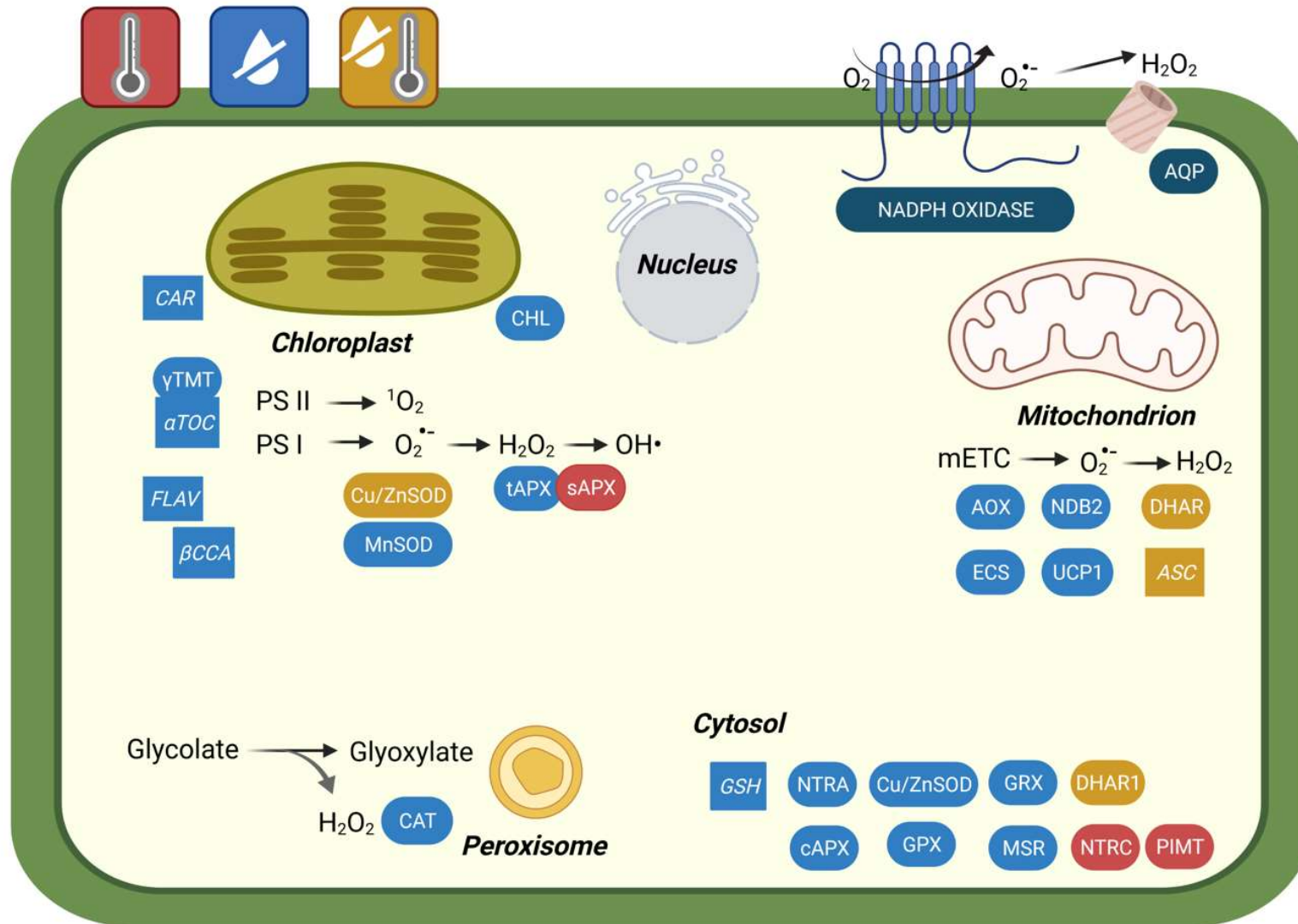


ROS generation sites

Overproducing ROS Scavengers provides increased tolerance

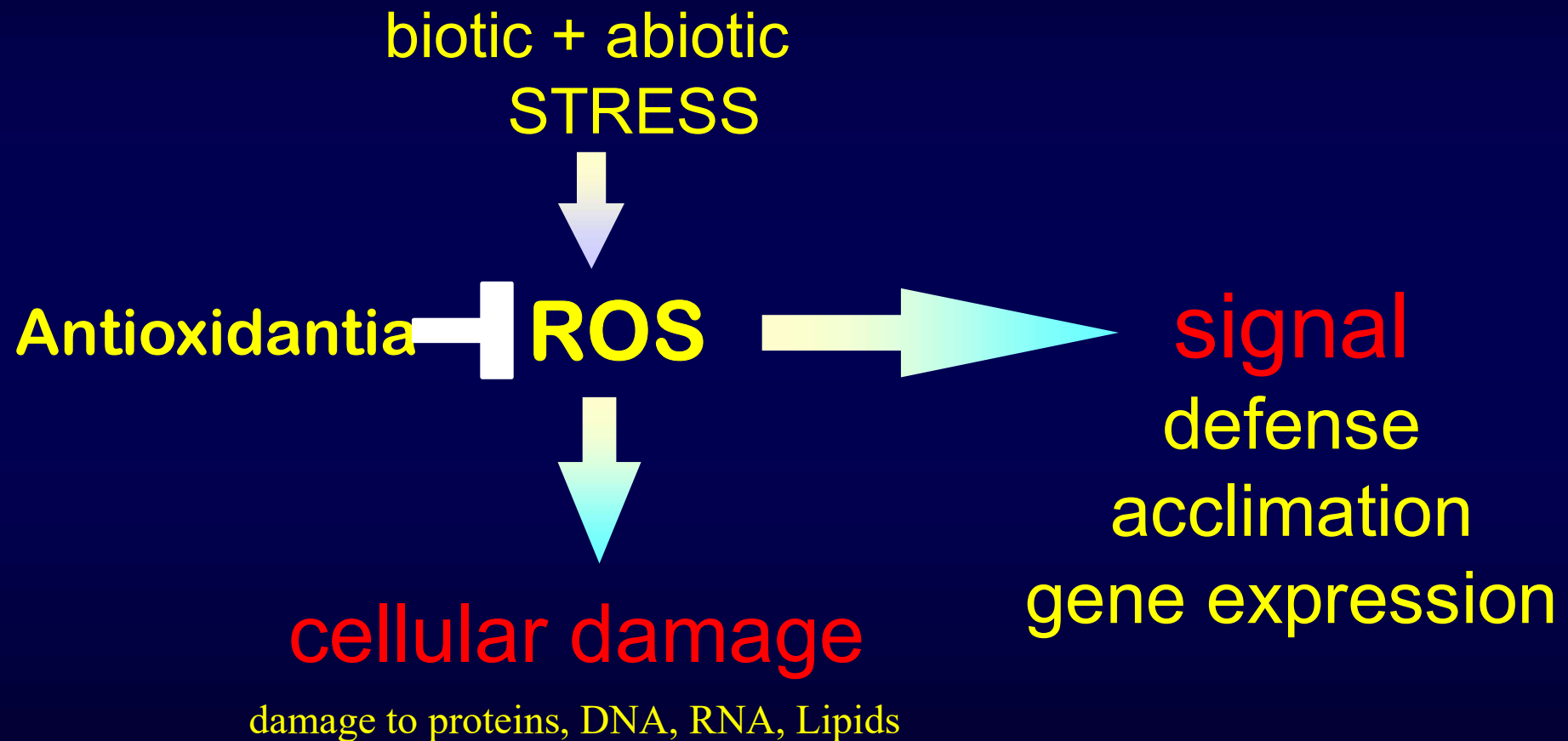
- Transgenic tobacco tolerance
 - *N. plumbaginifolia* MnSOD in chloroplast/mitochondria ozone
 - *A. thaliana* FeSOD in chloroplast paraquat
- Transgenic tomato
 - Petunia Cu/ZnSOD in chloroplast chilling
- Transgenic potato
 - Tomato cyt/chl Cu/ZnSOD in cytosol/chloroplast paraquat
- Transgenic alfalfa / maize
 - *N. plumbaginifolia* MnSOD in chloroplast/mitoch freezing/chilling

Improving Oxidative Stress Resilience in Plants



Review: Kerchev and Van Breusegem, 2021 *The Plant Journal*

Dual Face of Oxidative stress



Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes

T. Gechev^a, I. Gadjev^a, F. Van Breusegem^b, D. Inzé^b, S. Dukiandjiev^a, V. Toneva^a and I. Minkov^{a,*}

day
7

Herbicide (3-AT)



What doesn't kill you,
makes you stronger



Figure 2. H_2O_2 protects tobacco against oxidative stress. (A) H_2O_2

day
1

Primed with 5mM H_2O_2

MOCK

H₂O₂ triggers acclimatory responses (priming)

P. Kerchev, et al.

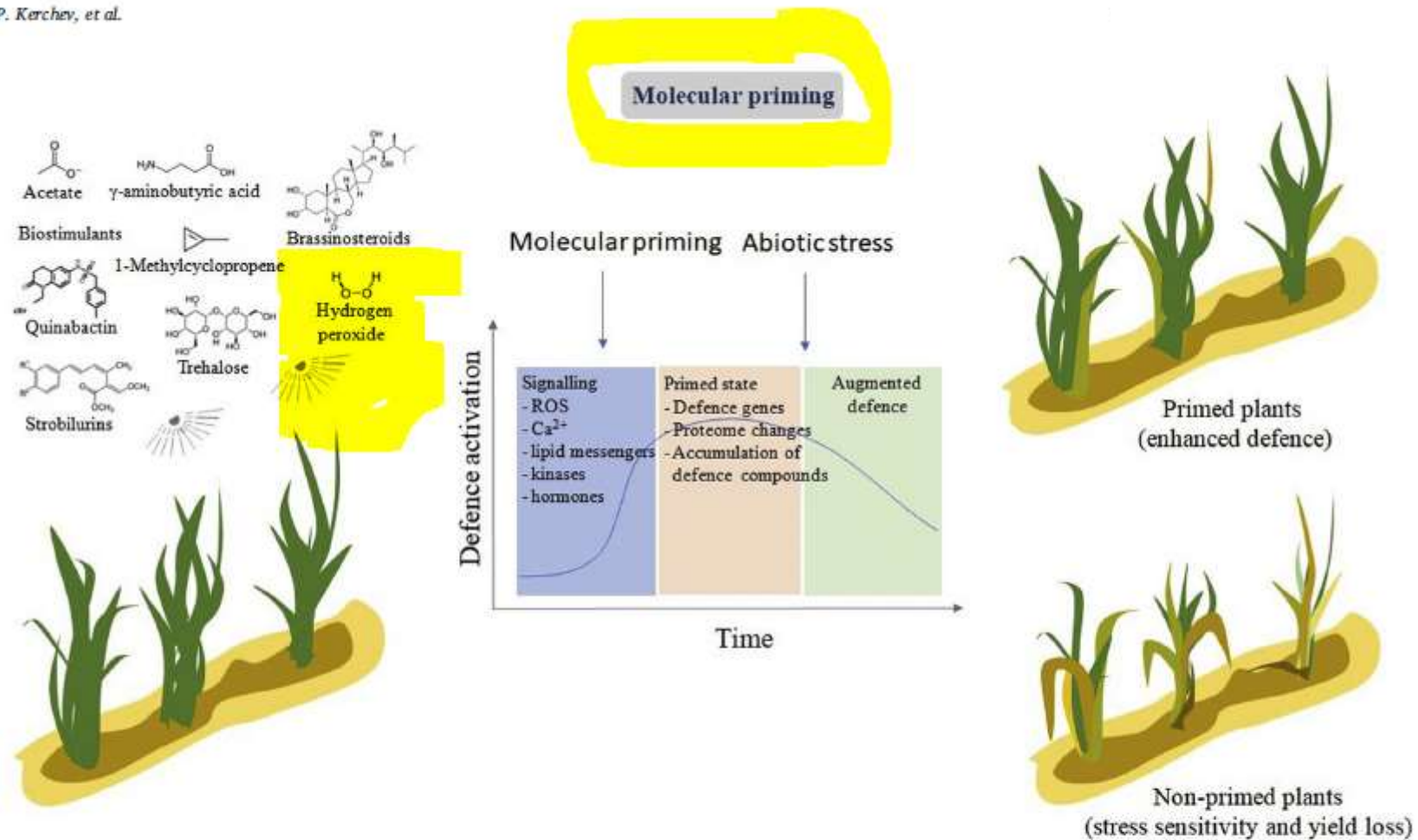
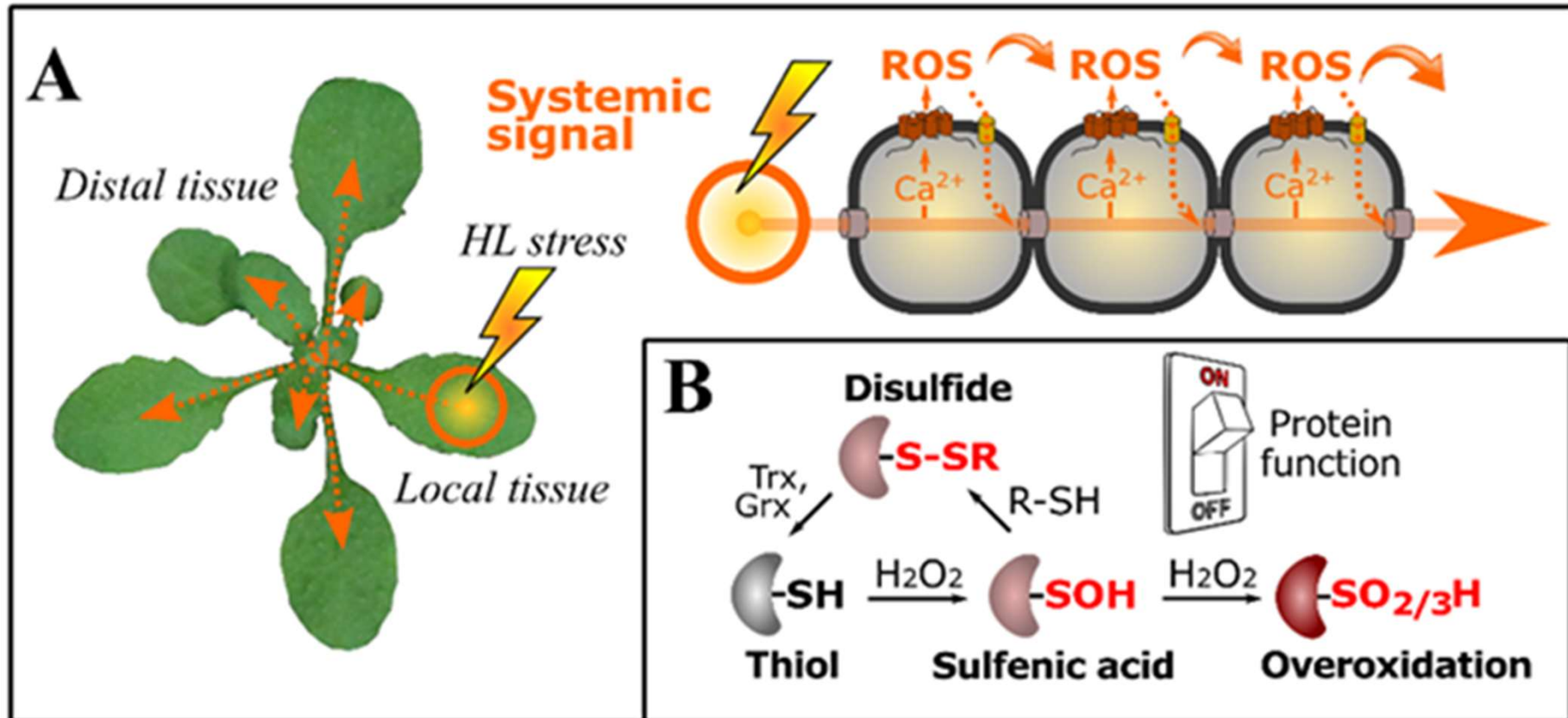
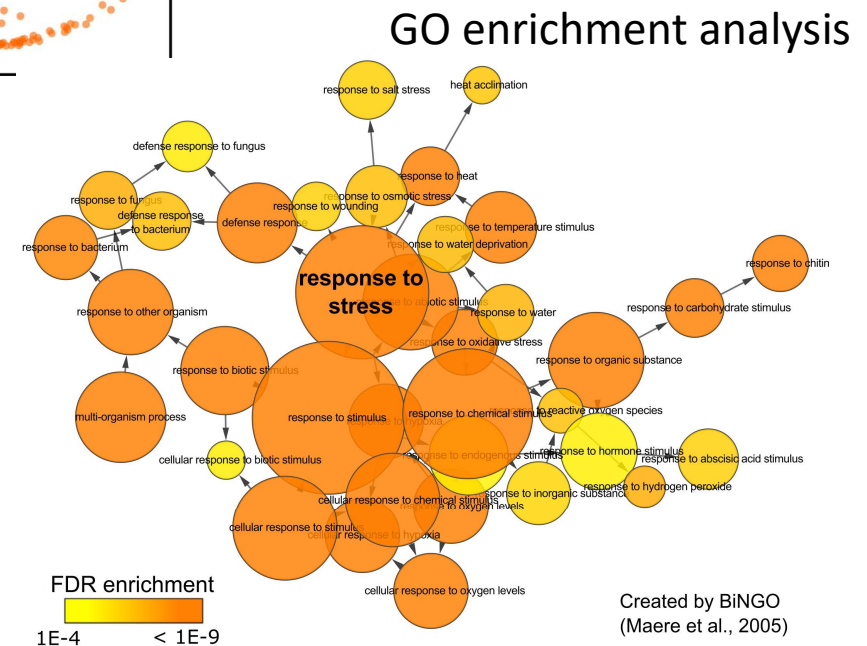
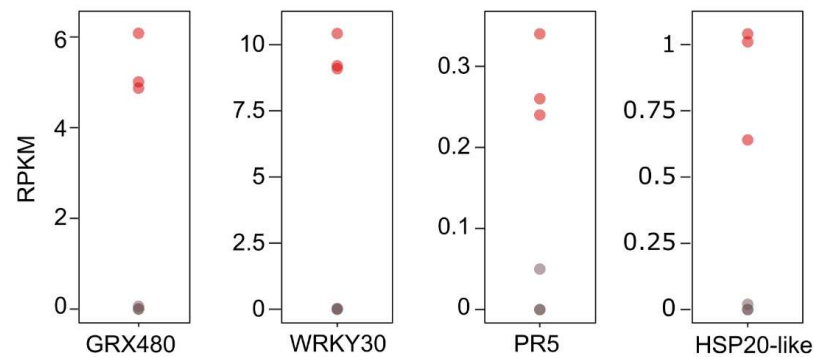
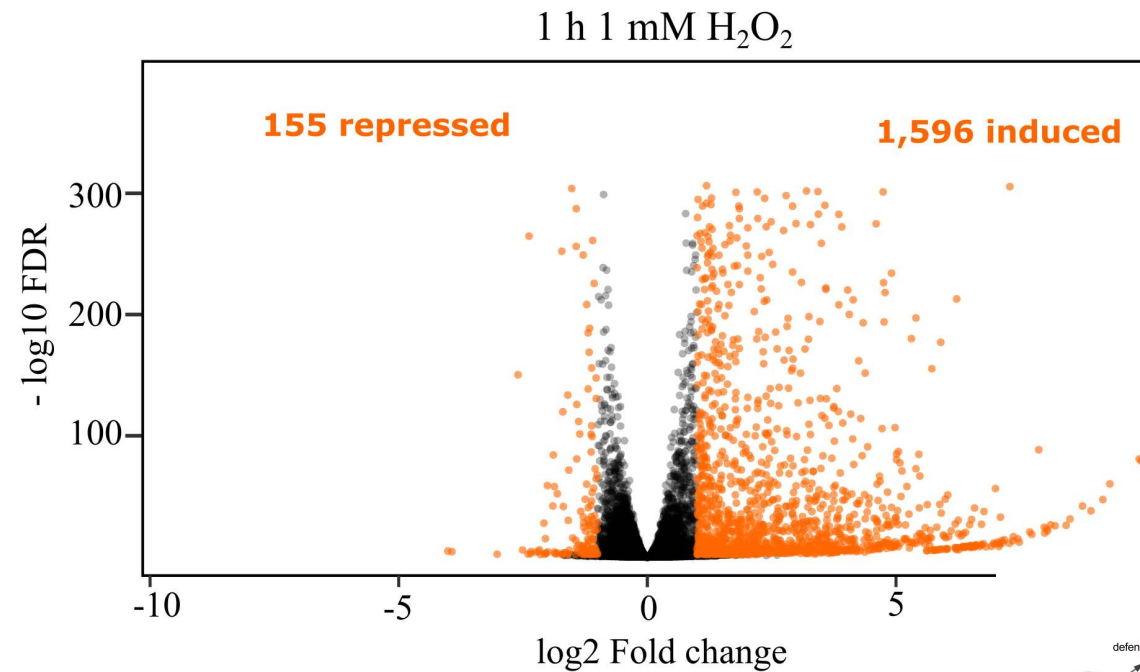


Fig. 1. Principle of plant defense activation by priming. Treatment with priming agents (left) induces endogenous plant defense mechanisms (middle), leading to subsequent stress tolerance in the primed plants. Agrochemicals are predominantly applied as a uniform spray solution (broadcast application) that can easily result in chemical waste, excessive carryovers, and damage to the vegetation. In contrast, directed application targets individual plants. Technological advances, such as computer vision and machine learning, make directed application more efficient than ever. Specific spraying of individual plants allows reduction of the dosage that can eliminate up to 90% of the used chemical and mitigate its environmental impact. Another exciting technology that will soon be available to the farmers combines agrochemicals with nanobodies as formulation agents that specifically target leaves or pests, improving retention and limiting the input of chemicals in the environment.

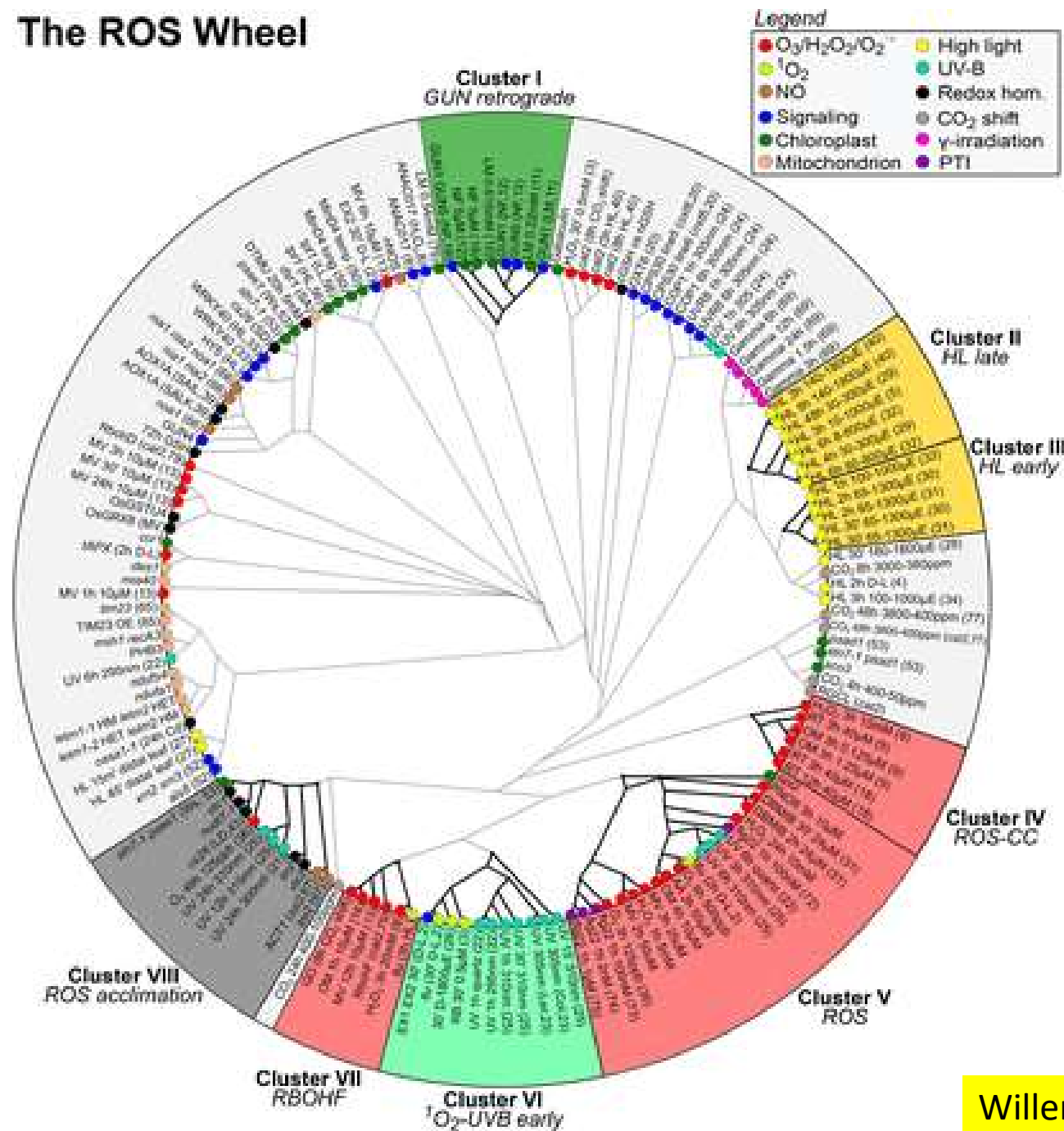
H₂O₂ in 'Systemic Acquired Acclimation'



H₂O₂ has a profound effect on nuclear gene expression



The ROS Wheel



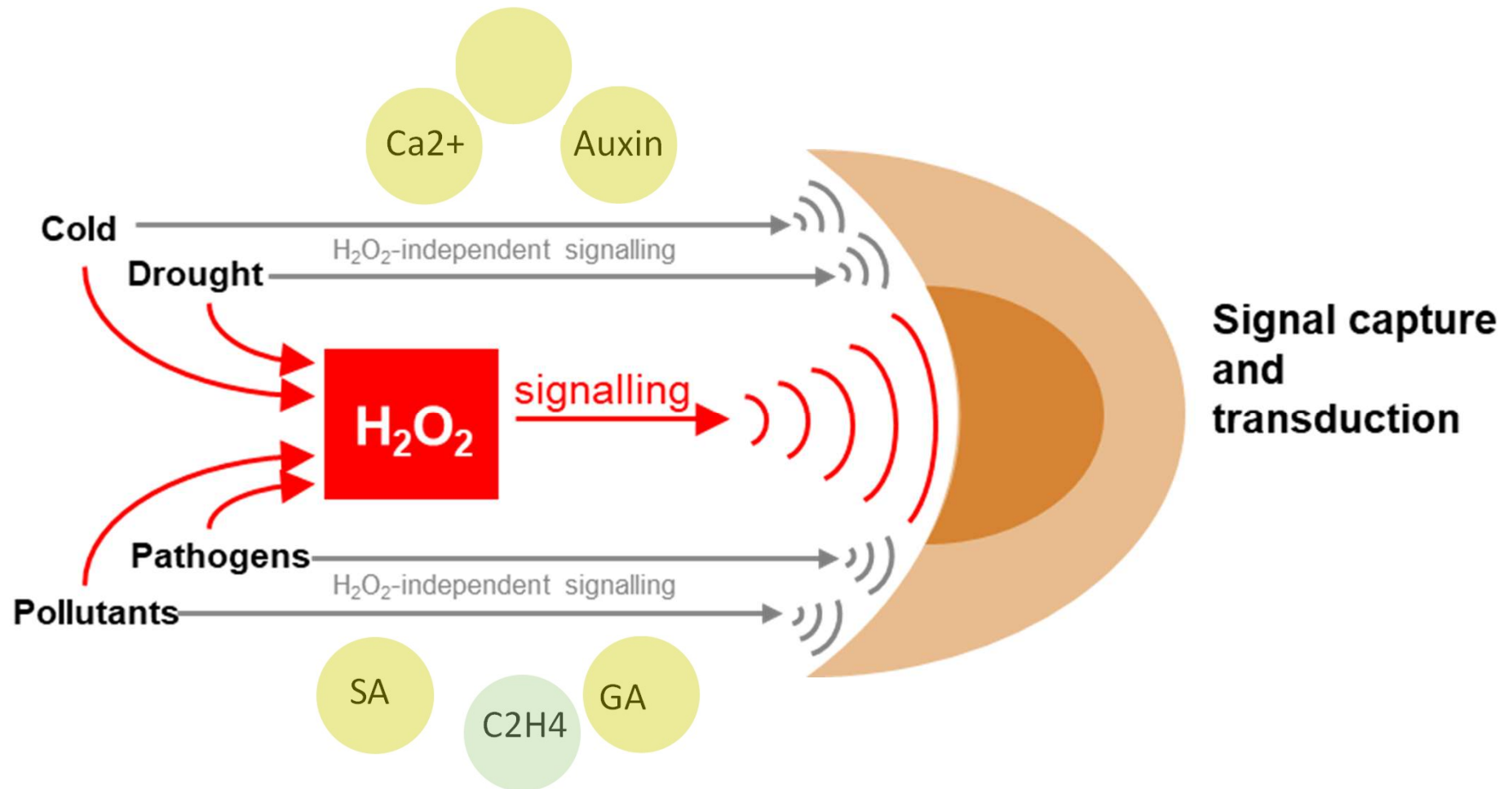
**Transcriptomic
footprints
disclose
specificity of
ROS signaling**

**~ROS
~ Organelle
~ Timing
~ Dose**



Willems et al. *Plant Physiology* 2016

H₂O₂ signaling contributes to the complex plant stress response



How plants sense H_2O_2 and transduce these stimuli into biological responses ?

What is the impact of increased H_2O_2 levels on plant cells ? How to perturb negative impact ?

ROS-related Organellar Signaling



Redox strategies for crop improvement ?

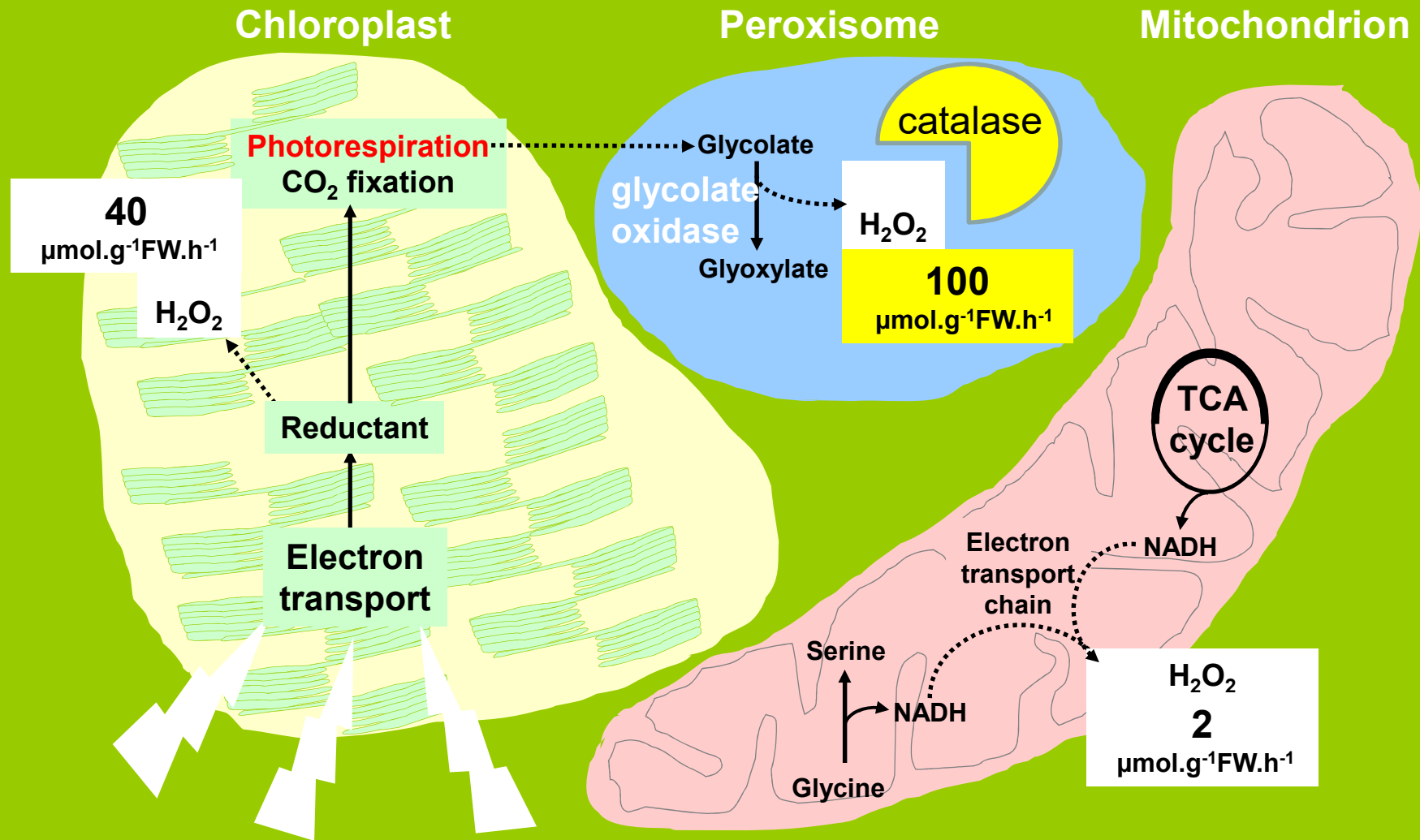
1. Forward and chemical genetics

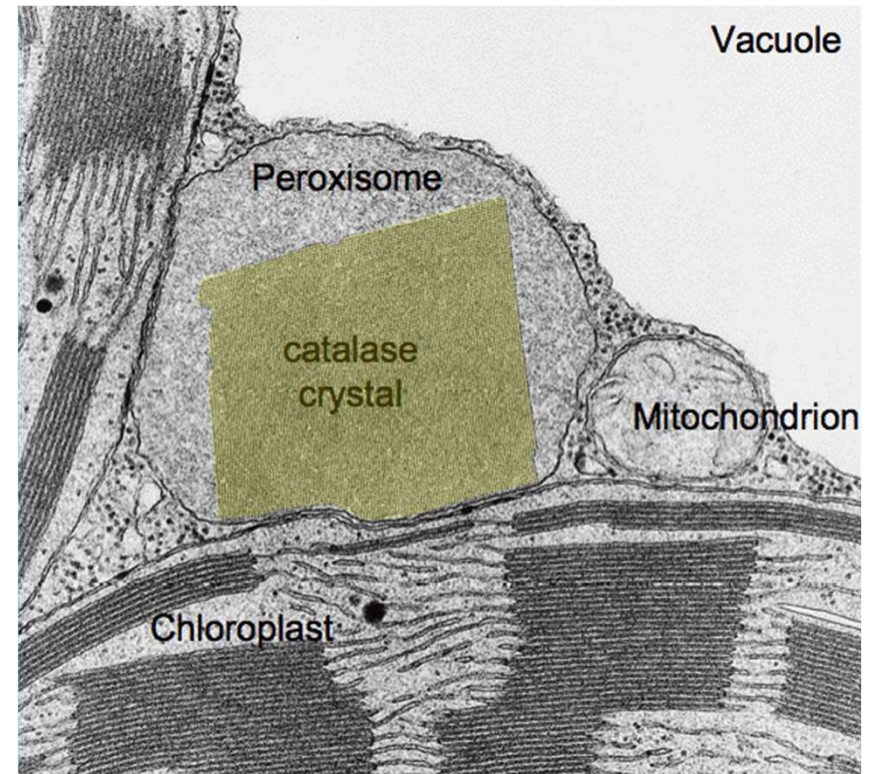
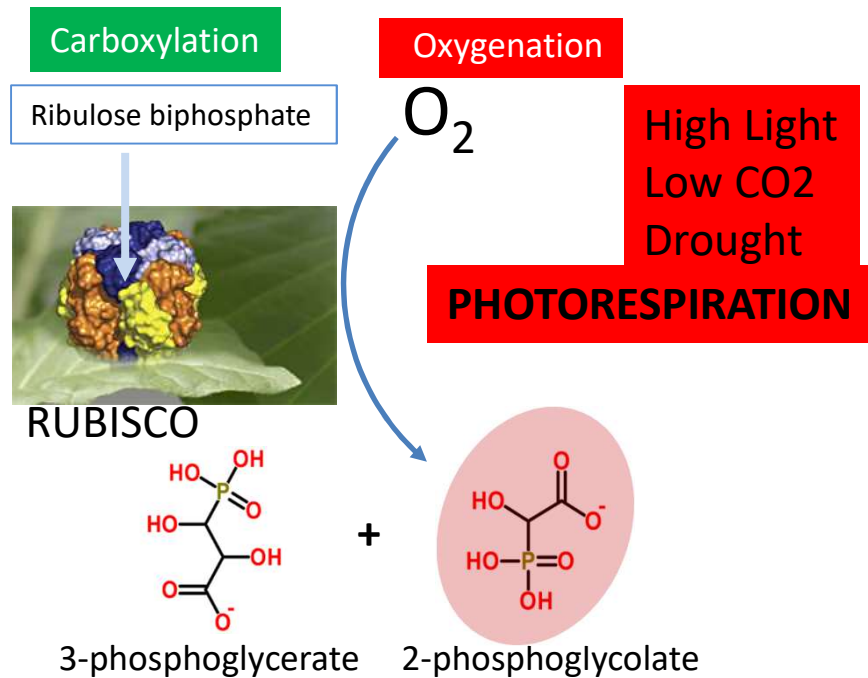
**GENETIC AND CHEMICAL PERTURBATIONS THAT
MITIGATE H_2O_2 INDUCED DAMAGE/CELL DEATH**

2. Redox Proteomics

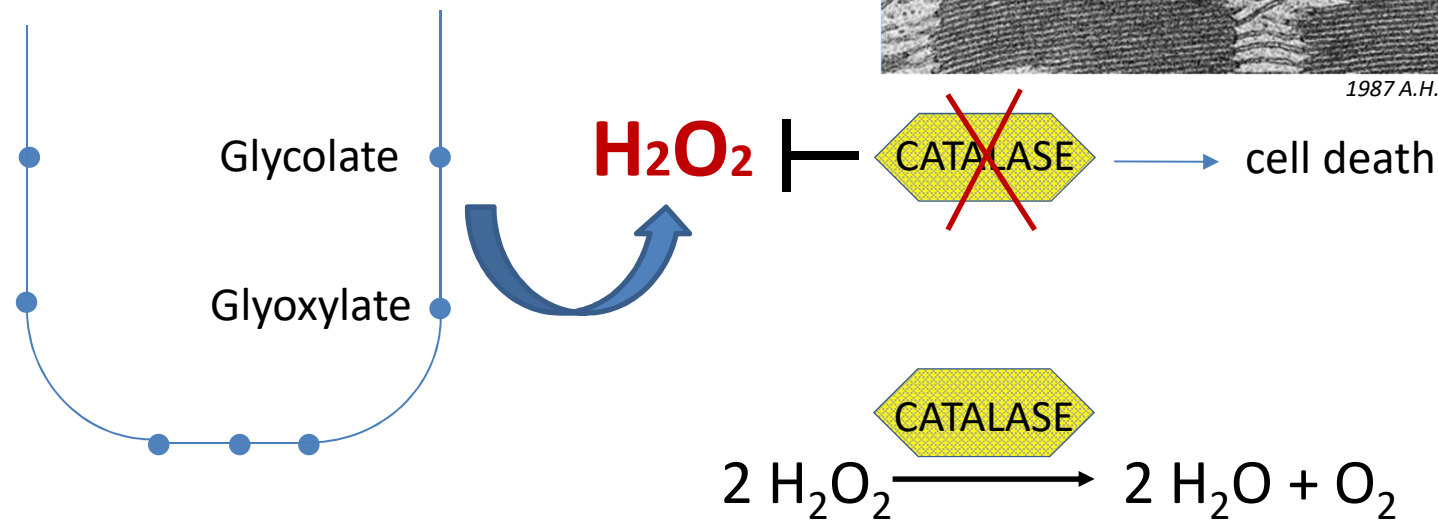
OXIDATIVE SULFUR SWITCHES IN PLANTS

Organelle H₂O₂ sources: the photorespiratory pathway is a major source of H₂O₂ in the light

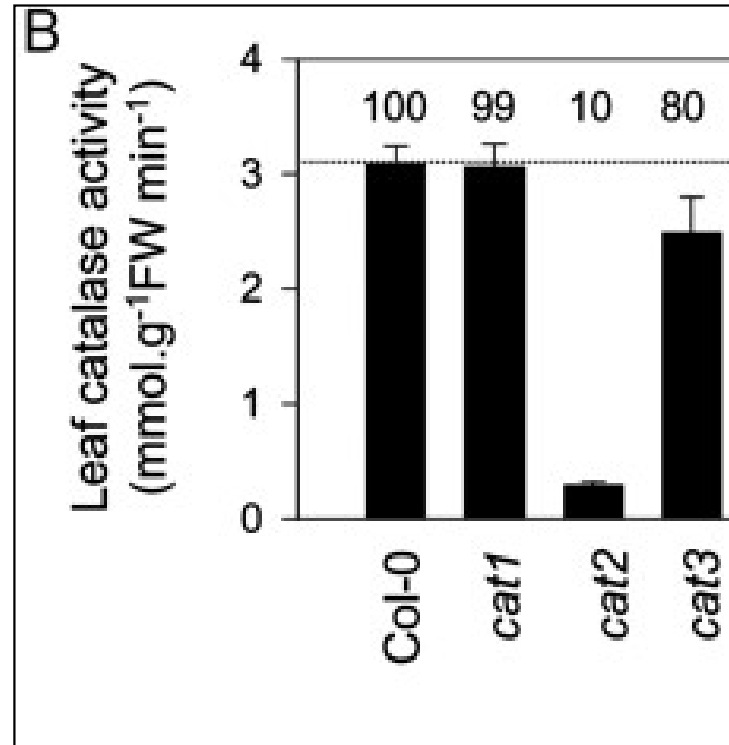




1987 A.H.C Huang, Academic Press, NY



CATALASE2 is responsible for ~ 90% of total catalase activities in green leaves



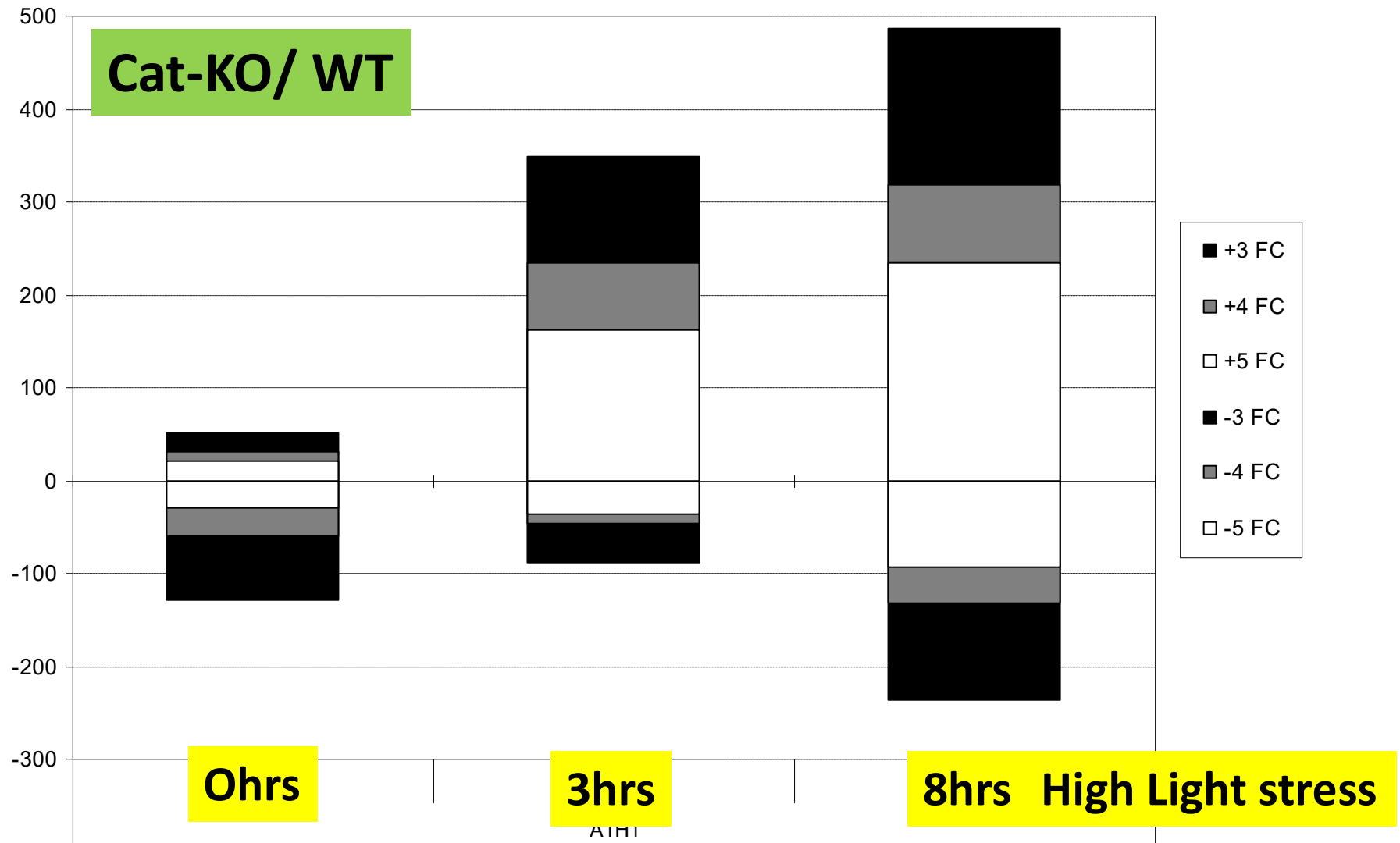
T-DNA knockout lines in *Arabidopsis* catalase isoforms

Plant Catalases: peroxisomal redox guardians



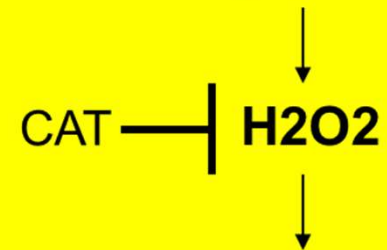
High CO₂; moderate light -----> ambient CO₂; high light (24hrs)

Increased H₂O₂ reprograms **nuclear gene expression**





Photorespiration (HL, low CO₂)

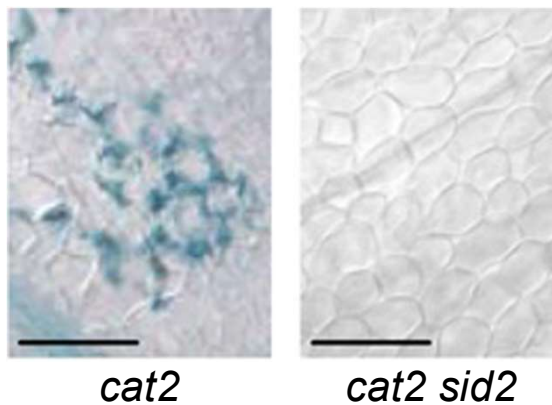
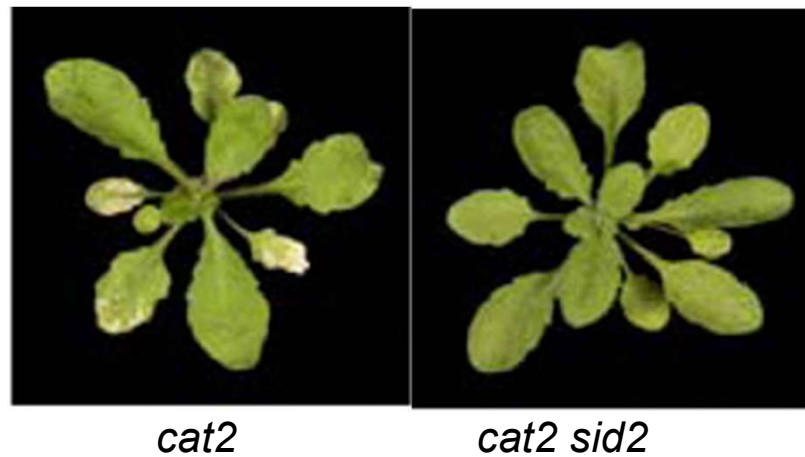


**defense response
cell death**

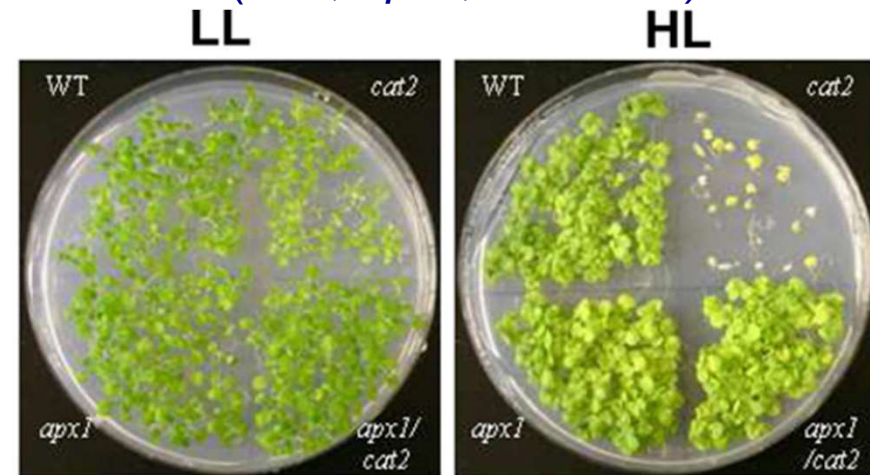
Vandenabeele et al. (2003; 2004)
Vanderauwera et al. (2005)
Queval et al. (2007)

The cell death phenotype is a genetically programmed process

cat2 phenotype is reversed by additional mutations (*sid2*, *apx1*, and *cad2*)

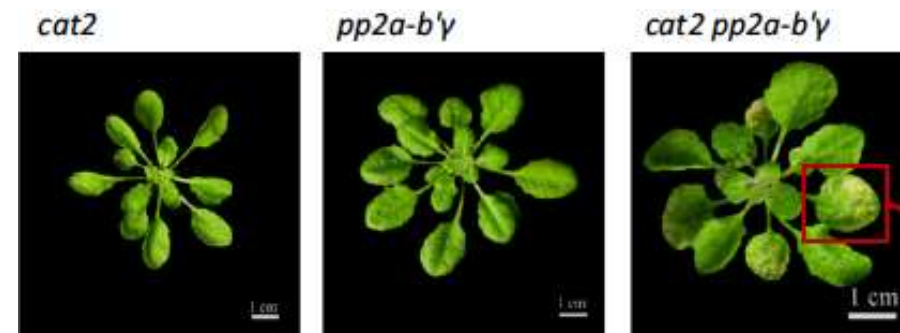


Chaouch et al., 2010



Vanderauwera et al., 2011

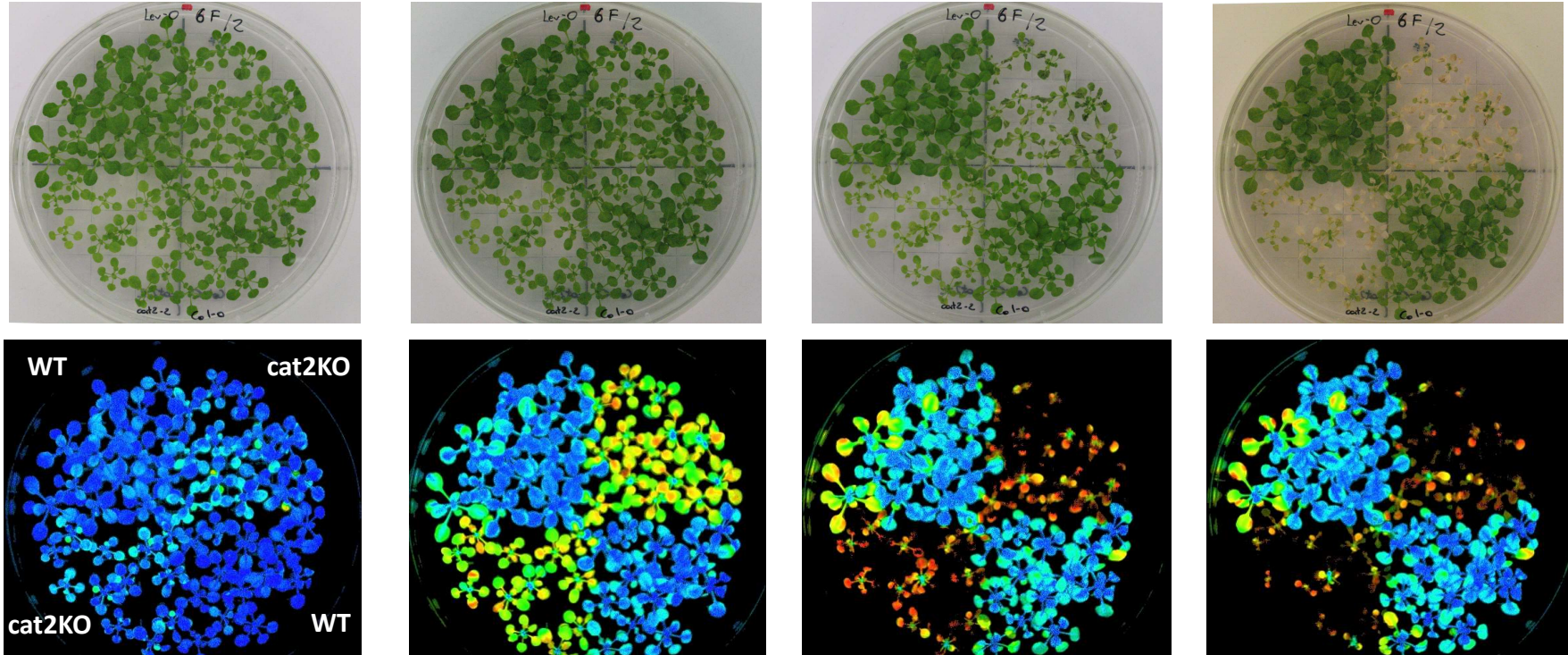
and enhanced by *pp2a-b'γ* mutation



Li et al., 2014

Photorespiratory H_2O_2 acts as a signal rather than as cytotoxic molecule

Bioassay: controlled induction of photorespiration



Restriction of gas exchange by sealing of petridishes + constant light regime:

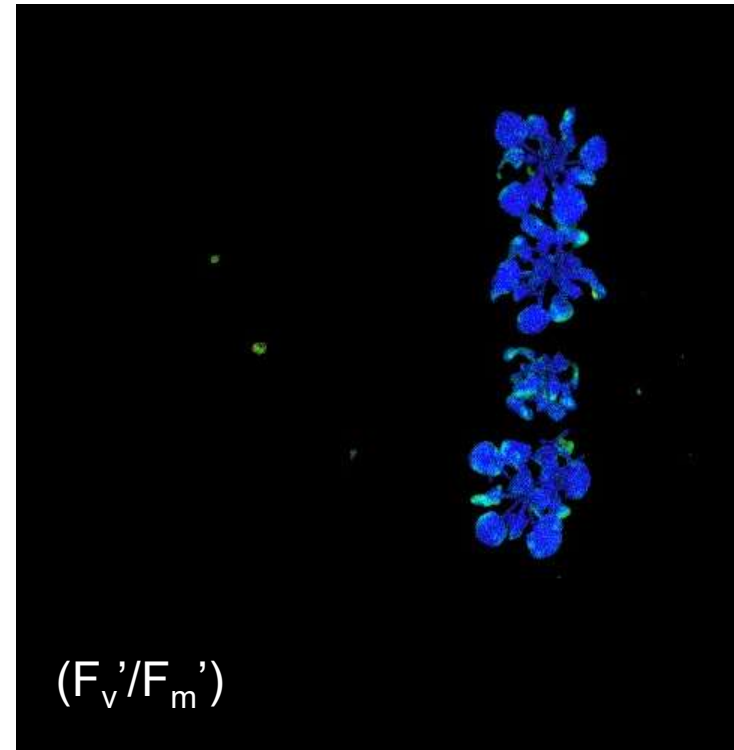
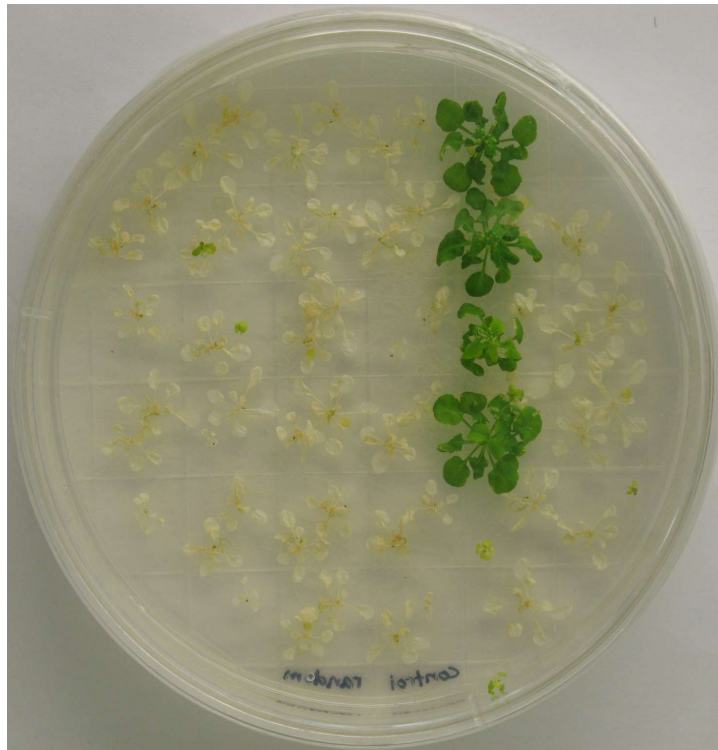
decreased CO₂ levels
controlled induction of photorespiration



In vitro photorespiratory bioassay

Restricted Gas exchange – Constant Light

Cell death induction in *CAT2* knock-out plants

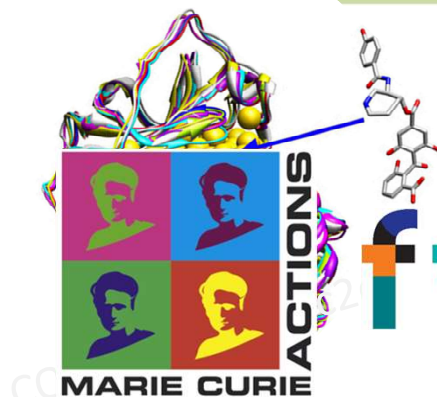
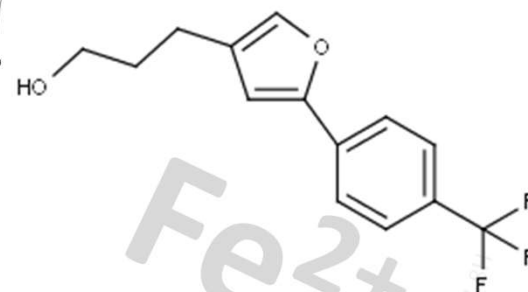
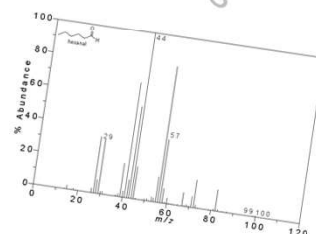


Catalase-deficient cell death phenotype within an in vitro plate assay (4 survivors are wild-type plants)

Forward screens for perturbations that mitigate the *negative* effects of photorespiratory H₂O₂

Genetic screen

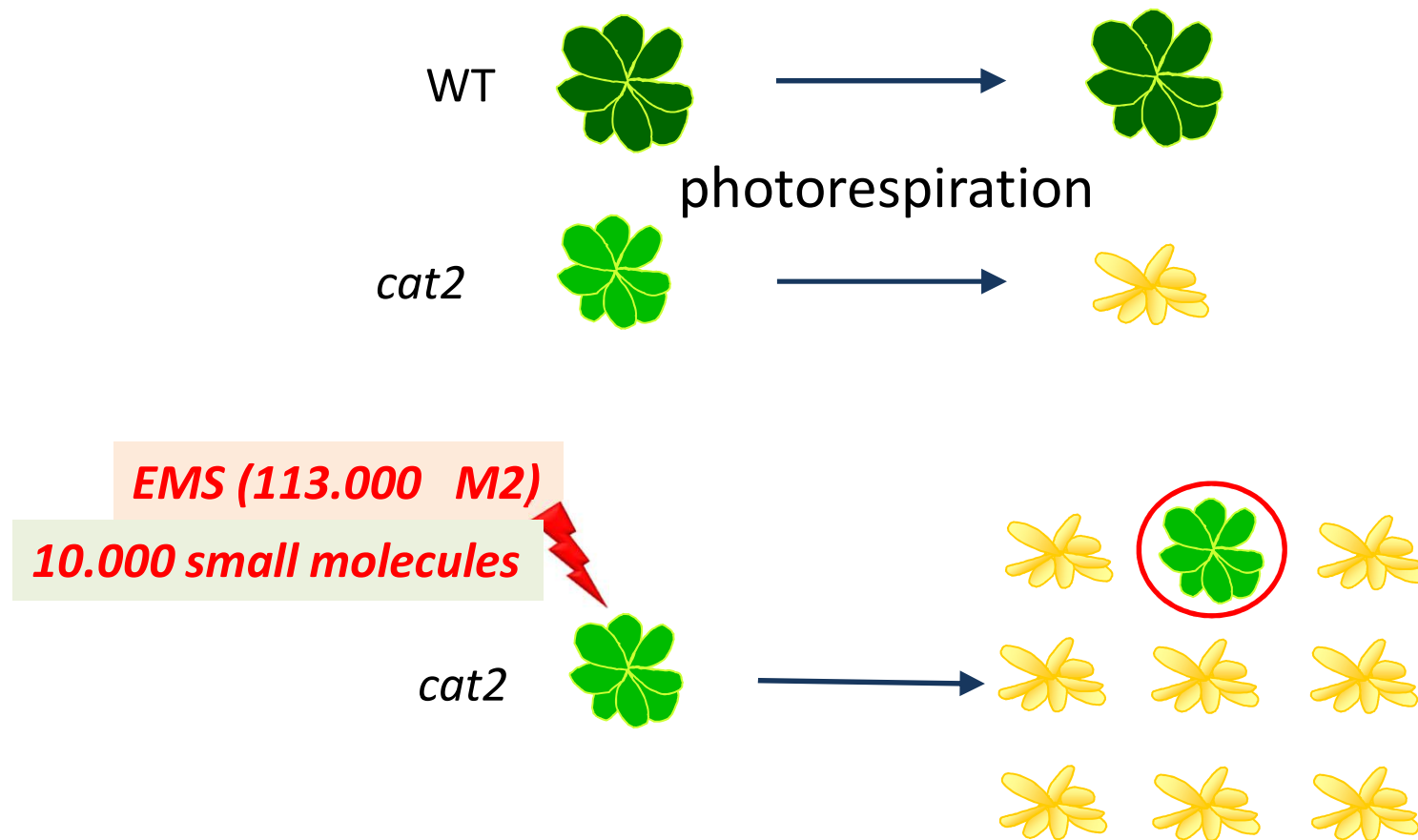
- ✓ Reduced photorespiration
- ✓ Improved resistance
- ✓ Translate to TILLING, Genome Editing strategies for crop improvement



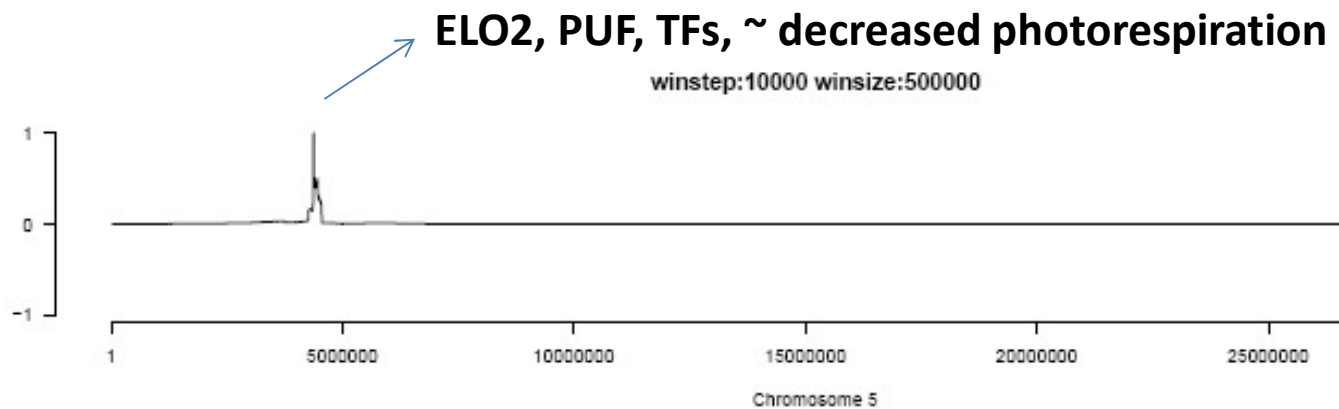
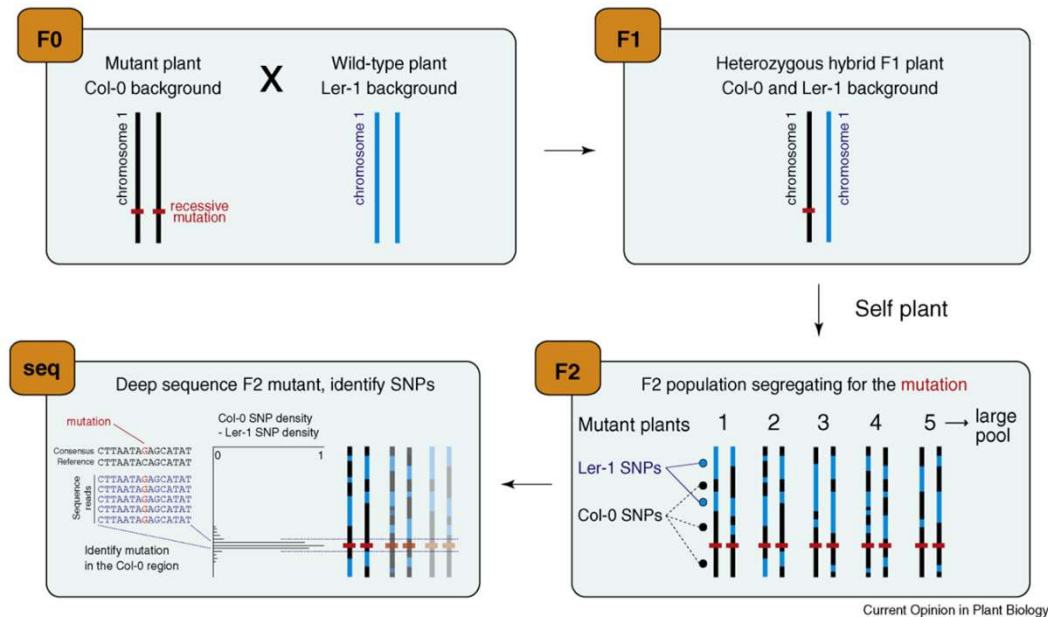
AGENTSCHAP
INNOVEREN &
ONDERNEMEN



Chemical and genetics screen to identify modulators of photorespiratory H₂O₂-induced cell death

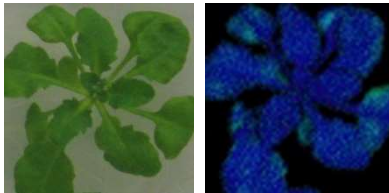


Causative mutation identification through deep sequencing

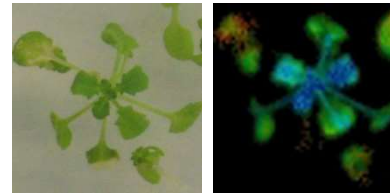


A suite of second-site mutations reverting *cat2*KO cell death

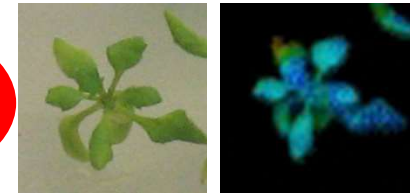
WT



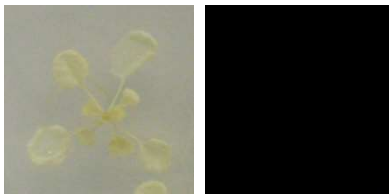
48-1



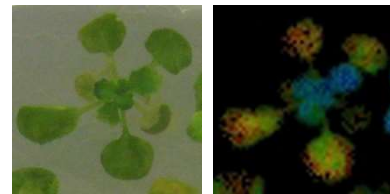
ELO



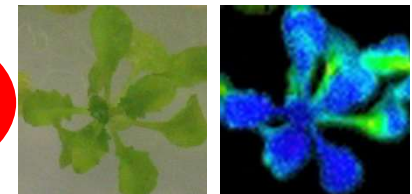
cat2^{-/-}



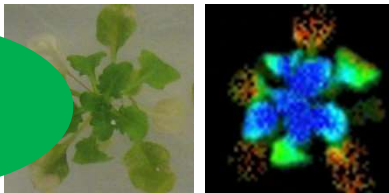
85-2



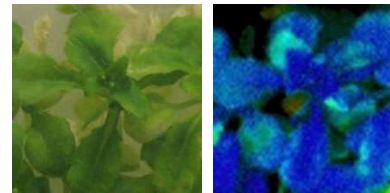
SHR



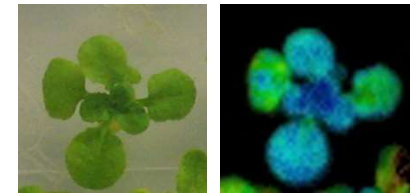
GLYR



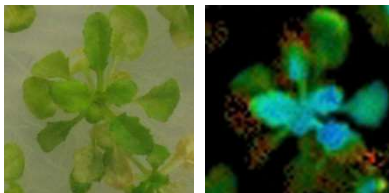
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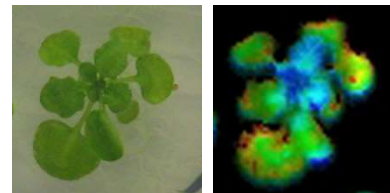
327-4



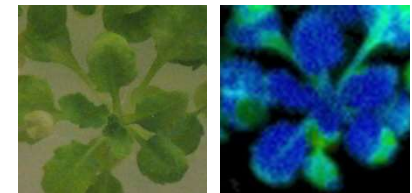
33-3



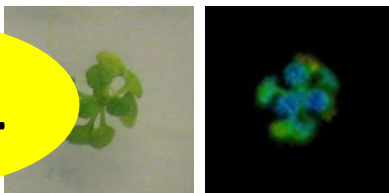
107-6



328-4



SME1



182-1

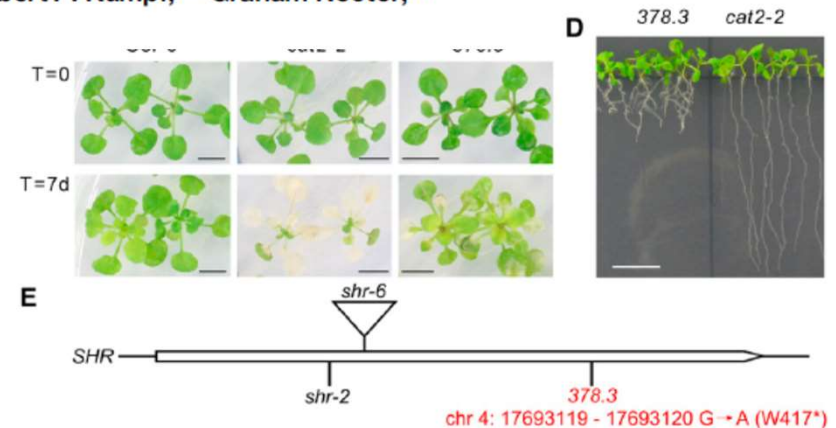


GOX1



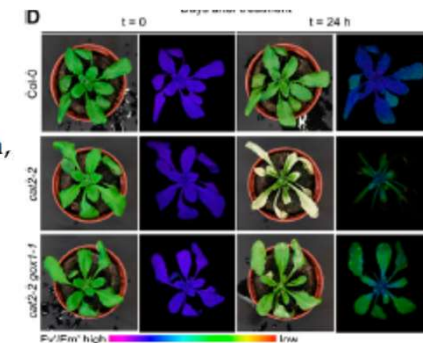
SHORT-ROOT Deficiency Alleviates the Cell Death Phenotype of the *Arabidopsis catalase2* Mutant under Photorespiration-Promoting Conditions

Cezary Waszczak,^{a,b,c,d,e,f,1} Pavel I. Kerchev,^{a,b,1} Per Mühlenbock,^{a,b,2} Frank A. Hoeberichts,^{a,b,3} Katrien Van Der Kelen,^{a,b} Amna Mhamdi,^{a,b,g,h} Patrick Willems,^{a,b} Jordi Denecker,^{a,b,4} Robert P. Kumpf,^{a,b} Graham Noctor,^{g,h} Joris Messens,^{c,d,e} and Frank Van Breusegem^{a,b,5}



Lack of GLYCOLATE OXIDASE1, but Not GLYCOLATE OXIDASE2, Attenuates the Photorespiratory Phenotype of CATALASE2-Deficient Arabidopsis^{1[OPEN]}

Pavel Kerchev², Cezary Waszczak², Aleksandra Lewandowska, Patrick Willems, Alexey Shapiguzov, Zhen Li, Saleh Alseekh, Per Mühlenbock, Frank A. Hoeberichts, Jingjing Huang, Katrien Van Der Kelen, Jaakko Kangasjärvi, Alisdair R. Fernie, Riet De Smet, Yves Van de Peer, Joris Messens, and Frank Van Breusegem*





bioRxiv

THE PREPRINT SERVER FOR BIOLOGY

bioRxiv posts many COVID19-related papers. A reminder: they have not been formally peer-reviewed and should not guide health-related behavior or be reported in the press as conclusive.

New Results

[Follow this preprint](#)

Mutation of Arabidopsis SMEI and Sm core assembly improves oxidative stress resilience

Patrick Willems, Valerie Van Ruyskensvelde, Takanori Maruta, Robin Pottie,
 Álvaro Daniel Fernández-Fernández, Jarne Pauwels, Matthew A. Hannah, Kris Gevaert,
 Frank Van Breusegem, Katrien Van der Kelen

doi: <https://doi.org/10.1101/2022.04.12.488072>

This article is a preprint and has not been certified by peer review [what does this mean?].



Abstract

Full Text

Info/History

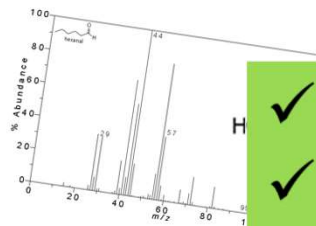
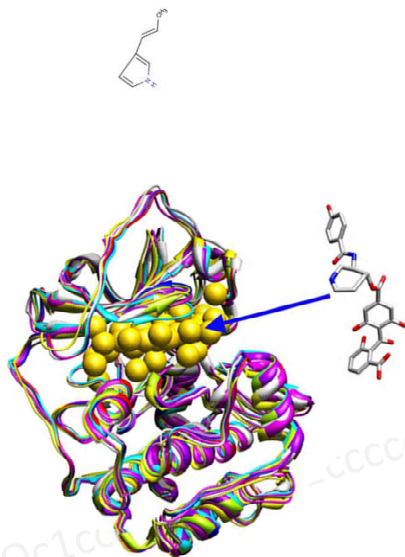
Metrics

[Preview PDF](#)

Small molecules:

yet another way to perturb the system

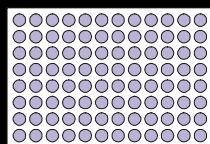
Chemical regulation of plant stress tolerance and yield



- ✓ Overcome gene redundancy
- ✓ Overcome loss-of-function lethality
- ✓ Inducible perturbations
- ✓ New leads to new agrochemicals

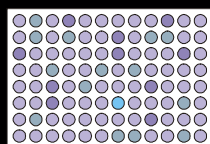
Chemical screen

Phenotype-based



*Plate of
cells/organisms*

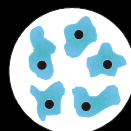
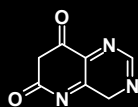
↓ Add compounds (10,000)



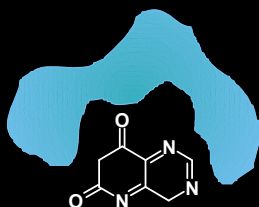
*Screen for
phenotypic changes*



**Compound that produces
phenotype of interest**

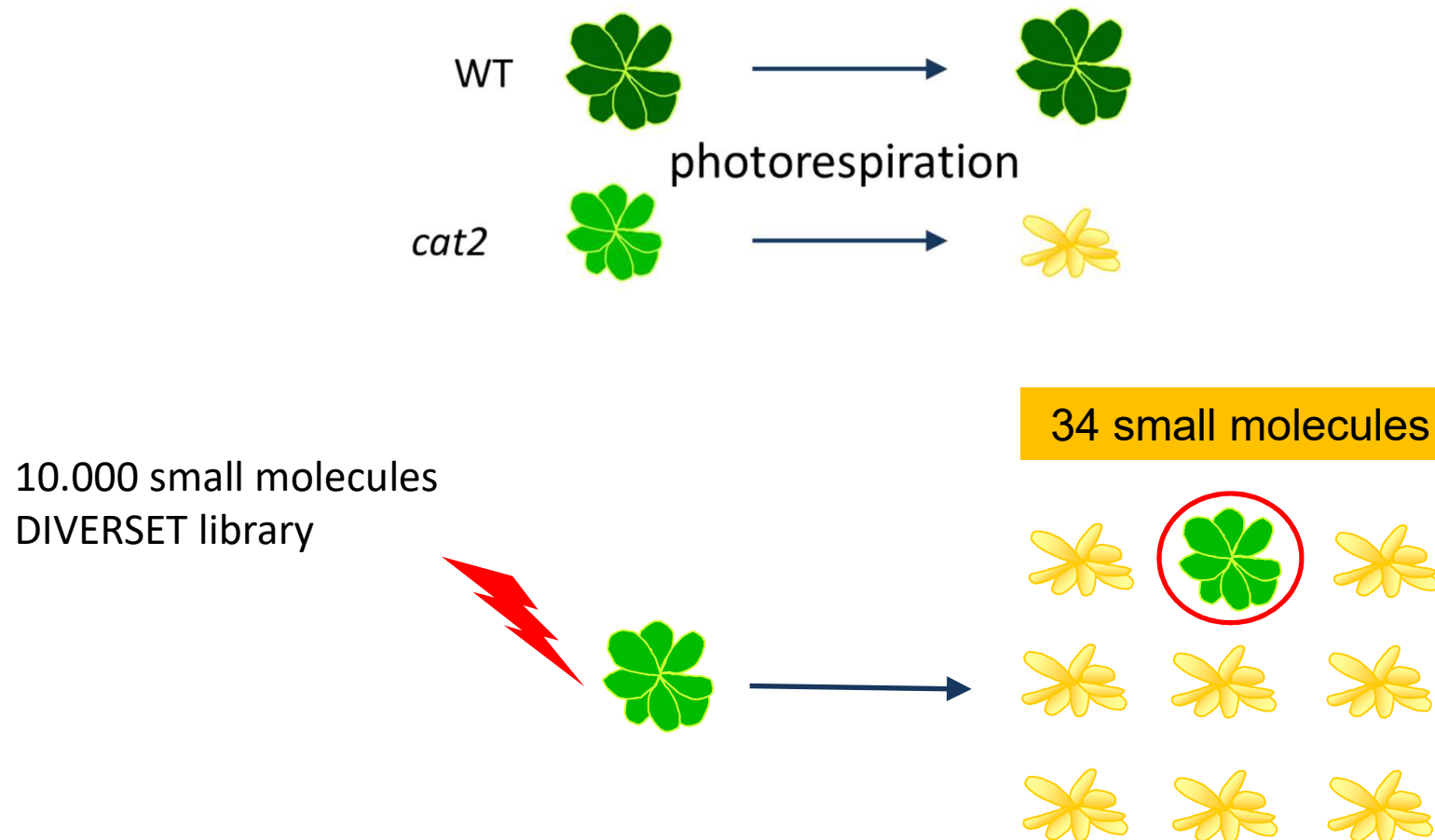


Identify protein target or Mode of Action



Target identification

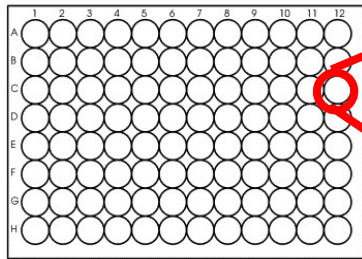
Chemical screen to alleviate photorespiratory H₂O₂-induced cell death



Miniaturised photorespiratory bio-assay



Plant seeds (0d)



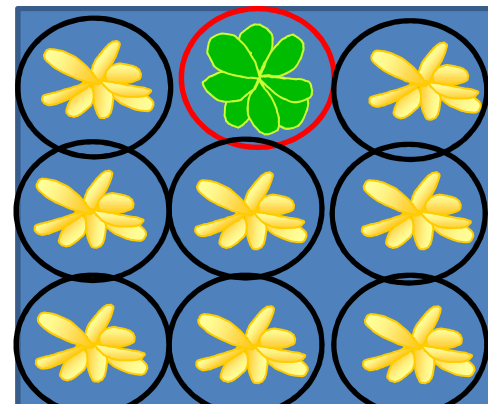
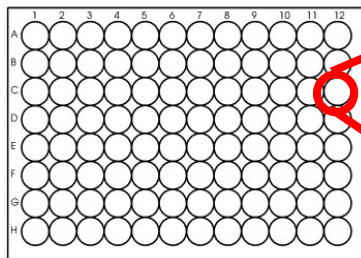
Analyze (14 - 21d)

Plate CD384aA0011

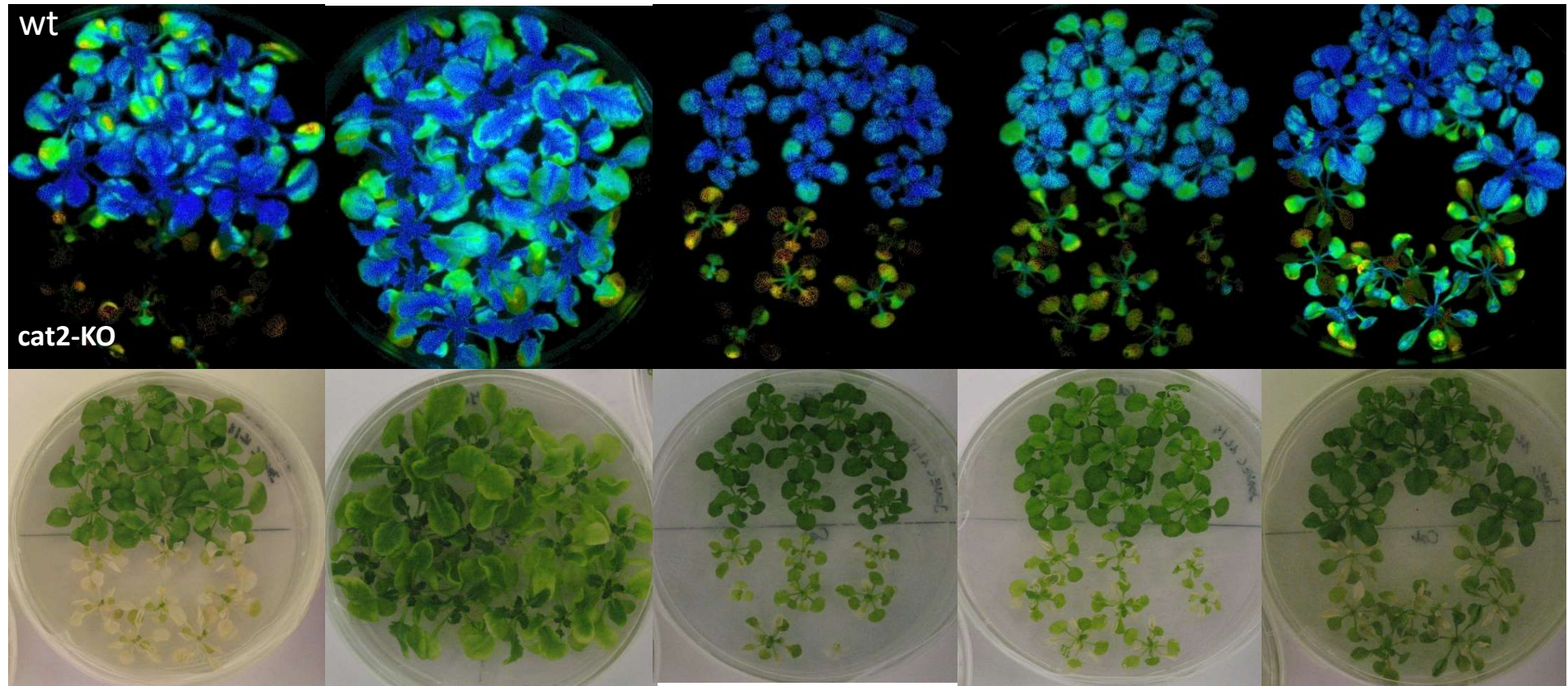
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
B	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
C	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
D	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
E	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
F	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
G	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
H	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
I	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
J	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
K	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
L	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
M	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
N	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
O	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
P	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X



+ stress + chemicals (7d)
10.000 molecules screened



34 different small molecules revert the *cat2*-KO phenotype



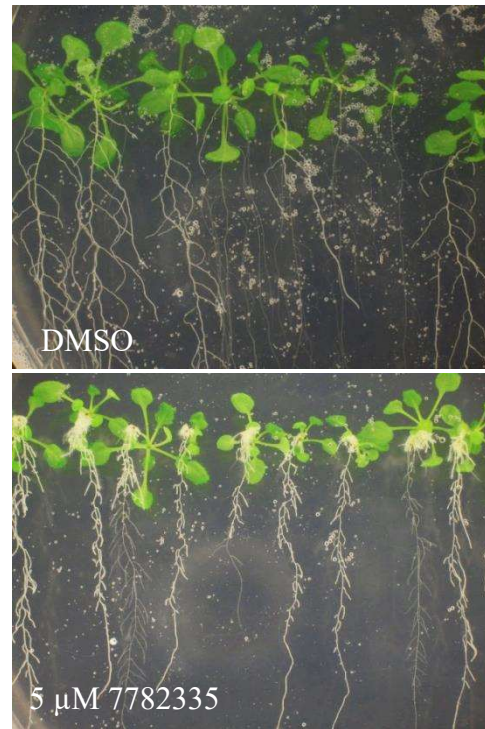
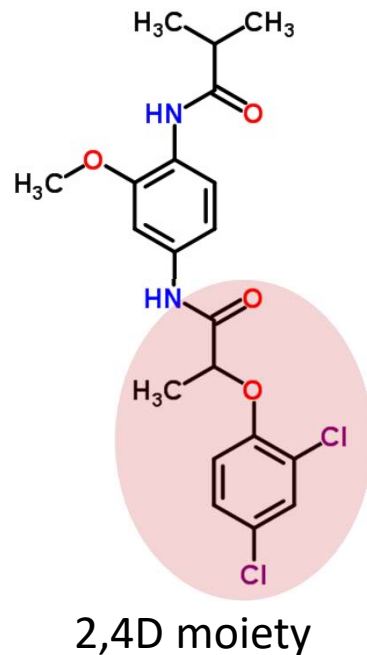
DMSO

"77"

- No antioxidant activities (ROS quenching assay).
- Target identification (photorespiration ?; defense response,...)
- Mechanism elucidation (gene expression, yeast ...)

Compound 77 triggers an auxin-like response

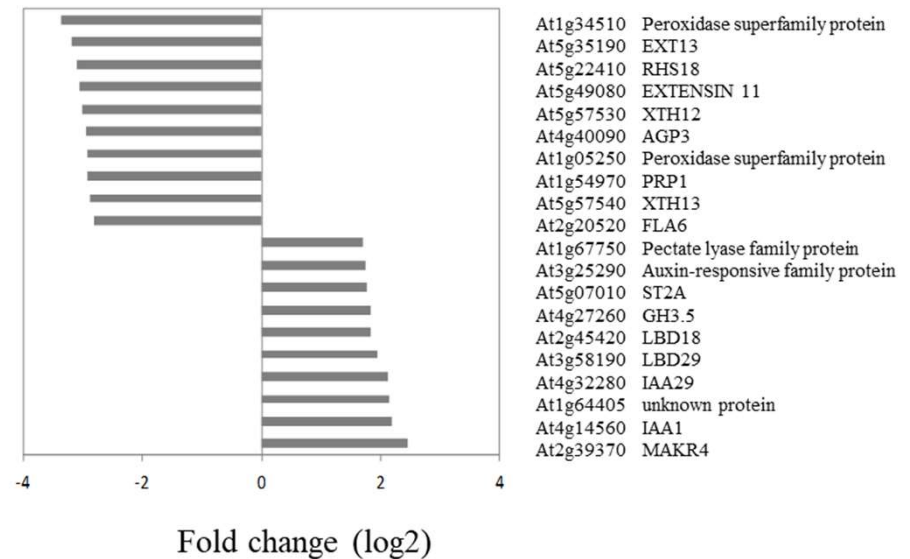
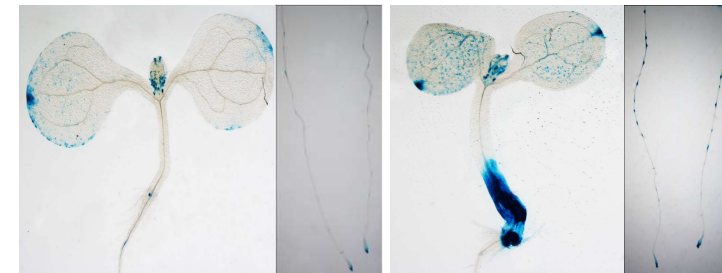
2-(2,4-dichlorophenoxy)-N-[4-(isobutyrylamino)-3-methoxyphenyl]propanamide



Increased Lateral Root formation
Shorter primary root

Kerchev, Muhlenbock et al., 2014

Induction of DR5:GUS



IAA, NAA transcriptome
TIR1 dependent

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