3rd TRAINING SCHOOL "PlasmaS FOR plant AND FOOD PROCESSING"

Advances and prospects in the understanding of seed germination and dormancy

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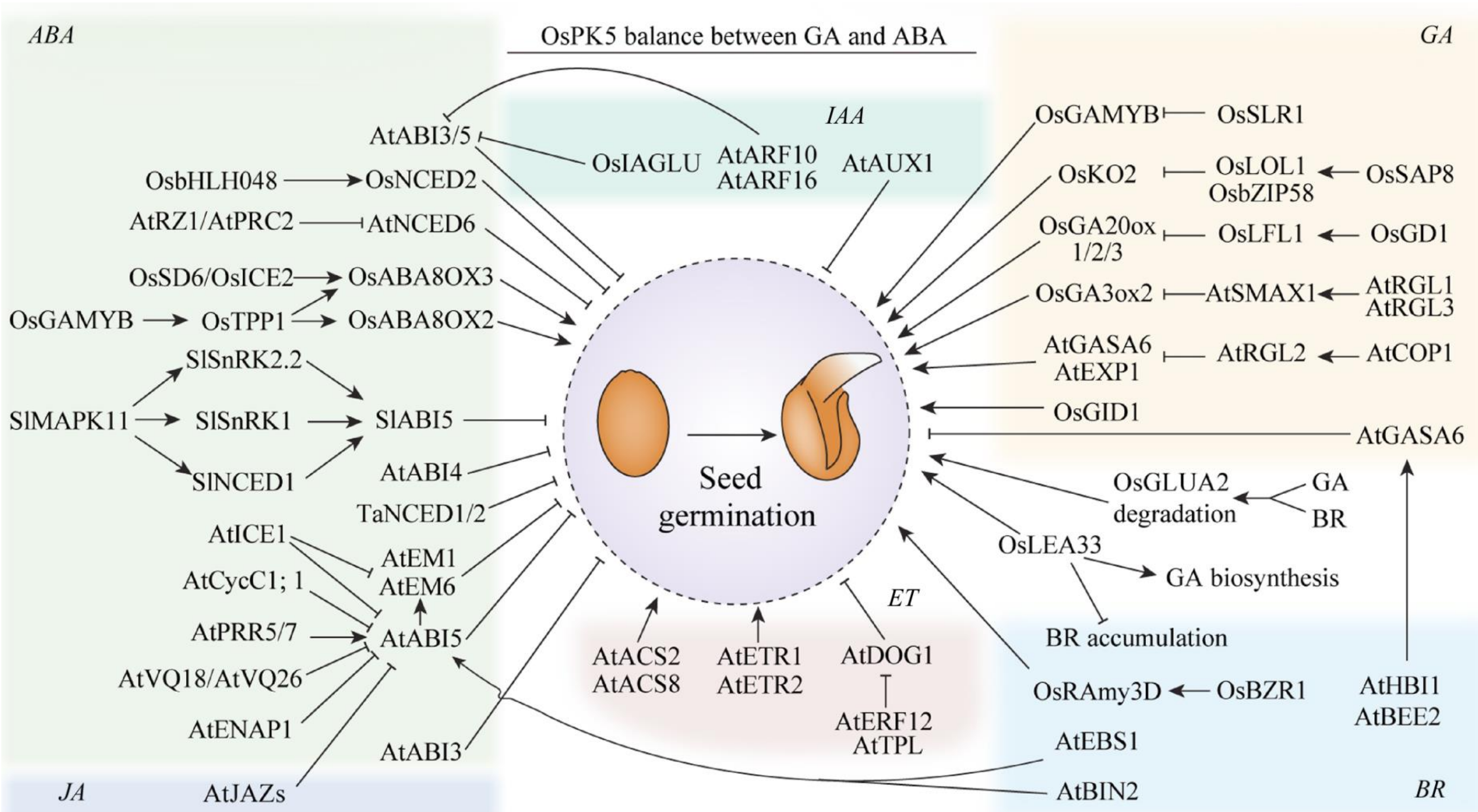
The molecular network regulating seed germination is complex....

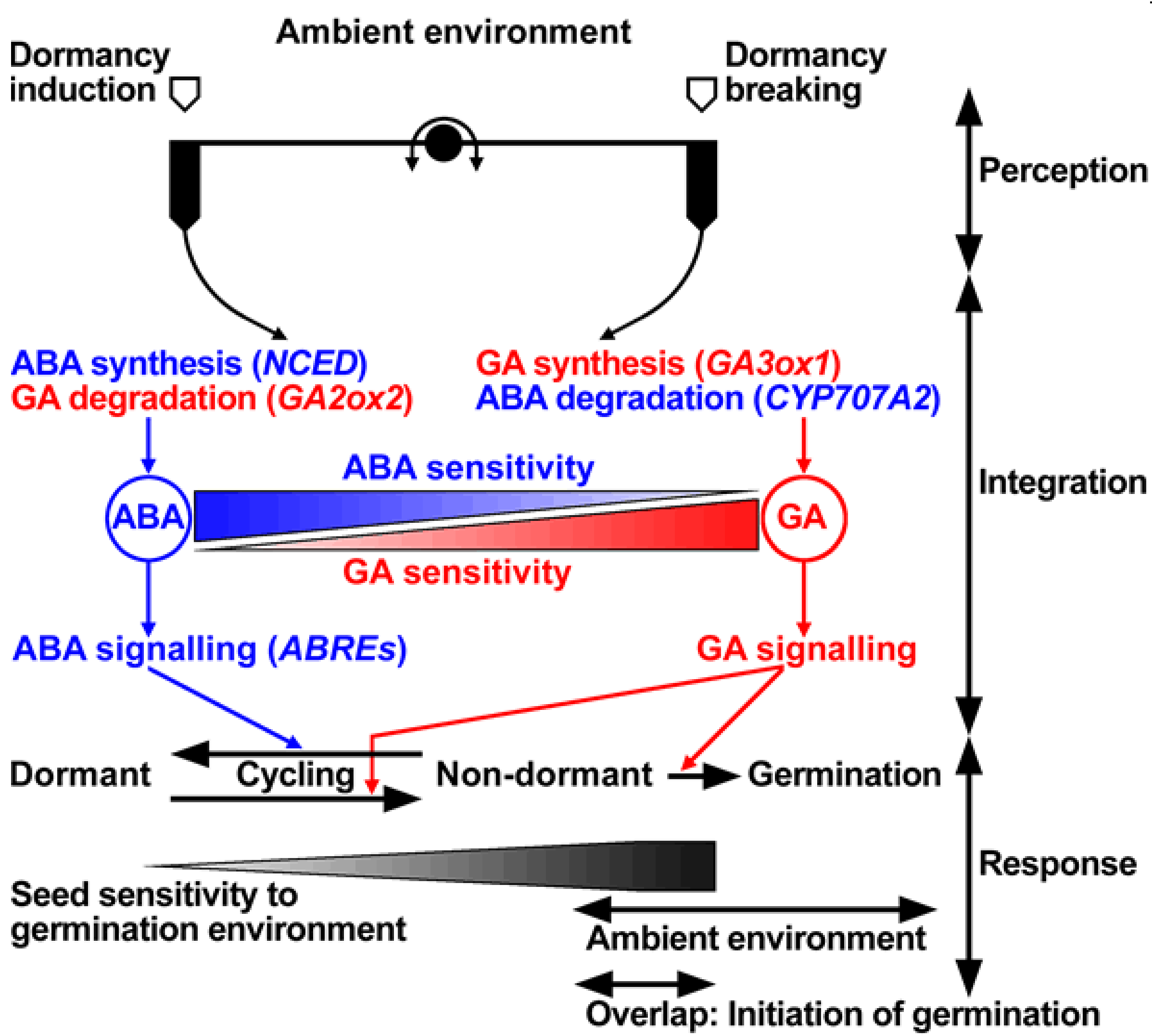


Jia Zhao, Yongqi He, Hongsheng Zhang, Zhoufei Wang

Advances in the molecular regulation of seed germination in plants

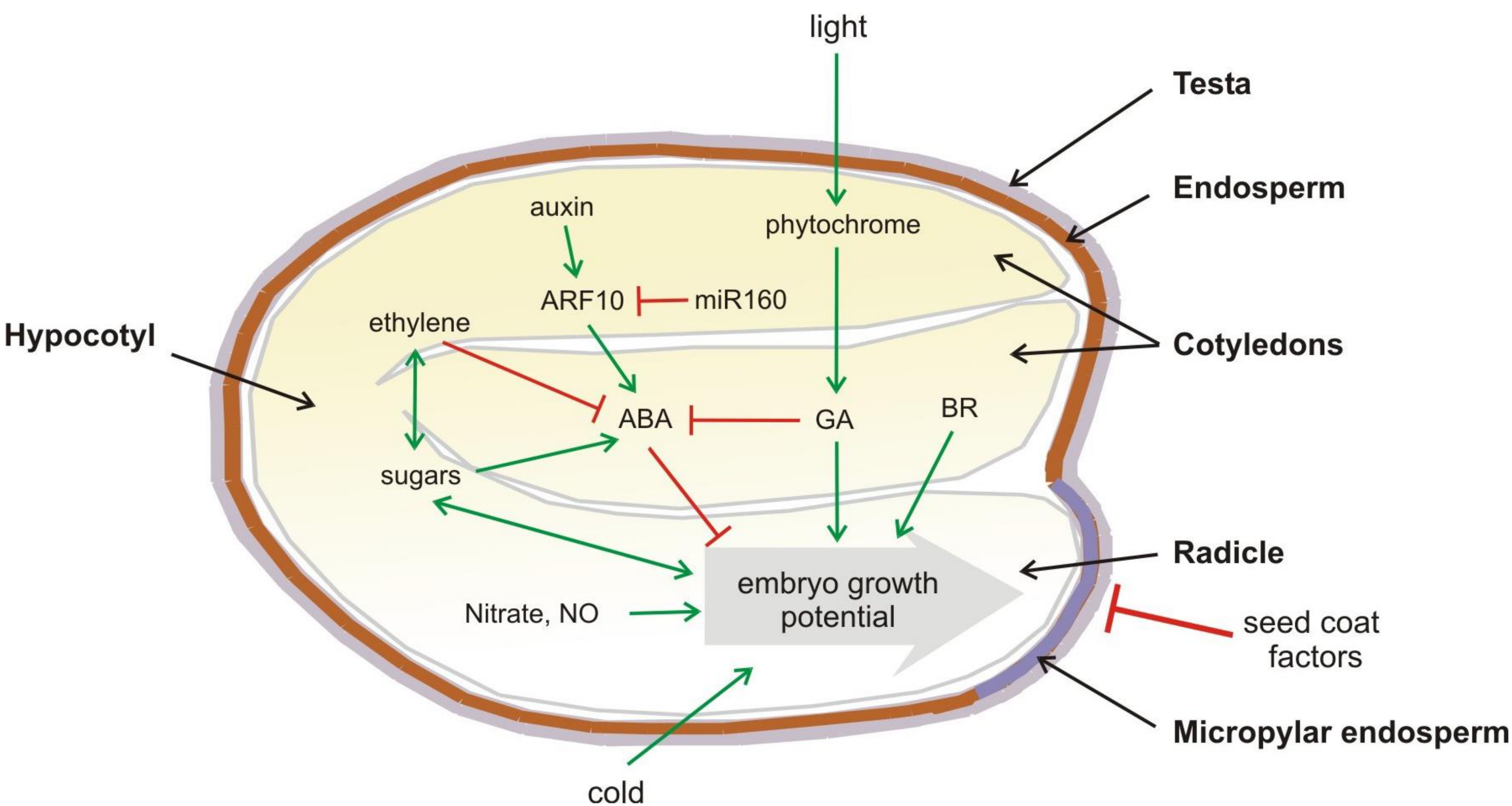
https://doi.org/10.48130/seedbio-0024-0005

....but mostly relies on a balance beween ABA and GA...



Tansley review 'Seed dormancy and the control of germination' by Finch-Savage and Leubner-Metzger (2006)
New Phytologist 171: in press - © Blackwell Science, http://www.newphytologist.org

....that responds to environmental and endogenous factors...



...including Reactive Oxygen Species (ROS)

ROS: candidates for being involved in the sensing of environmental conditions by seeds

Reactive Oxygen Species

Reactive oxygen species (ROS) are **highly reactive chemicals formed from O_2**. ROS are byproducts of the normal metabolism of oxygen

Free radicals:

- At least an unpaired electron

$O_2^{\cdot -}$	Superoxide
$\cdot OH$	Hydroxyl
$RO_2\cdot$	Peroxyl
$RO\cdot$	Alkoxyl
$HO_2\cdot$	Hydroperoxyl

Non radical forms:

- Highly oxidizing

H_2O_2	**Hydrogen peroxide**
$HOCl^-$	hypochlorique acid
O_3	Ozone
$^1O_2$	Singlet oxygen
$ONOO^-$	Peroxynitrite

H_2O_2: the most « stable », can cross membranes, secondary messenger

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Role of ROS in dry seeds

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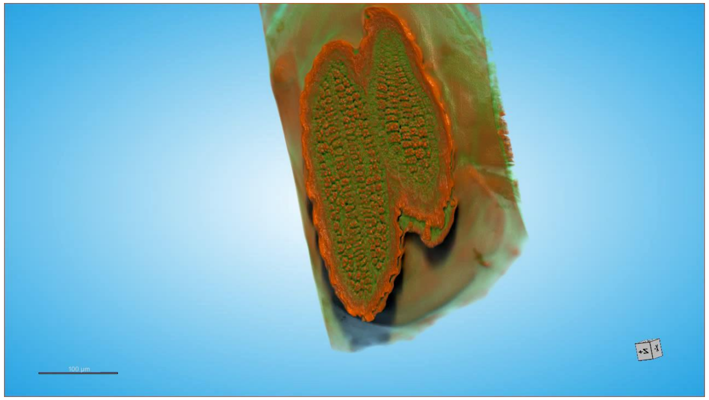
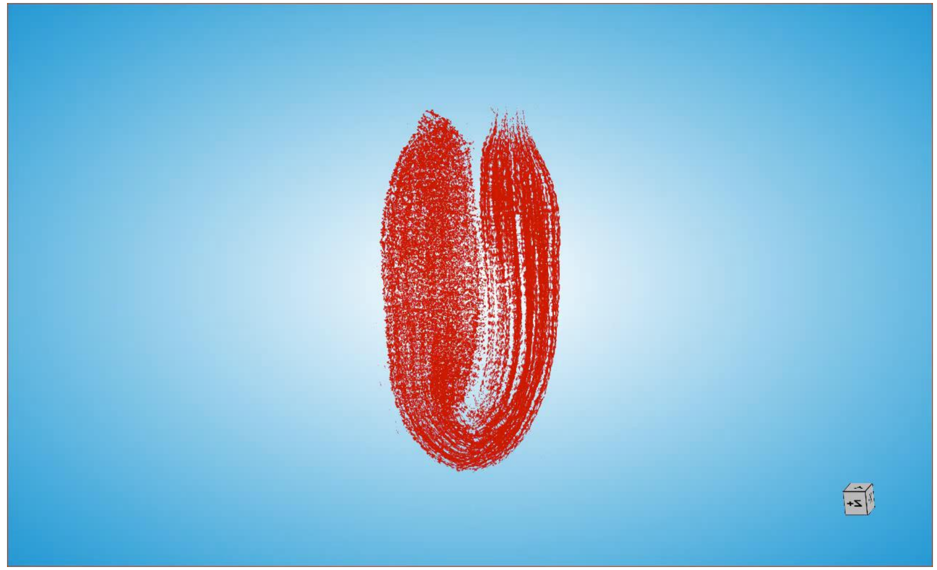
Orthodox seeds are anhydrobiotes (MC< 10%) where no metabolism occur

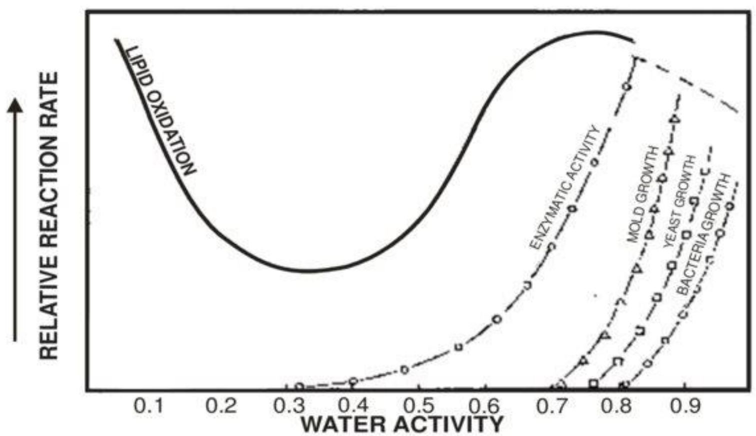




Tomography of Arabidopsis seed:
3D rendering of intercellular air space in the seed

But oxygen can diffuse within dry seeds…

…and continuously generates reactive oxygen species (ROS) through non-enzymatic reactions (eg. lipid oxidation)



Stability map of food as a function of water content
(modified from Labuza et al., 1972)

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Thus, ROS continuously accumulate in dry seeds with time

Ex: Seeds equilibrated at 33 % RH, stored at 5°C
Initial germination 100 %
Duration: 20 years (1987-2007)

Species	Germination (%) after storage in			
	air	vacuum	CO_2	N_2
Salvia	61	90	89	91
Pansy	32	83	84	74
Aster	0	62	31	67
Lettuce	81	98	99	99
Onion	7	95	91	94

Coll. HM Clause, M. Gaudillat

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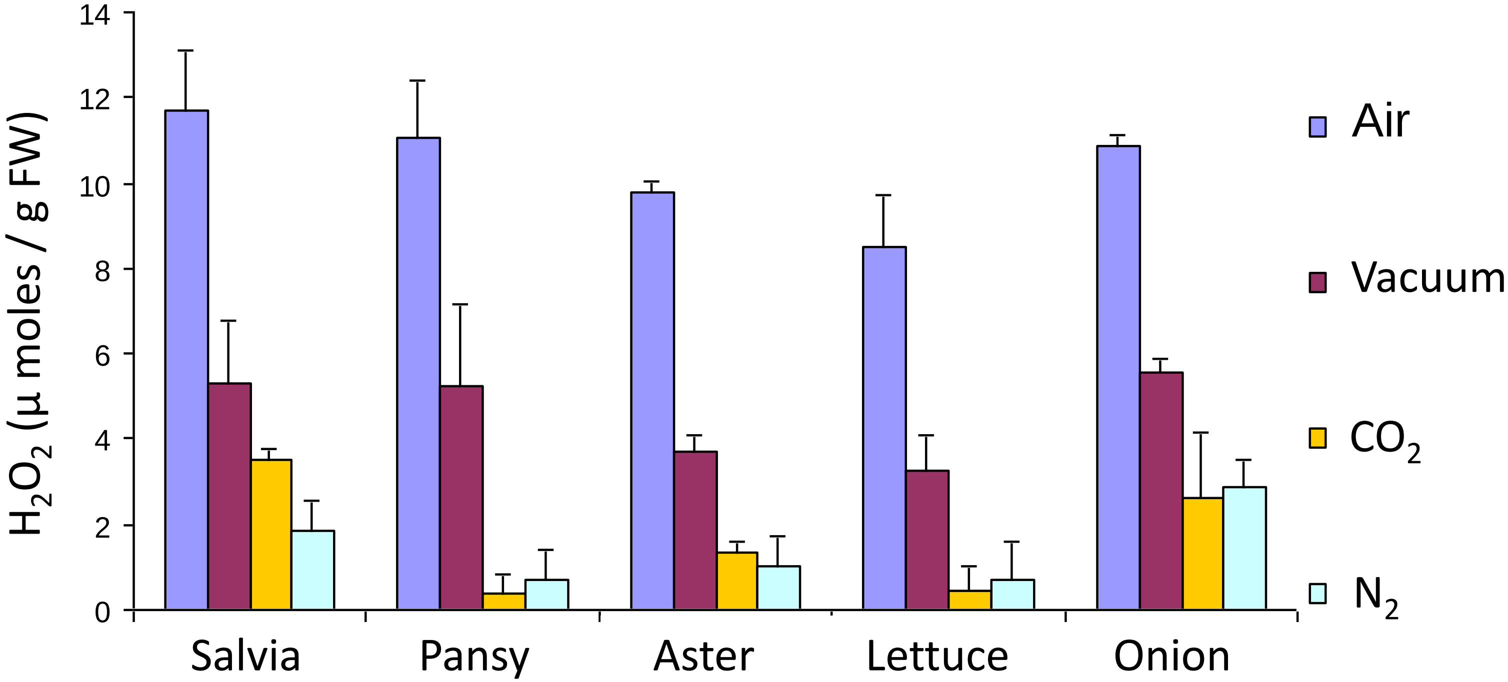
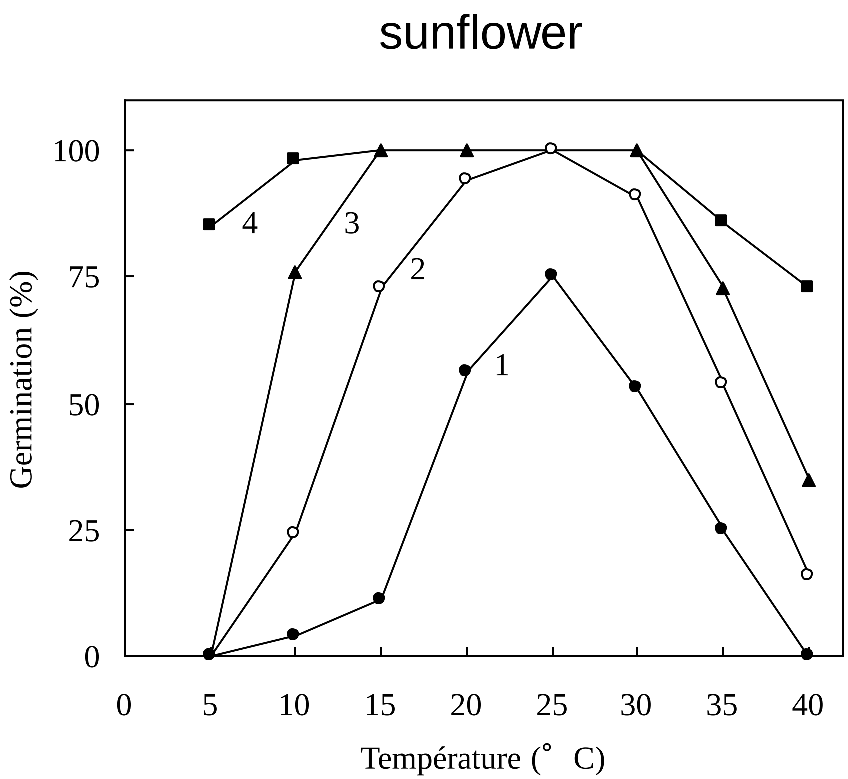
20 years of storage, 5°C, low MC (33 % RH)



Coll. HM Clause

Effect of after ripening on ROS accumulation

After-ripening: a dry storage period that allows the transition from a dormant to a non-dormant seed

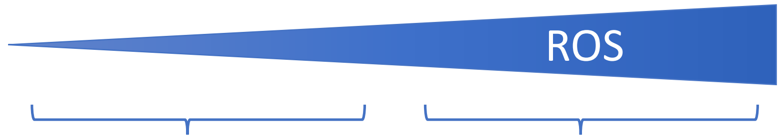


1: harvest
2: 1 month
3: 1.5 months
4: 2 months

Effect of after ripening on ROS accumulation

Sunflower seed

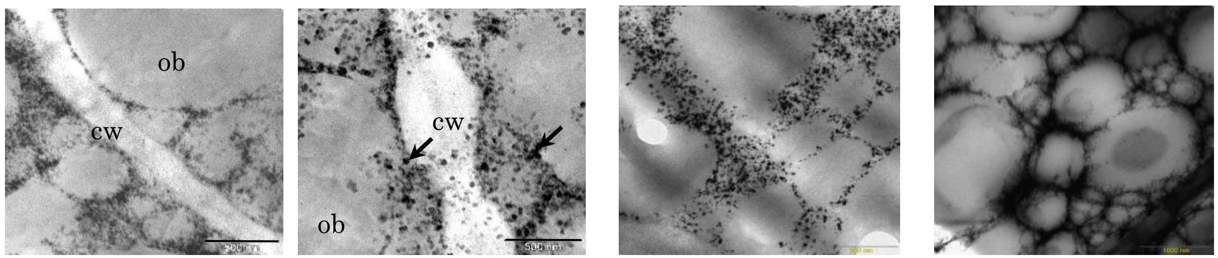
Harvest



Death

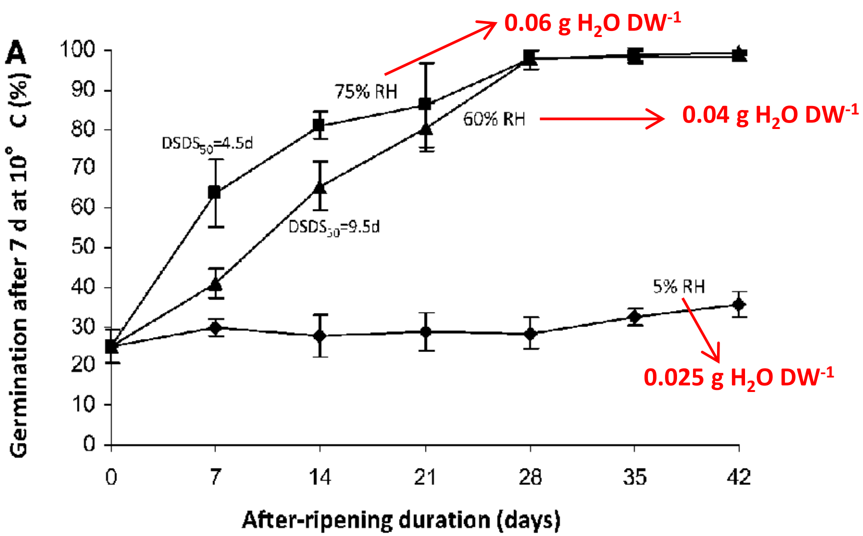
Dormancy alleviation

Aging



Sunflower seed axes – $CeCl_3$ staining of H_2O_2

Oracz et al. (2007), Bazin et al. (2011)



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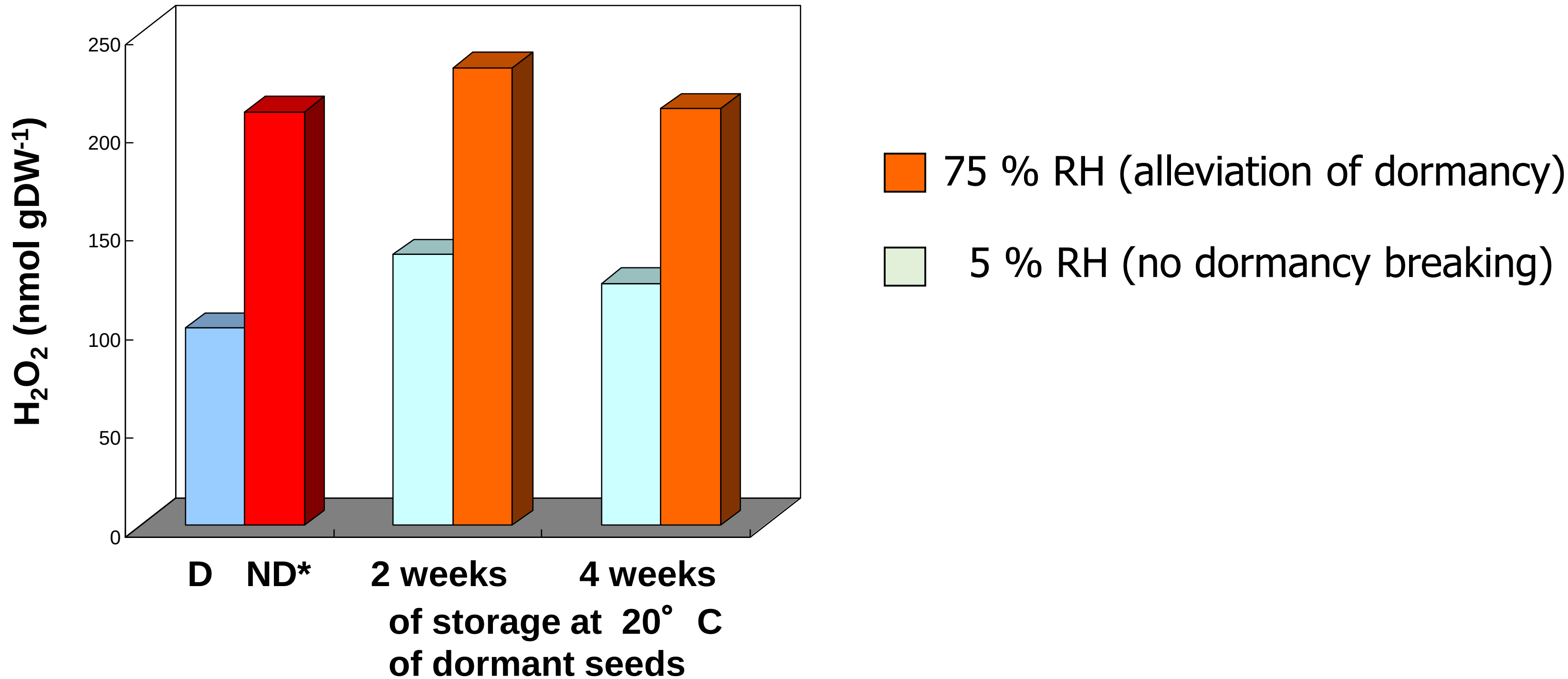
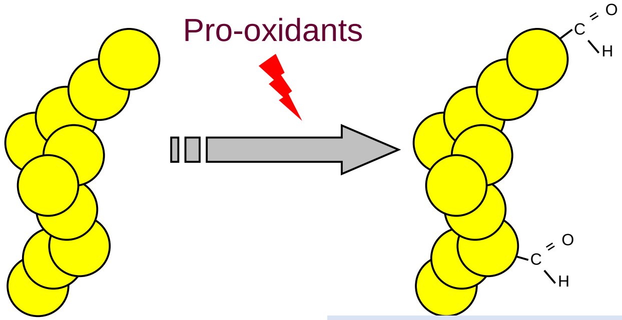
Changes in H_2O_2 content in axes during after-ripening

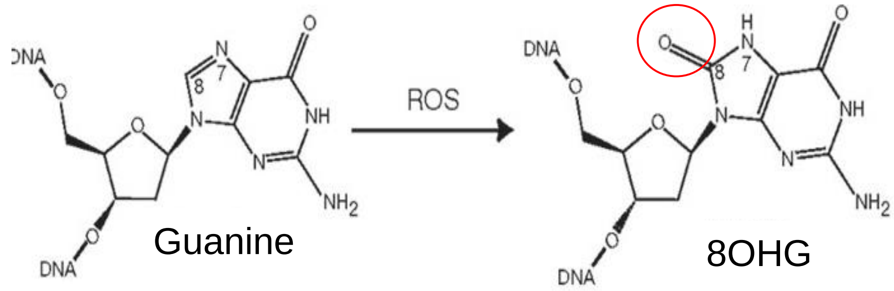


Alleviation of dormancy in the dry state is associated with ROS accumulation

*non dormant = stored for 6 months at room temperature/ MC = 4 % DW

Putative target for ROS : proteins and mRNA



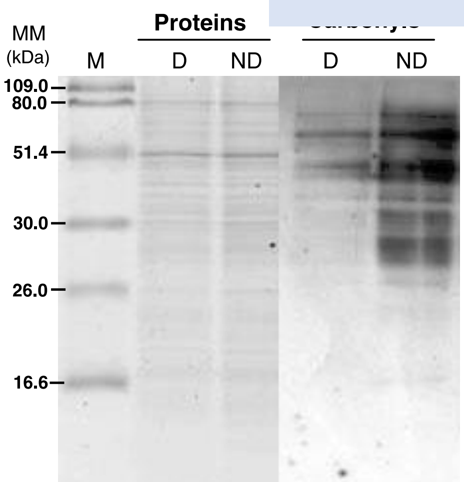


,8-dihydroguanine

Proteins and mRNAs that became specifically oxidized during after-ripening were negative regulators of germination (eg. ABA synthesis and signaling components)

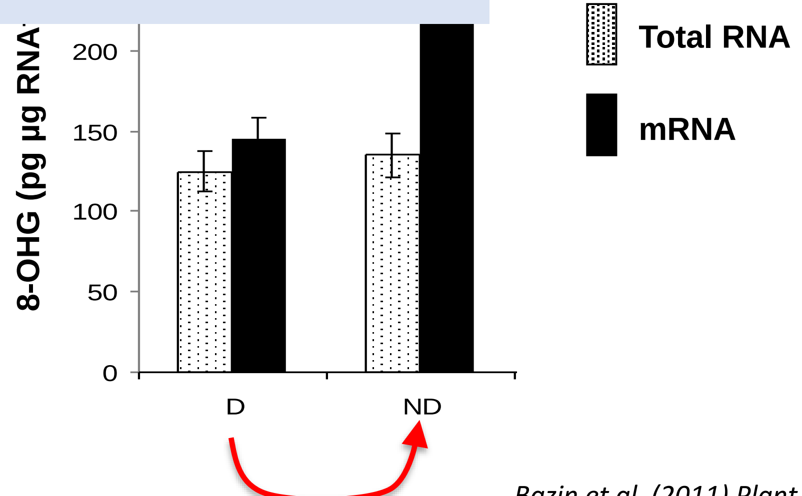
Degradation of oxidiz

dized mRNAs
nslation



Oracz et al. (2007) Plant J

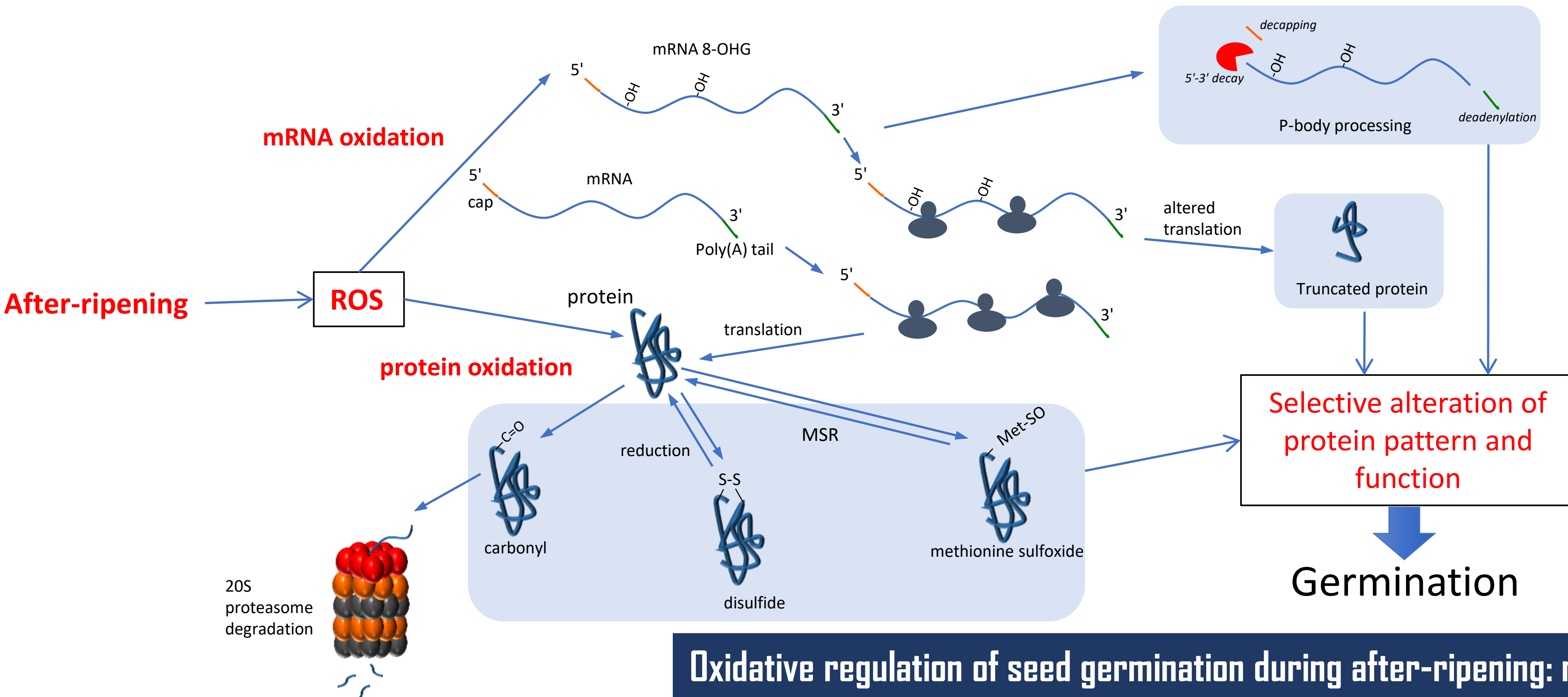
Sunflower seeds



after-ripening

Bazin et al. (2011) Plant Cell

ROS generation during dry storage : modification of cell signaling during subsequent seed imbibition



Oxidative regulation of seed germination during after-ripening: removal of positive regulators of seed dormancy

El-Maarouf-Bouteau et al., 2013

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Regulation of seed germination by ROS

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Effect of ROS on seed germination

Table 1 Reported effects of ROS on seed germination

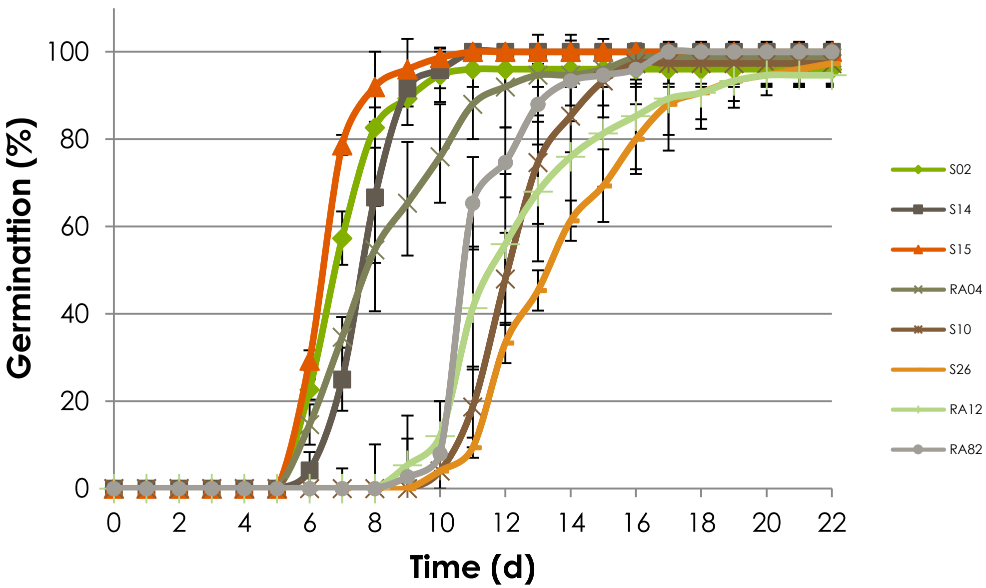
Context	Effect	Species
Zn and Arsenic stress	negative	*Anadenanthera peregrina* and *Myracrodruon urundeuva*
Germination	positive	apple
Dormancy alleviation (stratification)	positive	apple
Salt stress	negative	*Arabidopsis*
ABA cross-talk	ABA positive regulator of rboh and ROS	*Arabidopsis*
Cd Stress	negative	*Arabidopsis*
Mitochondrial functionning	positive	*Arabidopsis*
Salt stress	positive	*Arabidopsis*
Seed dormancy and iron deficiency	positive	*Arabidopsis*
Germination/ABA	negative	*Arabidopsis*
Salt stress/ethylene	negative	*Arabidopsis*
Germination/light	positive	*Arabidopsis*
Dormancy	positive	*Arabidopsis*
Germination/ABA/AIA	positive	*Arabidopsis*
Germination ABA GA	positive	*Arabidopsis*
Germination/ABA signalling	positive	*Arabidopsis*
Dormancy ABA GA	positive	barley
Seed germination and dormancy	positive	barley
Germination/ABA signalling	positive	barley
Dormancy alleviation	positive	barley
Germination/GA/NADPH oxidase	positive	barley
Germination/NADPH oxidase	positive	barley
Dormancy	positive	*Bidens pilosa*
Dormancy alleviation (stratification)	positive	*Bunium persicum*
Dormancy alleviation (stratification)	positive	*Hedysarum scoparium*
Germination/endosperm weakening	positive	lettuce
Mutagen agents	negative	maize
Dormancy alleviation by heat	positive	*Mesembryanthemum crystallinum*
Drought and salt stress	negative	Miscanthus
Germination/ABA	positive	pea
Germination	positive	Pea
High temperature, drought stress	negative	rice
Low phytic acid seed vigour	positive	rice

Negative effects of ROS often associated with stress/ageing

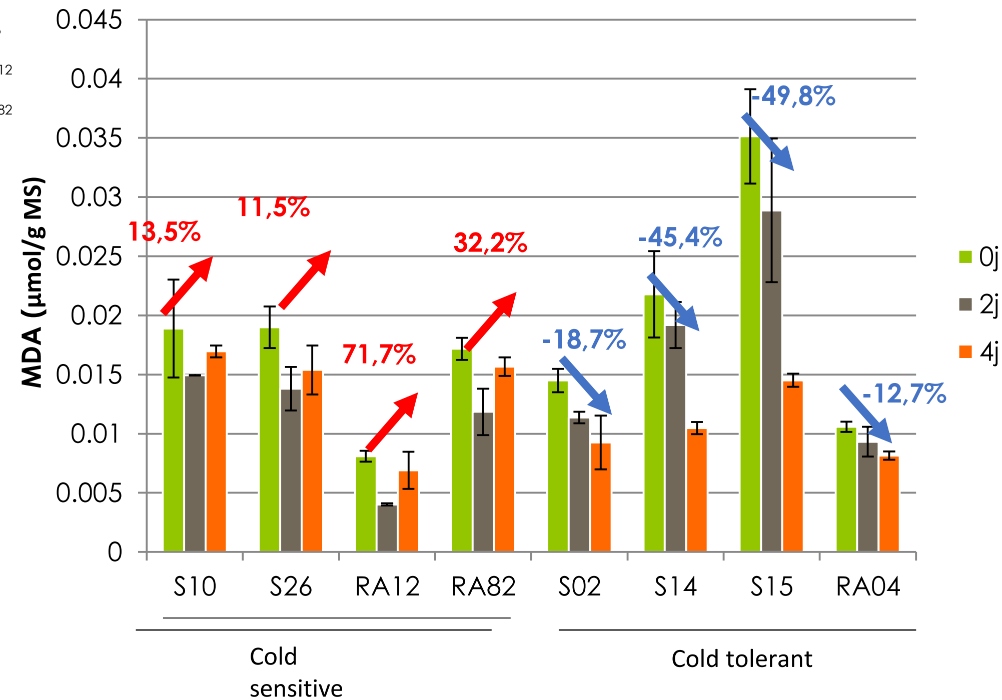
Bailly (2019) Biochem. J.

ROS are environmental sensors

Markers of seed quality/stress: sunflower germination at low temperature



ROS over production translates inappropriate conditions for seed germination



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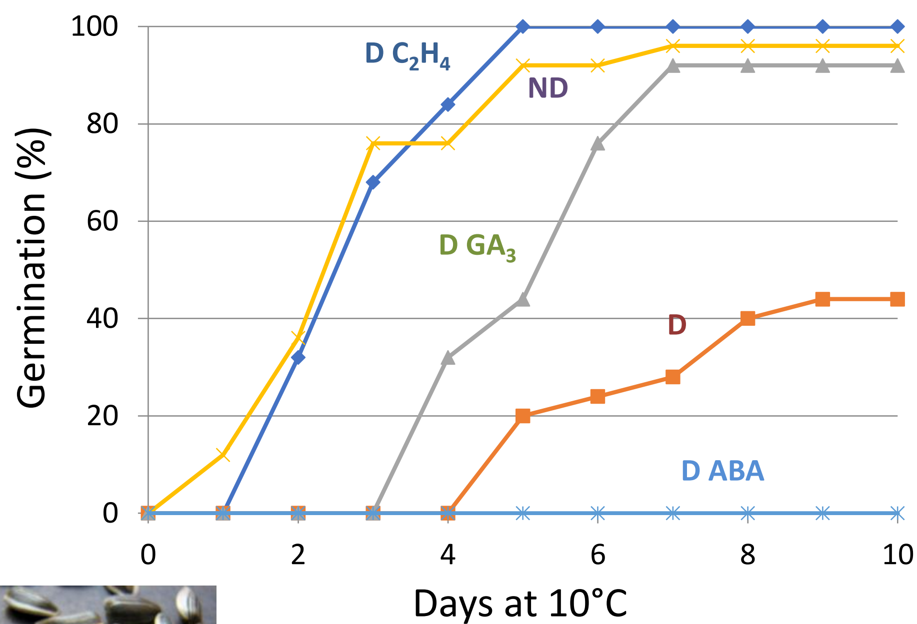
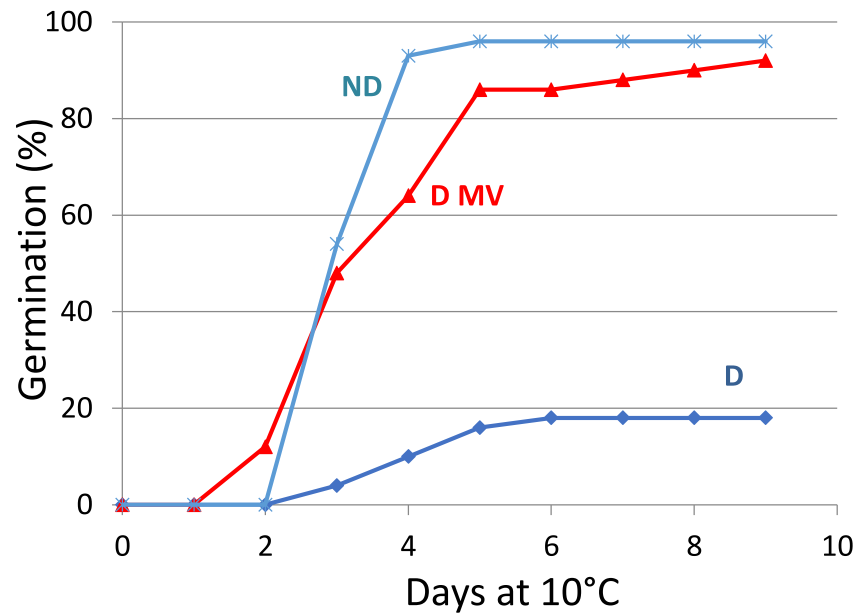
But ROS production is also a prerequisite for seed germination

Embryo dormancy of sunflower seeds

Effect of hormones



Effect of ROS: methylviologen



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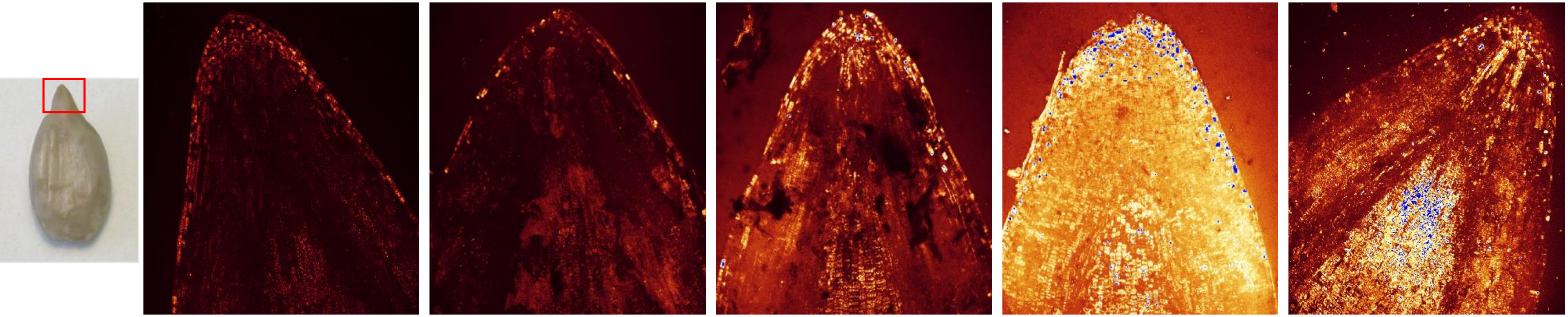
Crosstalk ROS x hormones

24 h 10 ° C

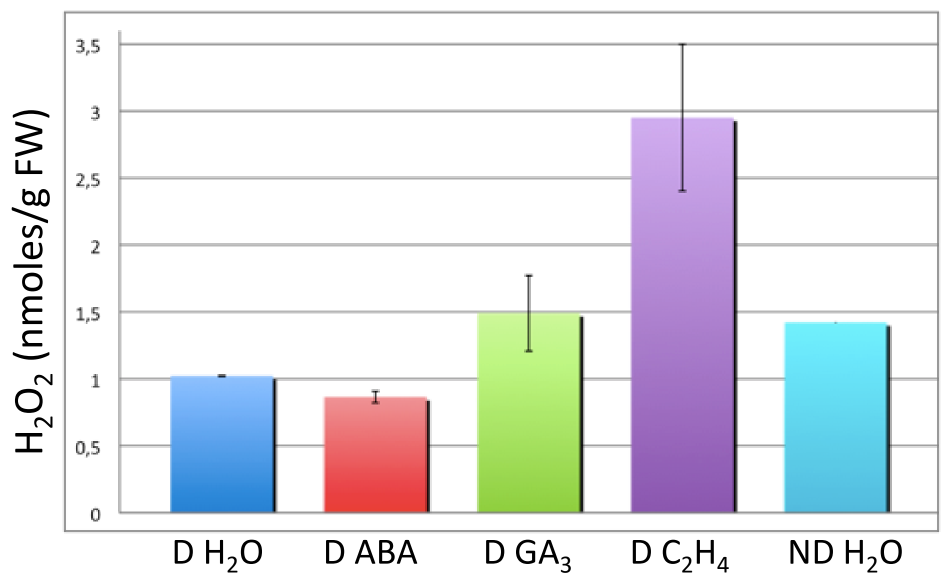
DCF (2′,7′-dichlorofluorescein)



Dormant H_2O | Dormant ABA | Dormant GA_3 | Dormant C_2H_4 | Non Dormant H_2O

No germination | Germination

Germination is associated with ROS generation
Hormones involved in germination modulate ROS generation



El-Maarouf-Bouteau et al. (2015) Plant Cell Environment

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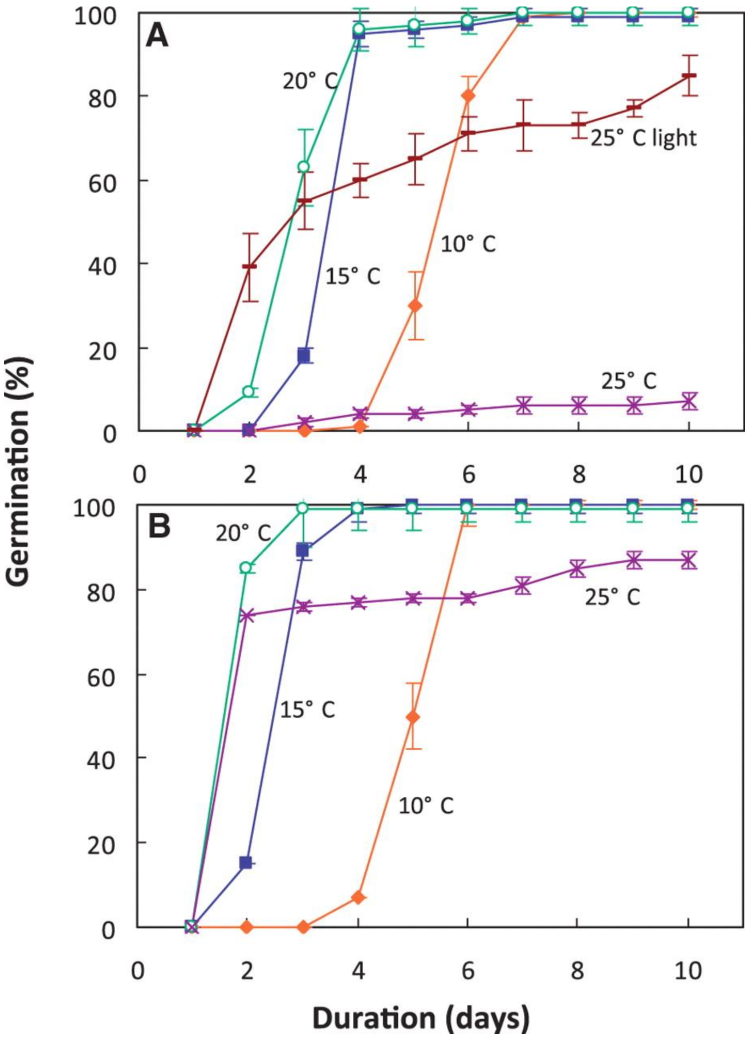
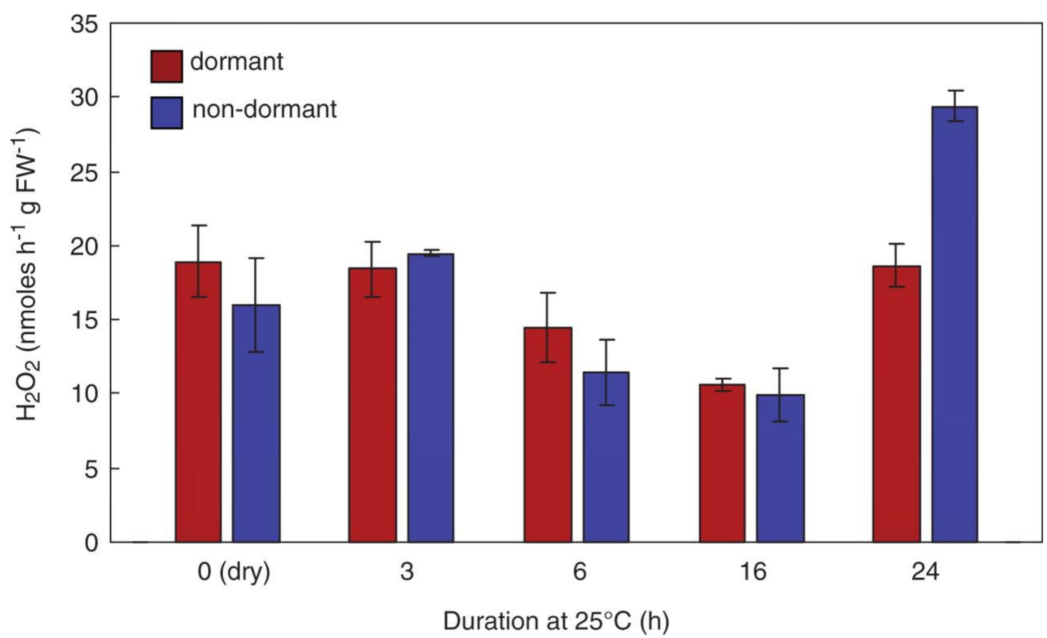
Arabidopsis: Germination of dormant and non-dormant Col seeds

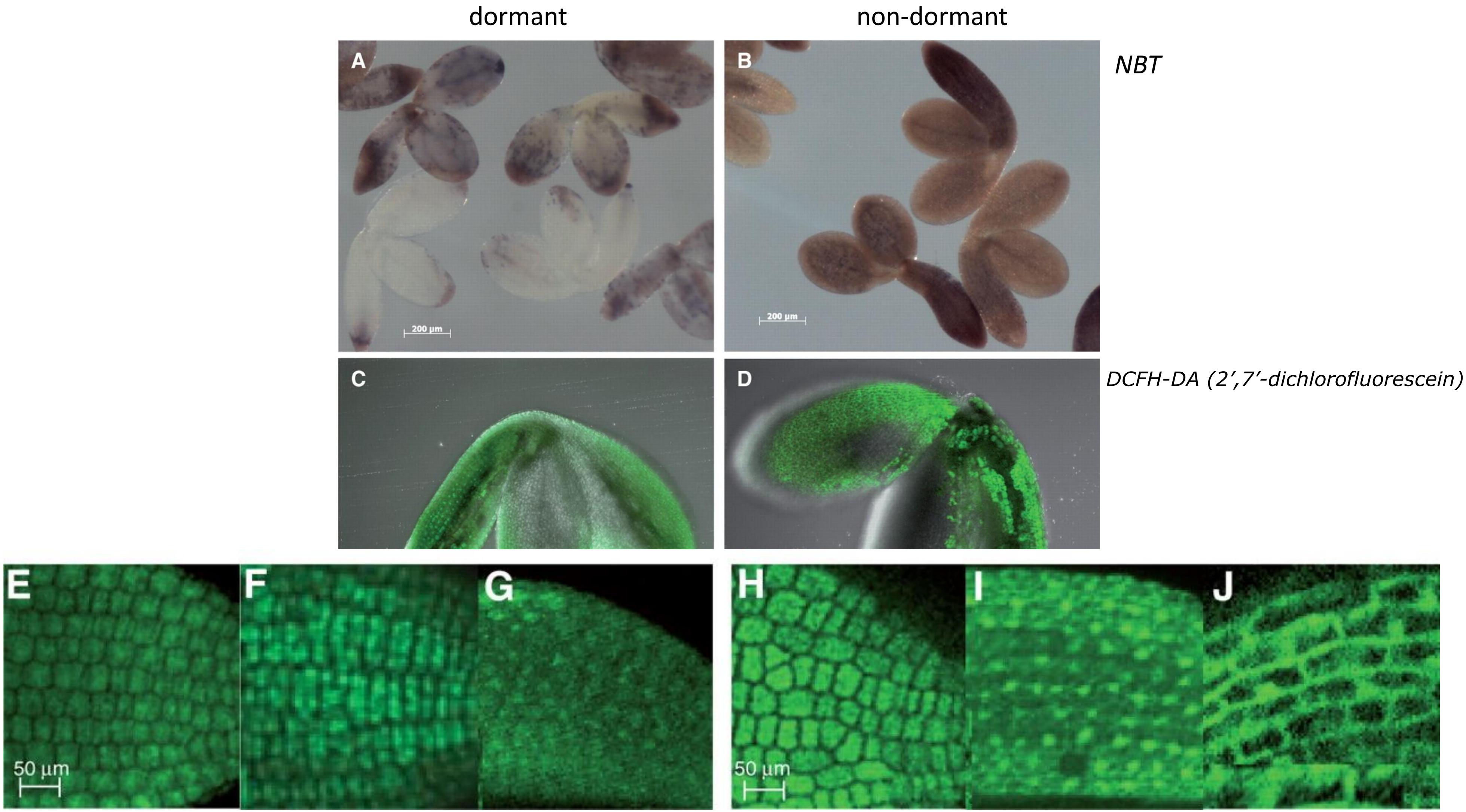


Leymarie et al. (2012) Plant Cell Physiology



Germination is associated with ROS production

In situ localization of superoxide anions and ROS in dormant and non-dormant Col seeds



Leymarie et al. (2012) Plant Cell Physiology

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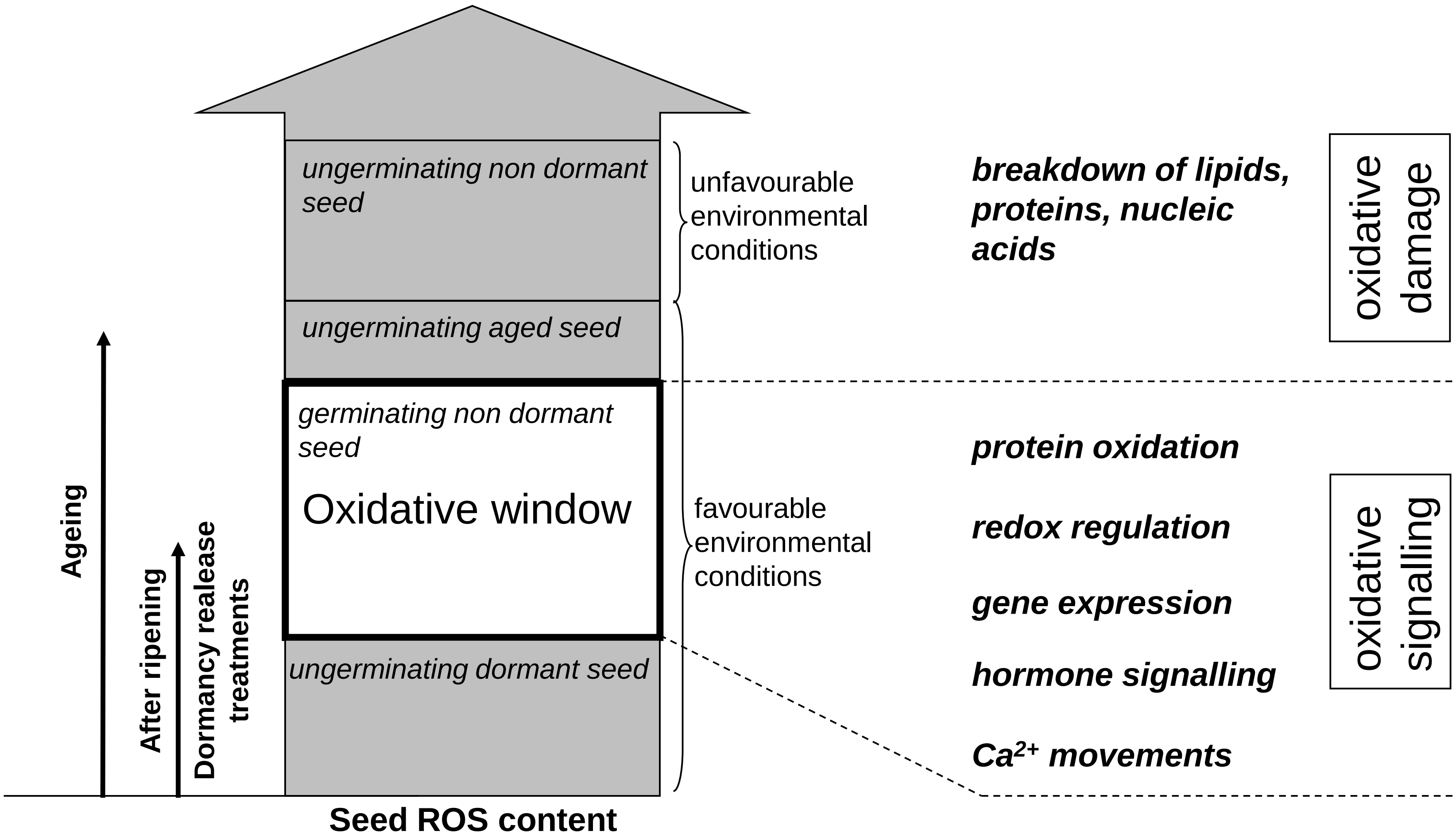
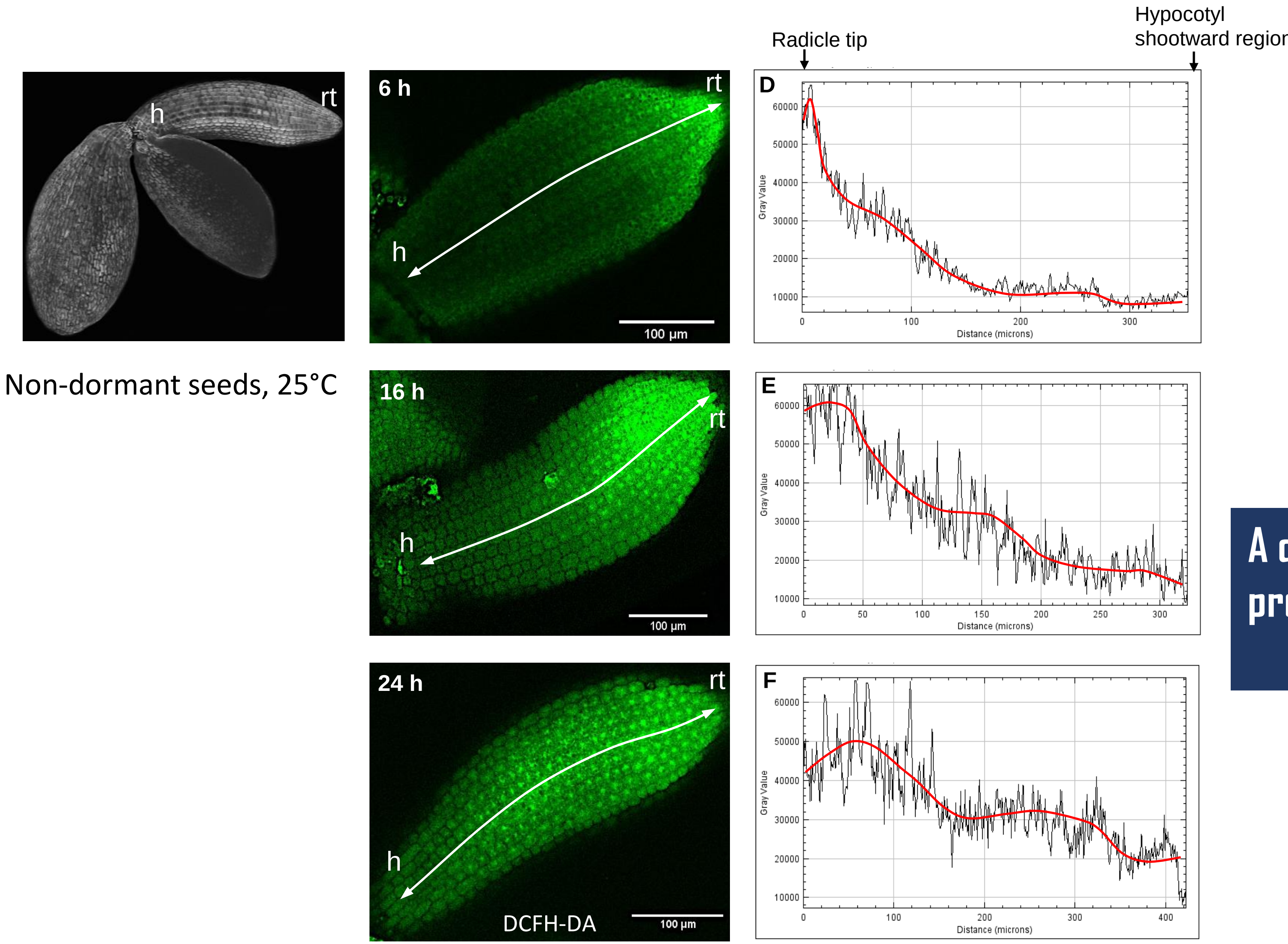
The oxidative window: a model for explaining the role of ROS in seed germination



Bailly et al. (2008) CR Biol

Dynamics of ROS production during seed germination



Bailly (2019) Biochem. J.

A dynamic regulation of ROS production at the tissue level

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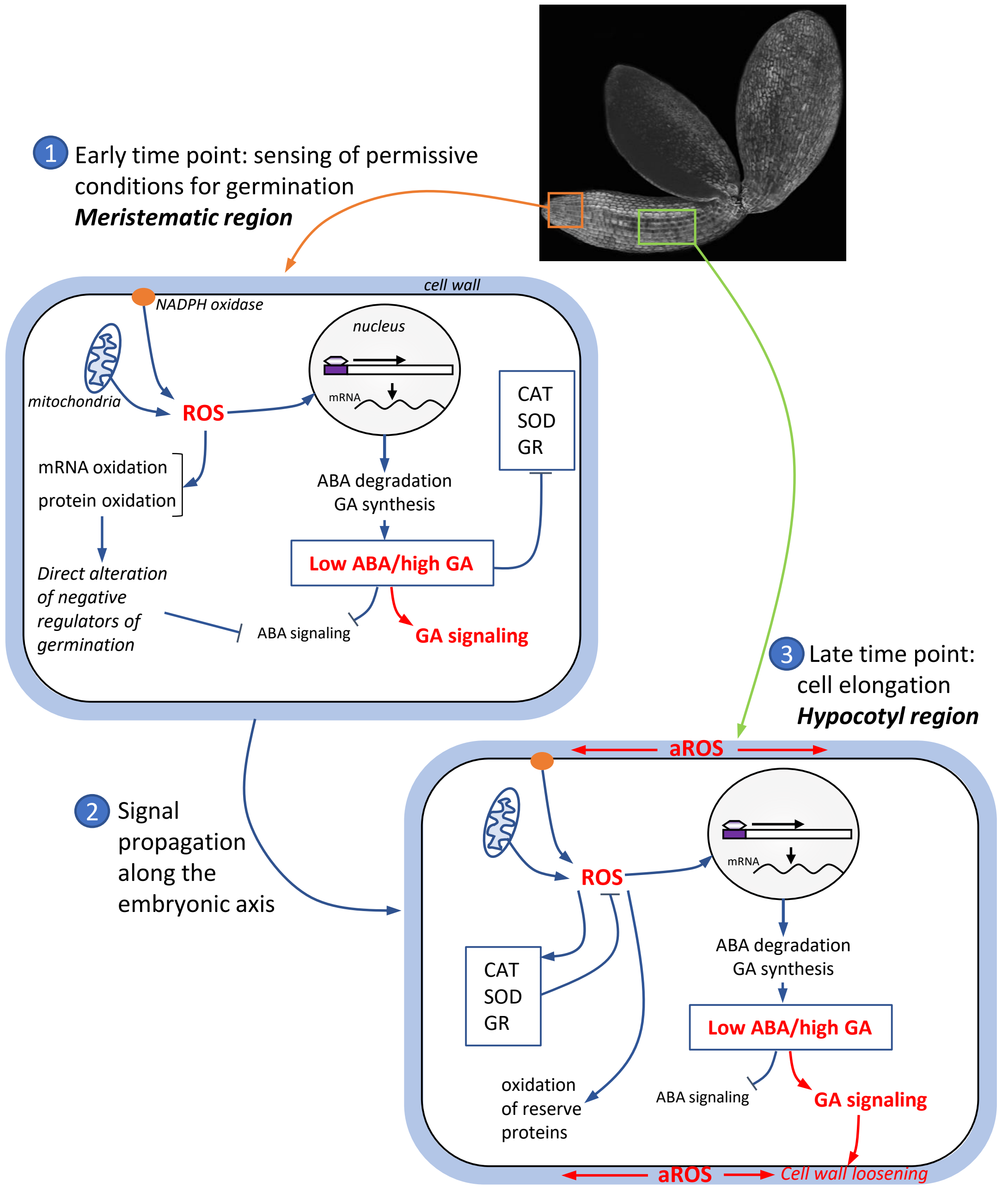

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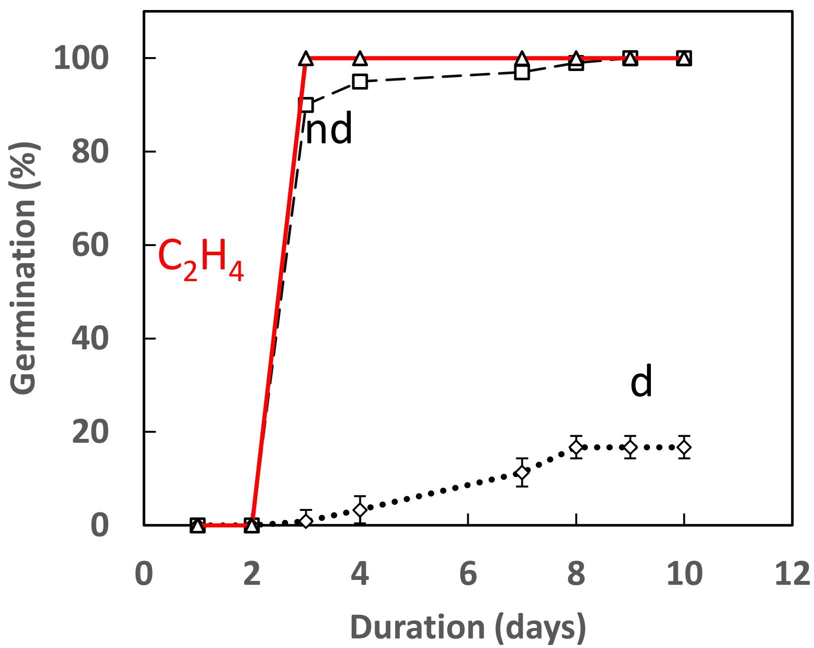
Bailly (2019) Biochem. J.

Deciphering the role of intracellular ROS trafficking in the regulation of seed germination

Subcellular mechanisms of ROS signaling: where are ROS coming from (NADPH oxidases ? Mitochondria ?) and where « do they go » ? (what are their targets) ?



Jurdak et al. (2021) New Phytol.

Effect of ethylene on Arabidopsis seed dormancy release

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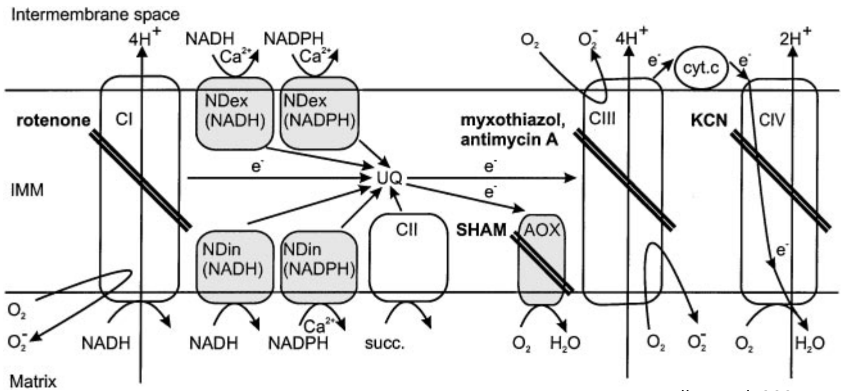
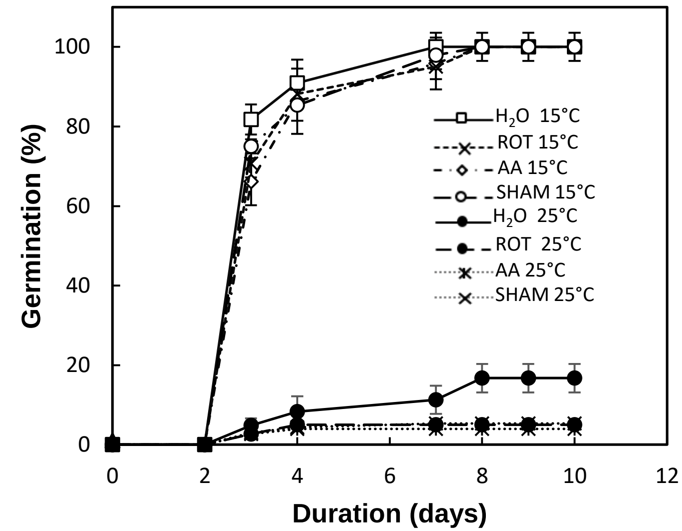
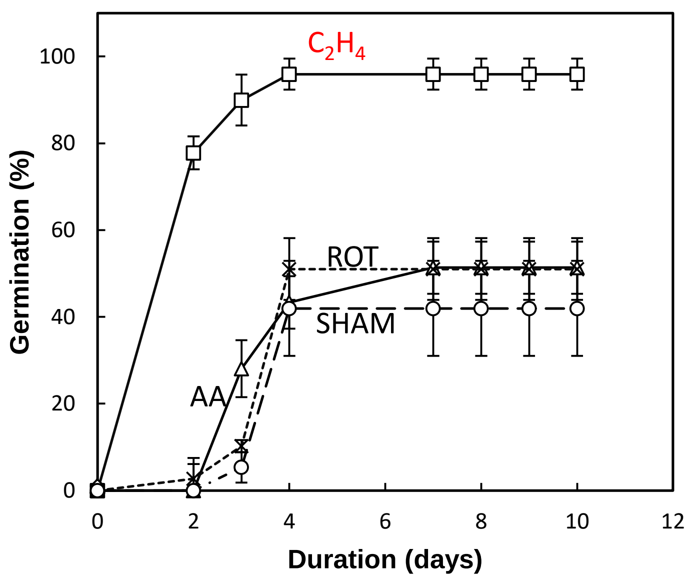
Role of mETC in response to ethylene

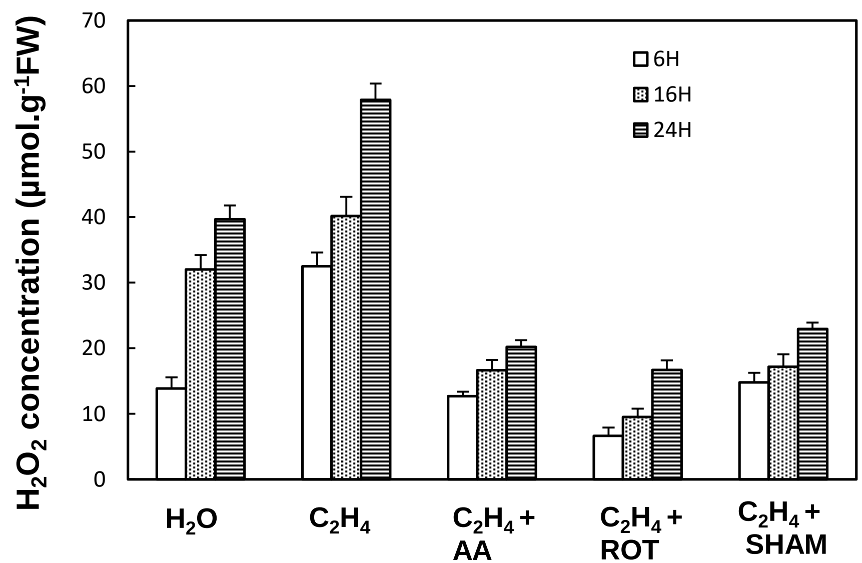


Moller et al. 2001

rotenone (ROT, 5.10^{-5} M) complex I inhibitor
salicylhydroxamic acid (SHAM, 10^{-4} M) inhibitor of the mitochondrial alternative oxidase
antimycin A (AA, 5.10^{-6} M)complex III inhibitor

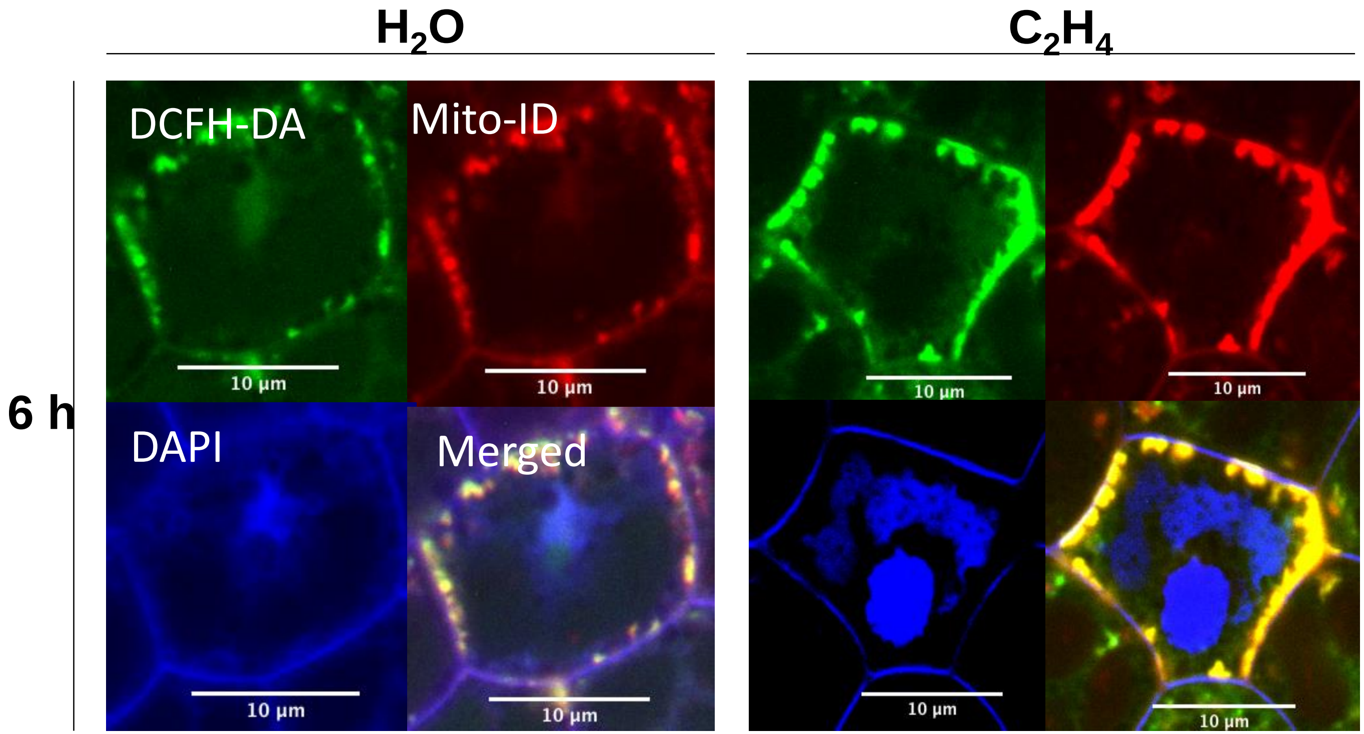






Ethylene effect on seed germination requires H_2O_2 production by mitochondria

Intracellular ROS localization



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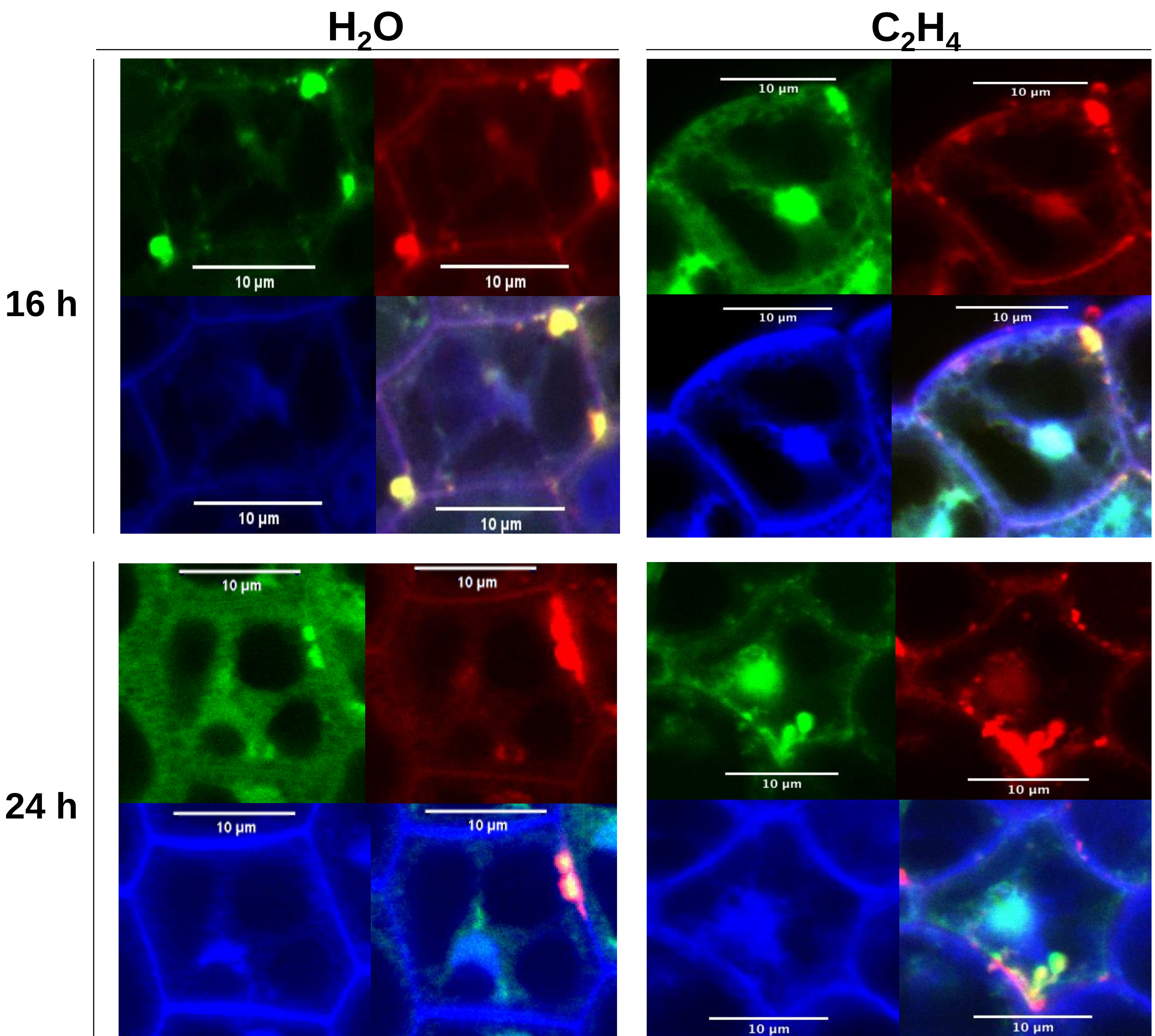
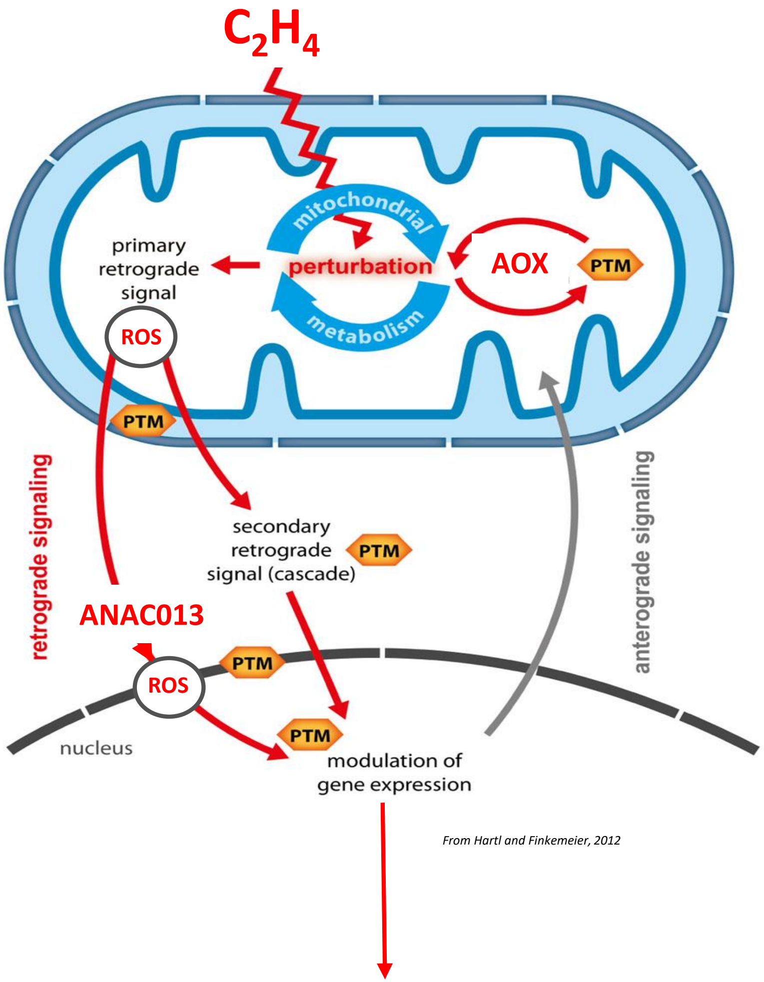
Jurdak et al. (2021) New Phytol.



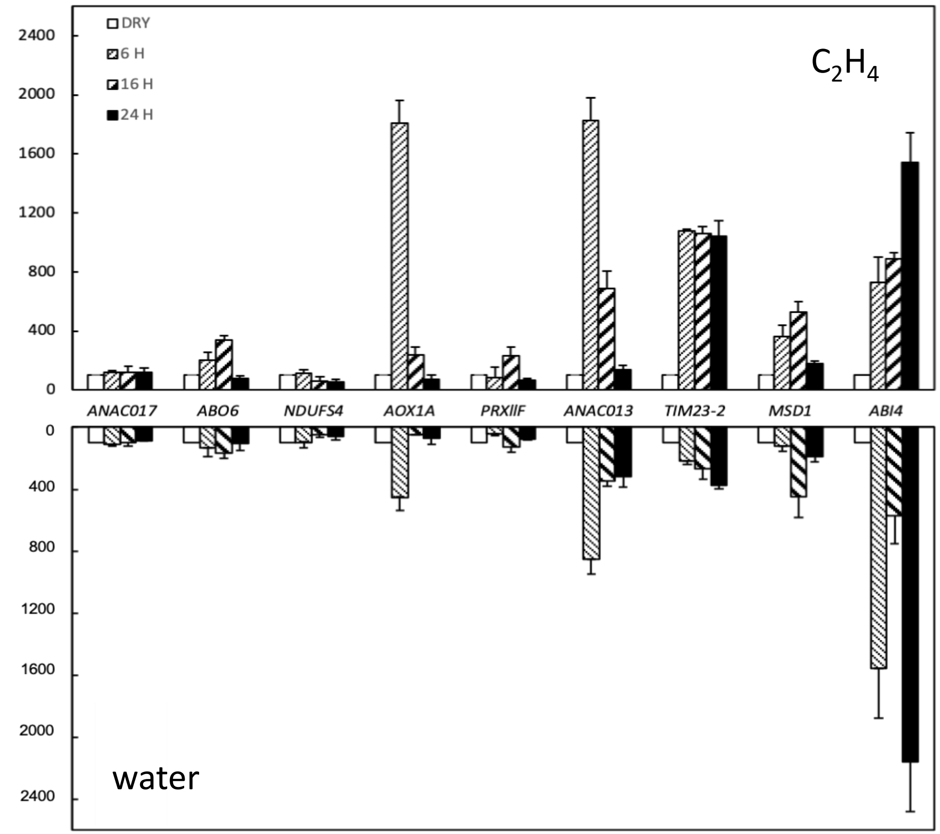
ROS production is mitochondrial then nuclear in ethylene treated seeds only, suggesting retrograde signaling

Jurdak et al. (2021) New Phytol.

Expression of known markers of retrograde signaling in plants



Seed germination



Ethylene triggered expression of AOX (mitochondrial alternative oxidase) and ANAC013 (TF)

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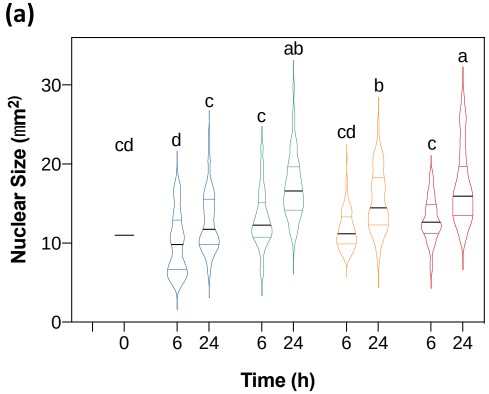
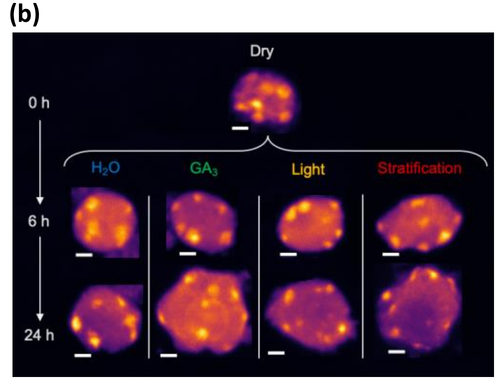
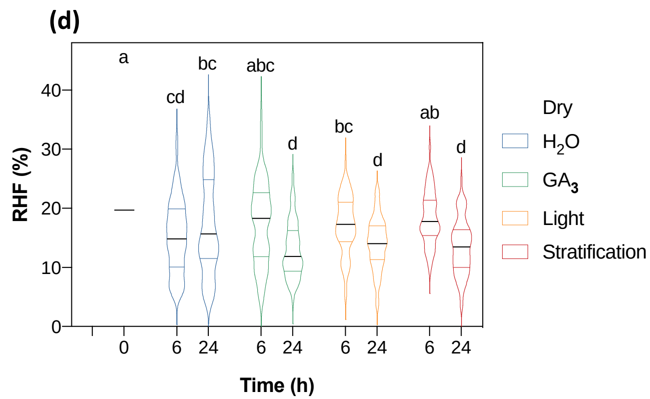
Jurdak et al. (2021) New Phytol.

What are the effect of ROS accumulation on chromatin organization ?

GA, light and stratification

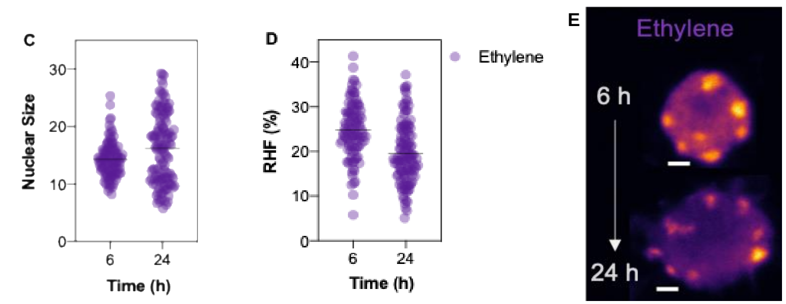






RHF: relative heterochromatin fraction

C_2H_4



Nuclear ROS modify chromatin organization: chromatin decompaction

Jurdak et al. (2022) New Phytol.

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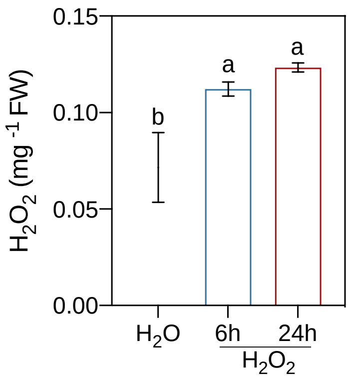
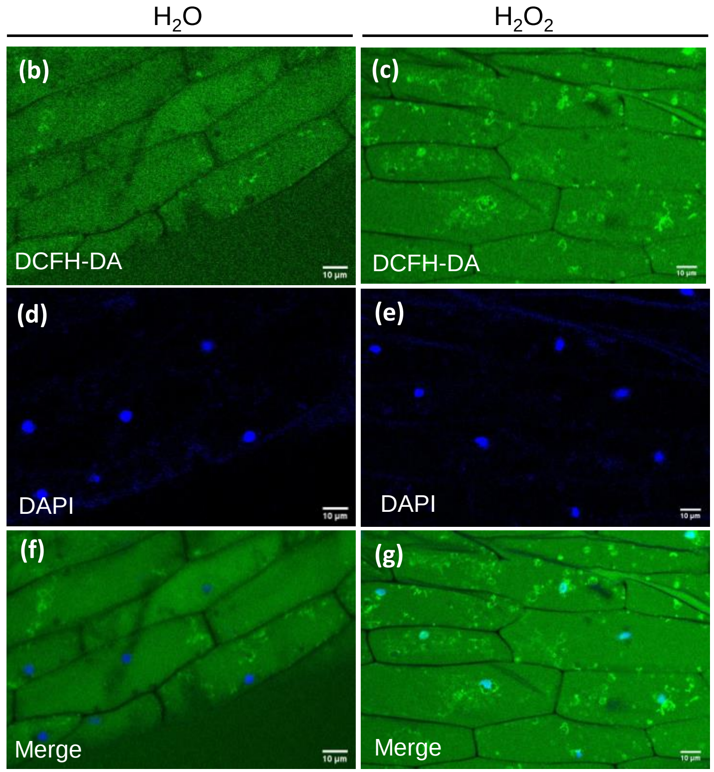
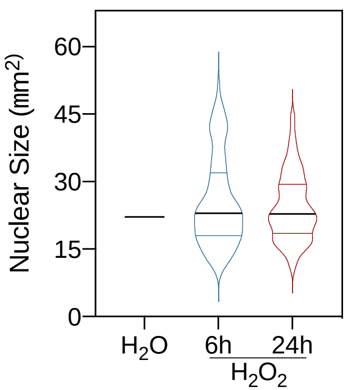
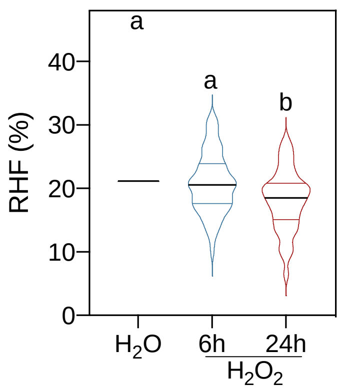
Proof of concept: artificial induction of ROS accumulation within nucleus

Treatment of 7 d old seedlings by H_2O_2









Whatever the organ, nuclear ROS alter chromatin organization
Which in turn can modify gene expression

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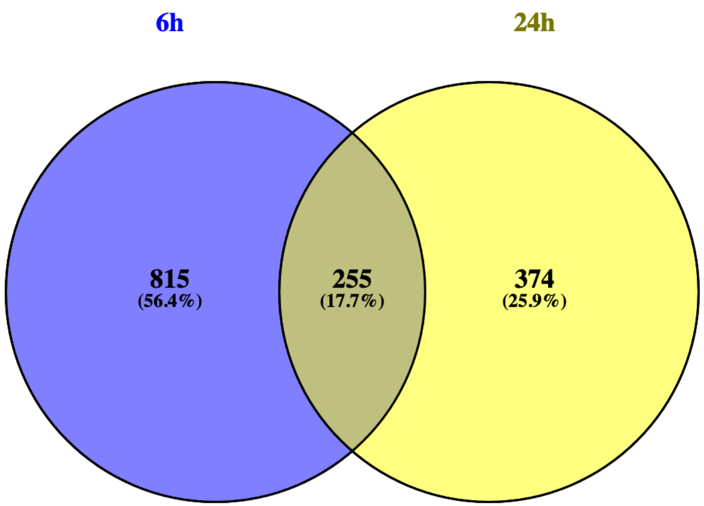
ROS accumulation within the nucleus/change in chromatin organization/change in gene expression

RNA-seq study of H_2O_2 treated seedlings

UP

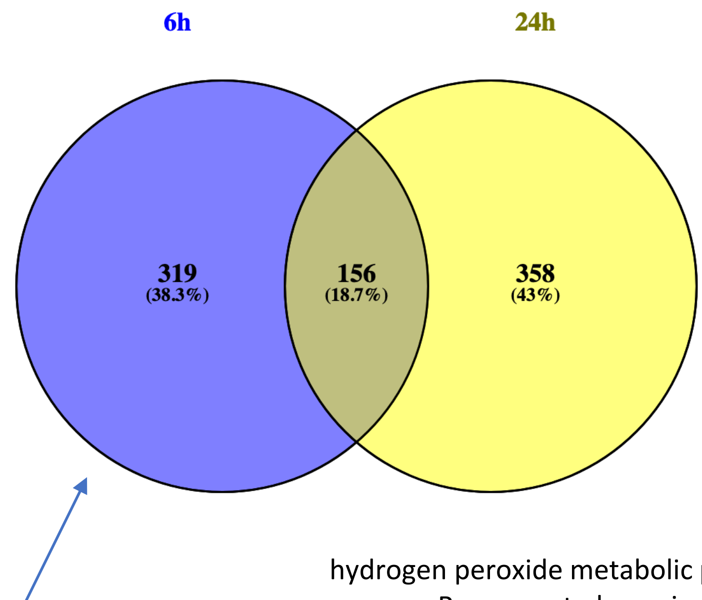


Response to heat
Response to hypoxia
Response to H2O2
Glutathione transferase

Response to heat
Response to hypoxia
Response to H2O2
Proteasome
HSP20-like chaperone

DEGs

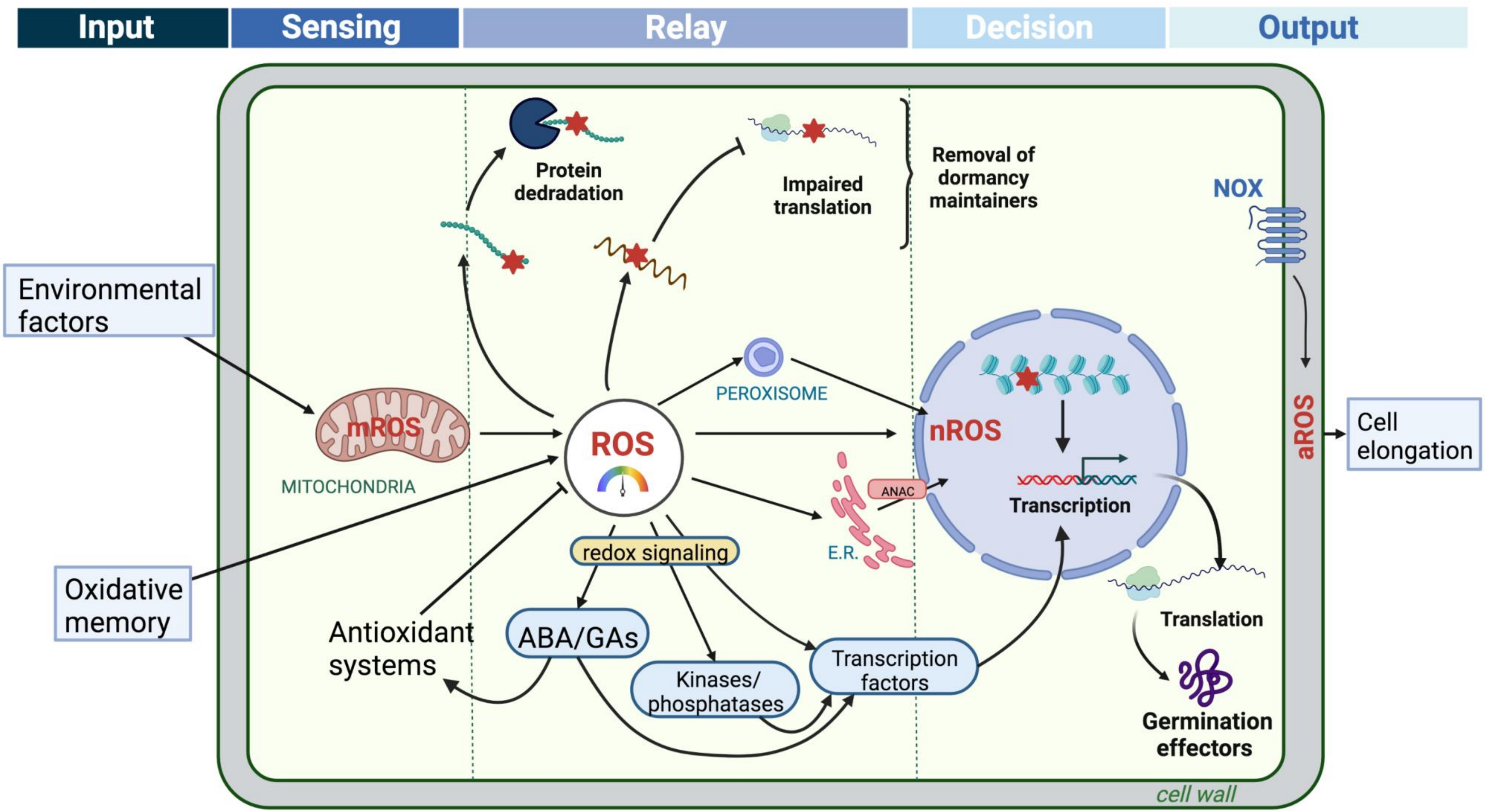
DOWN



hydrogen peroxide catabolism
(12 peroxidases)
Photosynthesis
Cell wall

hydrogen peroxide metabolic process
Response to hypoxia

A putative model of ROS trafficking that controls seed germination



Bailly (2023) Advances in Botanical Research (Oxidative Stress Responses in Plants)

To sum-up

ROS play a key role in all steps of seed life, including dry storage

Their homeostasis translates internal and external message into a germinative response: the oxidative window

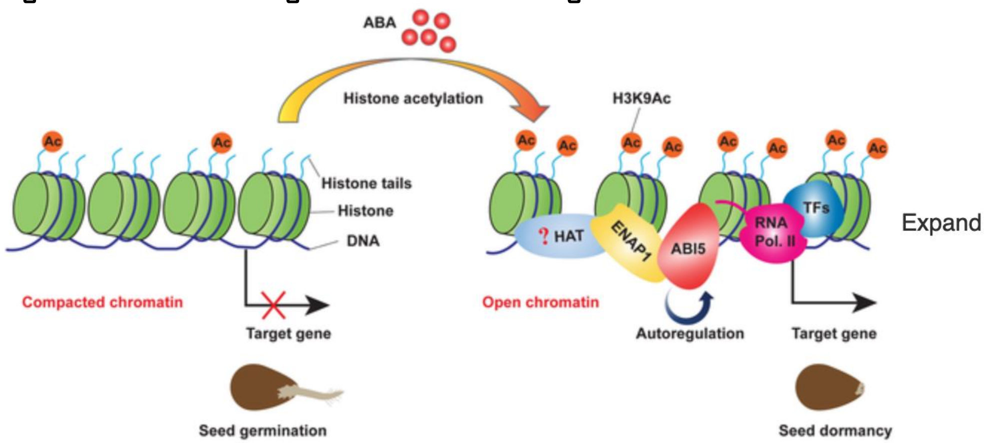
Regulation of seed germination by ROS relies on a complex interplay between cellular compartments

ROS downstream mode of action, has to be considered with regards to the other signaling pathways (ie. plant hormones) but is poorly known

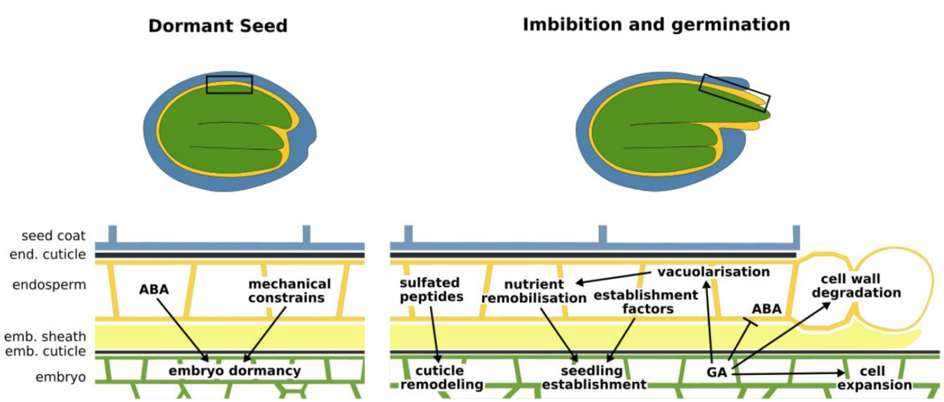
The specificity of cellular response towards the various ROS is not known

Challenges, among others

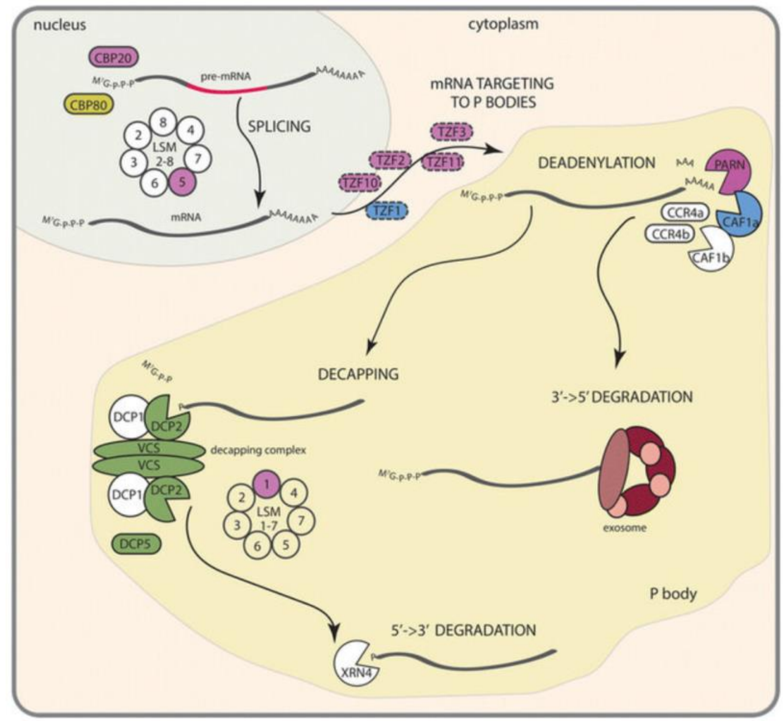
Epigenetic and seed germination, transgenerationnal effects



Crosstalk endosperm-embryo



RNA metabolism



Deciphering the biological mechanisms involved in Arabidopsis seed dormancy release by cold atmospheric plasma

Co workers

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Context

Seeds are at the core of agriculture since the critical step of plant life and crop productivity is seed germination

But global warming and pesticide restriction will dramatically alter this step and in turn crop productivity and food safety

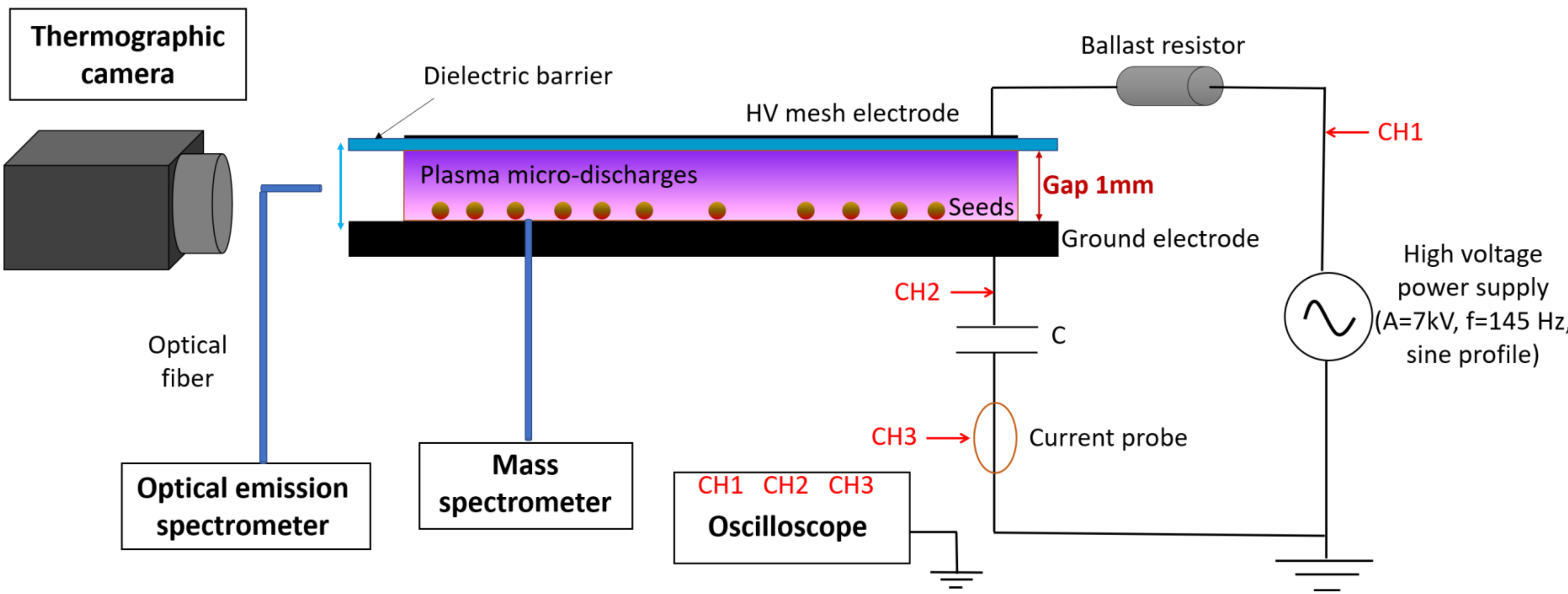
New solutions for sustaining seed germination in a changing environment are required:

Dry plasma approach (also single-step approach) which consists to expose seeds, seedlings or plants directly to a discharge or post-discharge is increasingly used in agriculture

An urgent need to better understand how can plasmas modify seed biological properties

Experimental set-up

Treatment of dry seeds using an Air Di-electric Barrier Device at P_{atm}

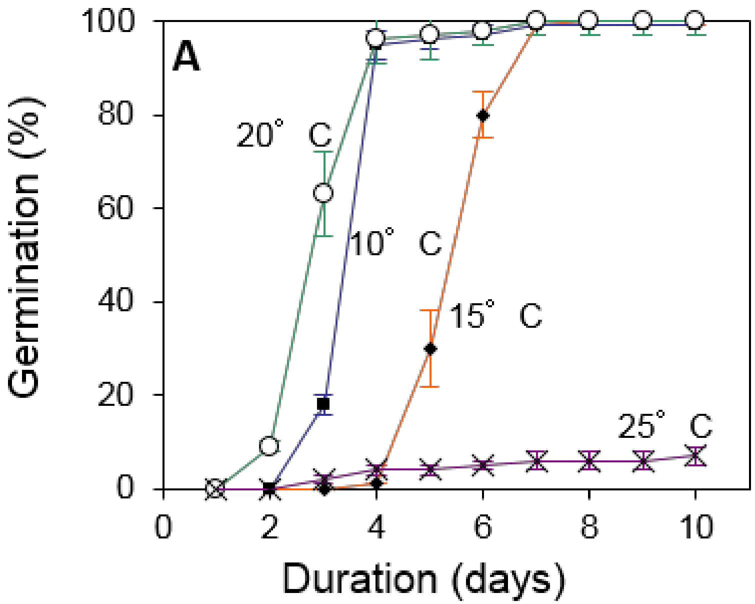


Duration the treatment: 15 min, dry seeds
Peak amplitude of the voltage : 7kV at 145 Hz
Power 11.6 W

Biological model: Arabidopsis seed dormancy

At harvest Arabidopsis seeds are dormant
No/low germination at 25°C in the dark

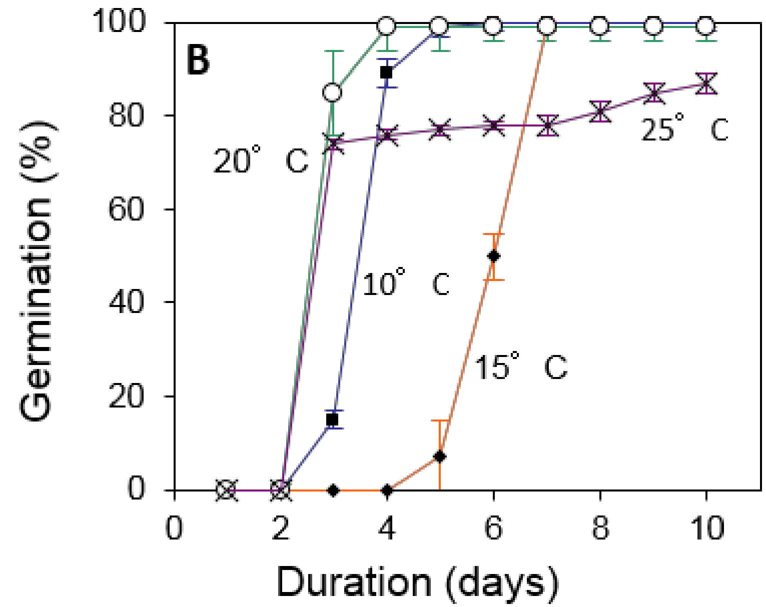
Dormant seeds



Dry after ripening
weeks-months

Cold Plasma Treatment
Minutes

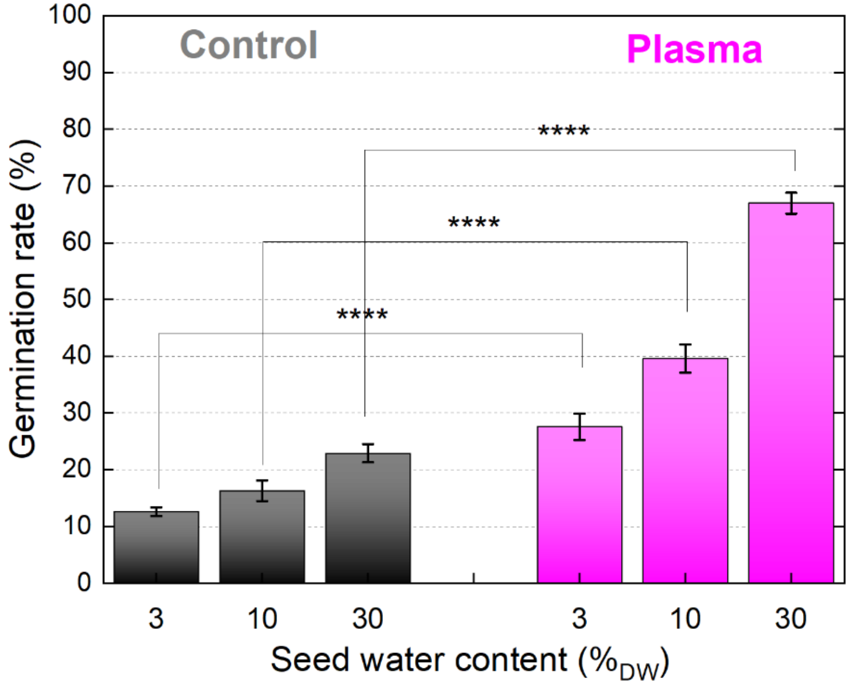
Non-dormant seeds

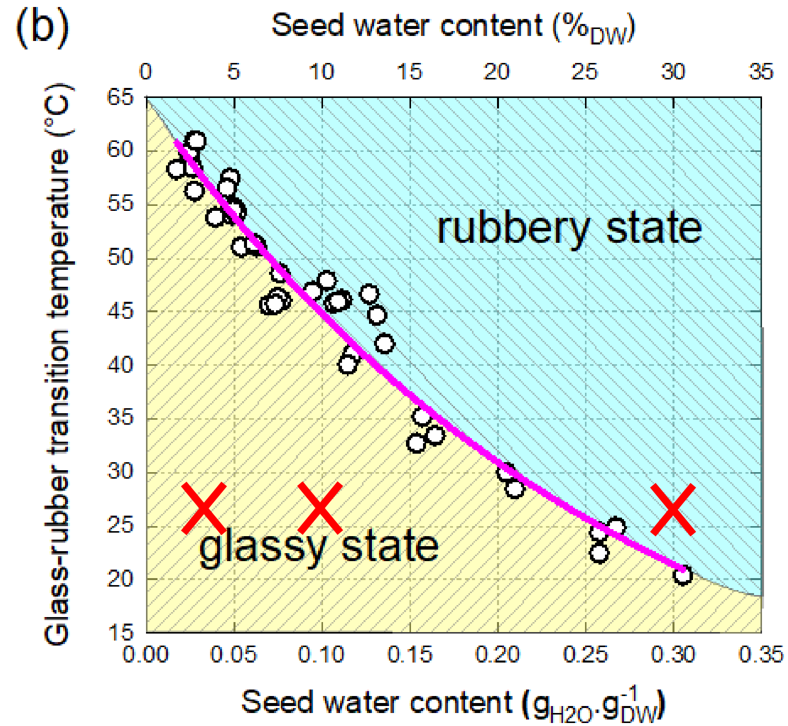


The mechanisms of seed dormancy alleviation in the dry state (anhydrobiosis) are poorly known: can be studied by using plasmas

Leymarie et al., *Plant Cell Physiol*, (2012)

Dormancy alleviation by CAP treatment





CAP treatments release Arabidopsis seed dormancy within 15 min

The efficiency of the CAP treatment relies on seed MC and cytoplasmic viscosity

August et al. (2023) J of Physics D

What are the biological processes triggering this change of seed physiology (alleviation of dormancy)

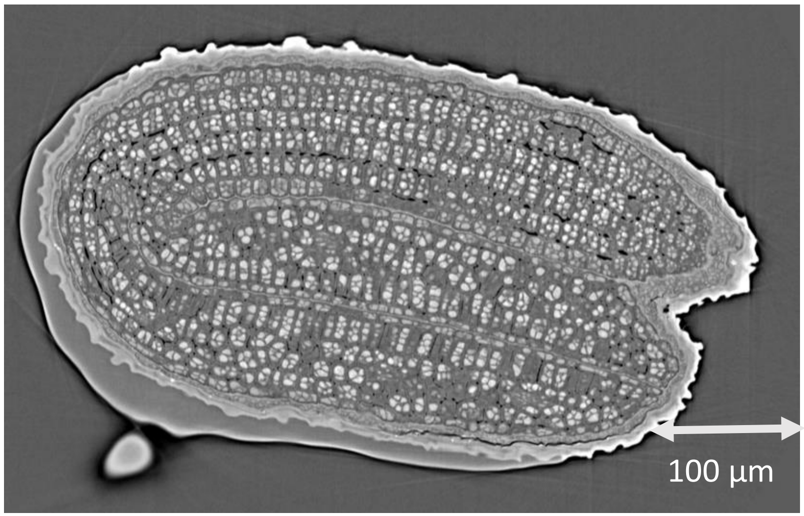
How can plasmas have an effect in anhydrobiosis ?

- Structural changes (change of seed physical structure)

- Biological changes: oxidative processes and gene expression

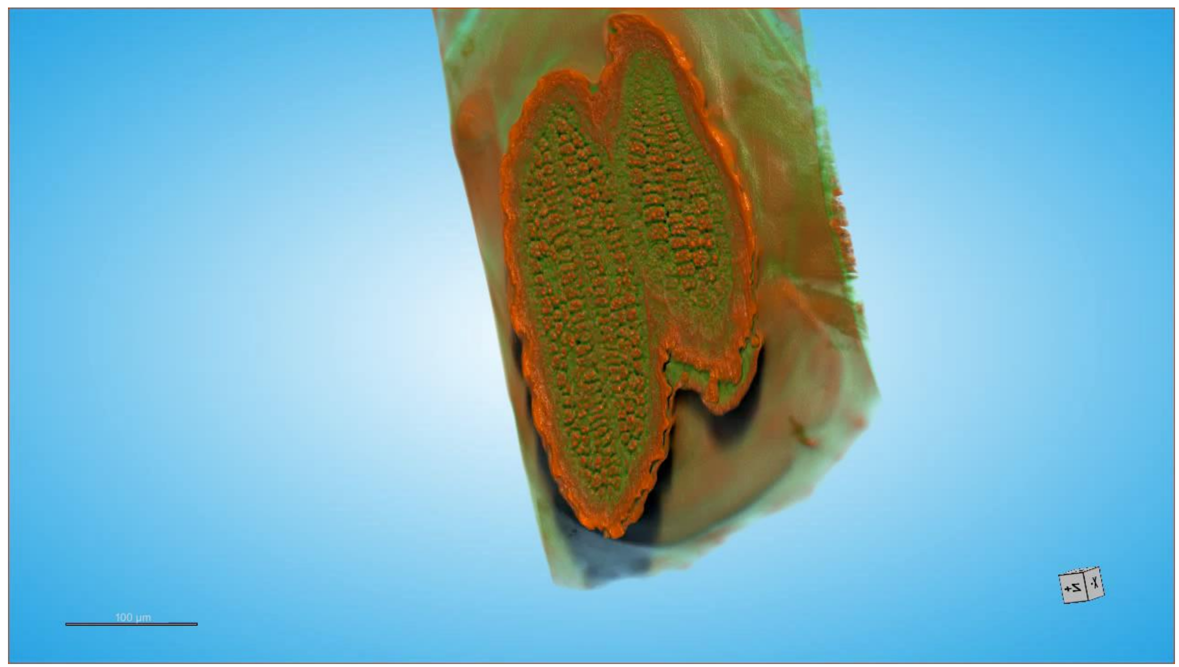
1. Effect of CAP on seed physical properties

X-ray microtomography on the ANATOMIX beamline of SOLEIL synchrotron (Orsay, France)



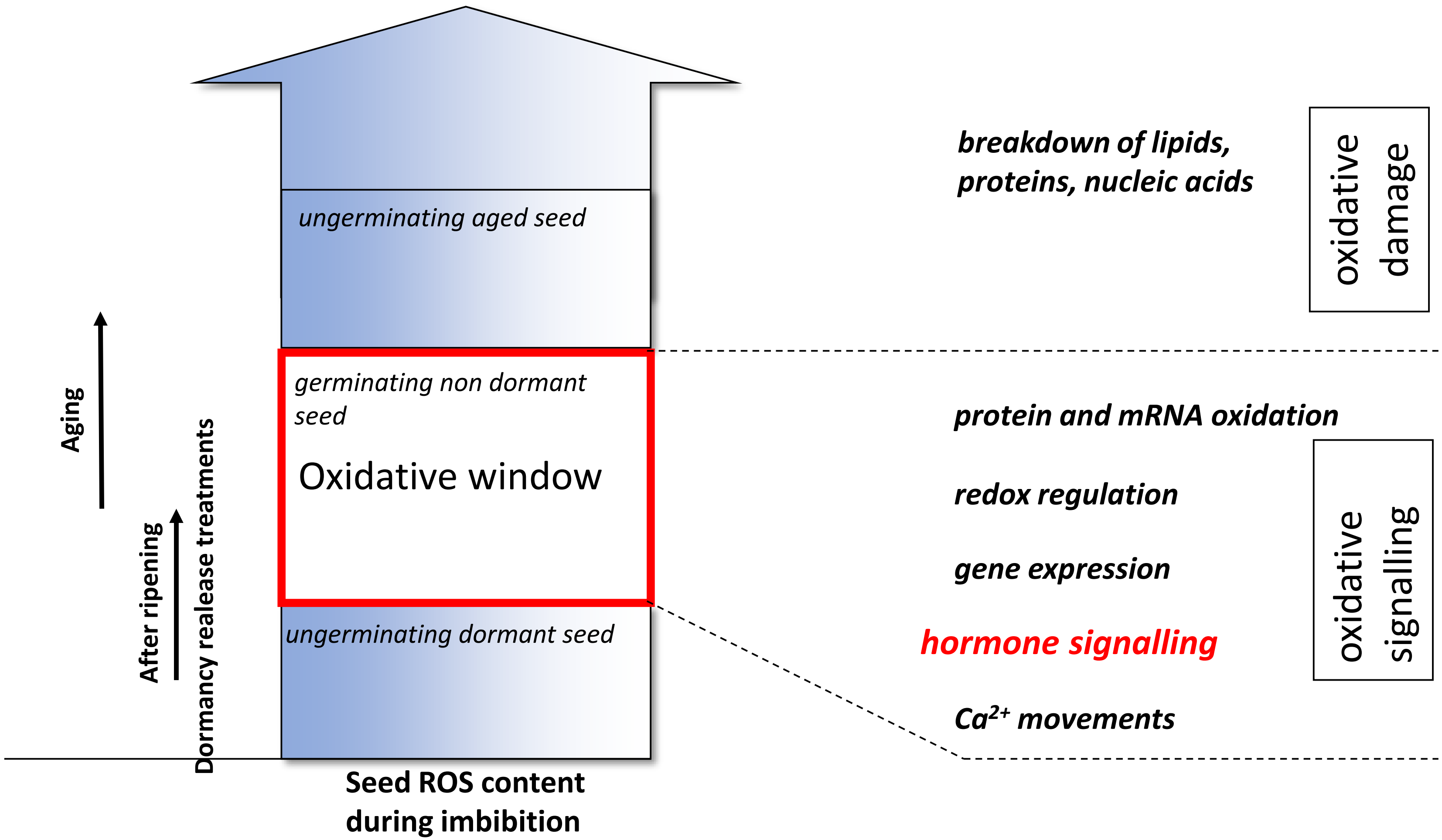
Seed Cross Sections

3D reconstruction



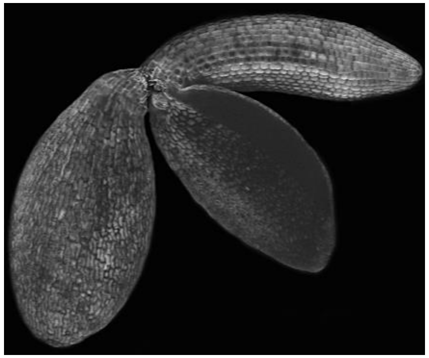
2. Effect of CAP on seed biological properties

CAP alter oxidative processes within seeds: the oxidative control of seed germination

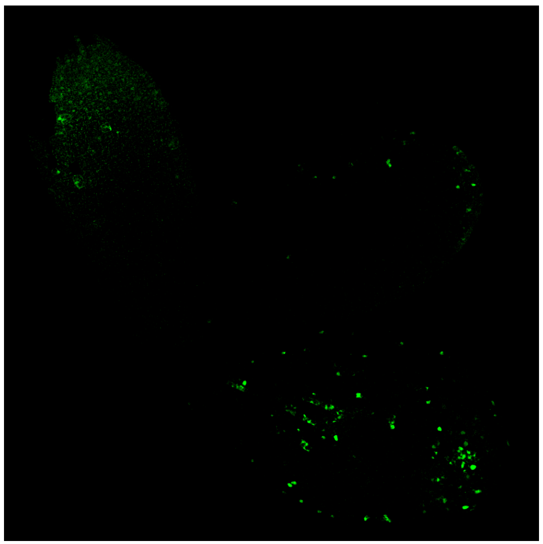


Bailly et al. (2008)

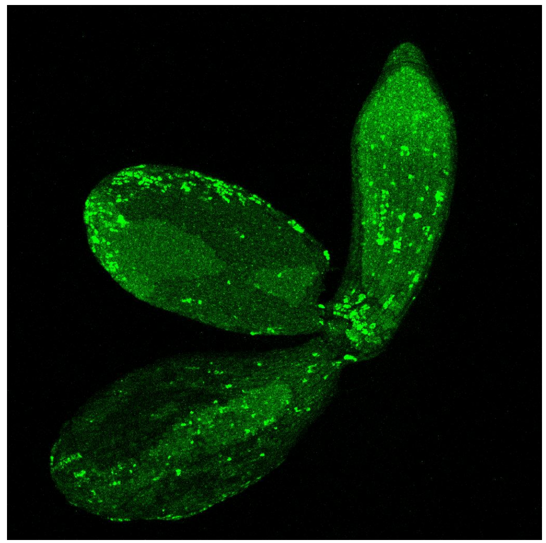
2. Effect of CAP on seed biological properties



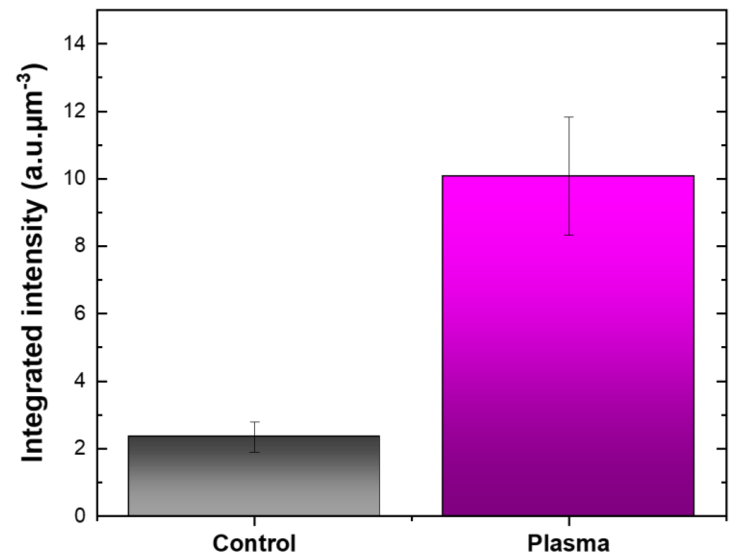
Evaluation of ROS in embryos by confocal microscopy



Embryo « CTRL »



Embryo « Plasma »



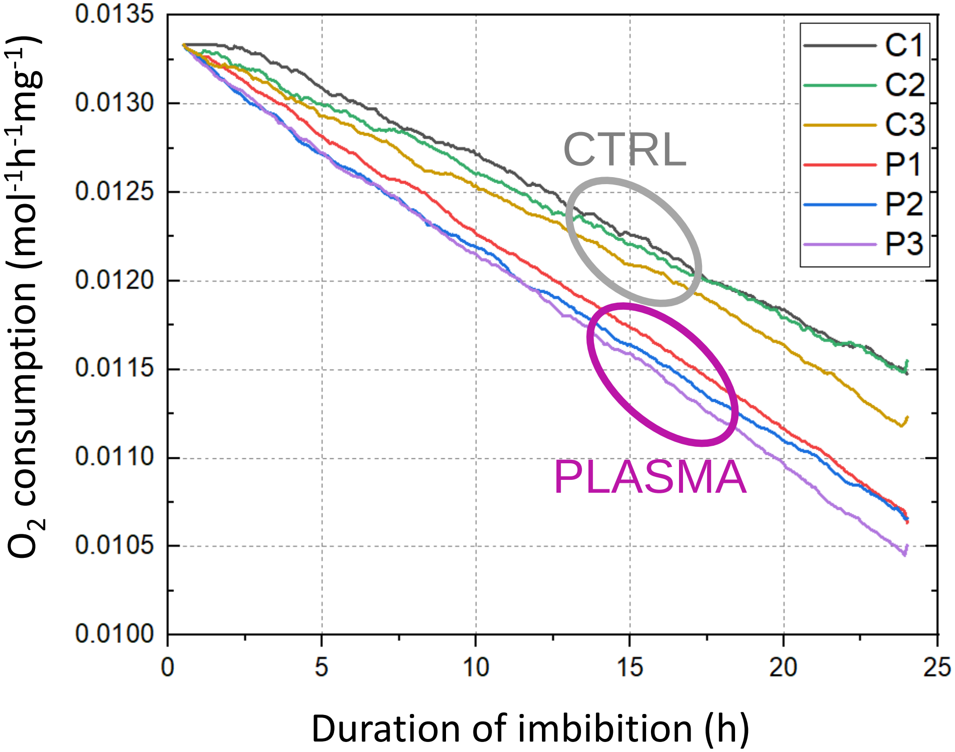
Mean over 50 embryos/group

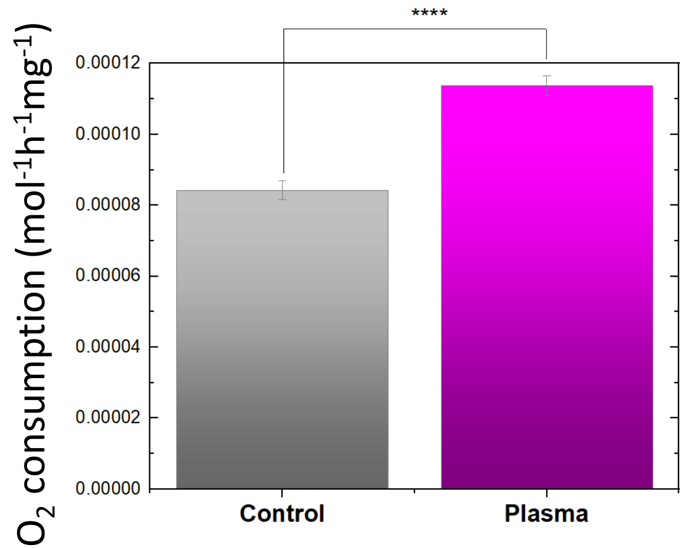
ROS fluorescence with DCFH-DA

CAP induced ROS accumulation within dry seeds : confirmed the role of ROS in the release of dormancy in anhydrobiosis

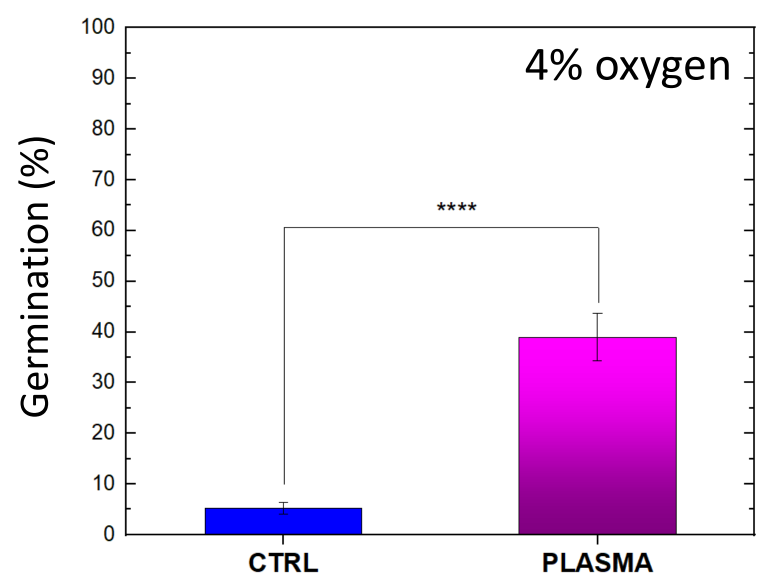
2. Effect of CAP on seed biological properties

Relationship CAP/oxygen-dependent processes (respiration, germination in hypoxia) ?





CAP also modified oxygen-related processes during germination



Conclusion

- Seed dormancy release by cold plasmas is linked with structural changes, oxidative processes and gene regulation

- Transcriptomic analysis allowed to highlight the biological processes triggered by plasma on seed dormancy release and confirmed role of ROS and oxygen in plasma response

- Opportunity to determine molecular markers and pathways related to plasma treatment, that can be used to evaluate its efficiency

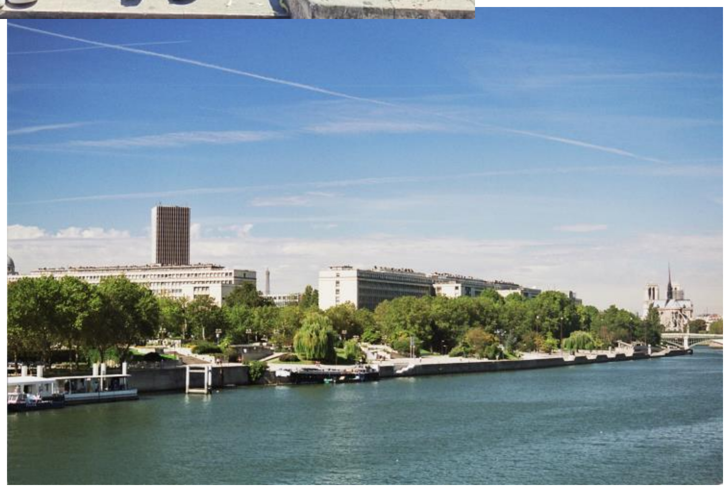
- Our results should help improving the use of CAP treatments on crop species

To sum-up

Mechanisms of seed germination are far from being all known and climate change issues require a better knowledge of this process

Plasma treatments are promising techniques for improving seed germination but they will require:

- an adaptation of techniques (scale-up)
- better interactions between physicists and seed biologists



Seed Biology Group

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