



UNIVERSITÀ DI PAVIA



Molecular perspectives into seed priming

The seed repair response as a key player in the pre-germinative metabolism

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*international society
for seed science*



ISTA
Seed Quality Assurance



Outline...



Brief overview on Seed Priming research



Seed pre-germinative metabolism



DNA Damage Response (DDR) as a seed repair response




Systems developed to investigate seed priming at the molecular level

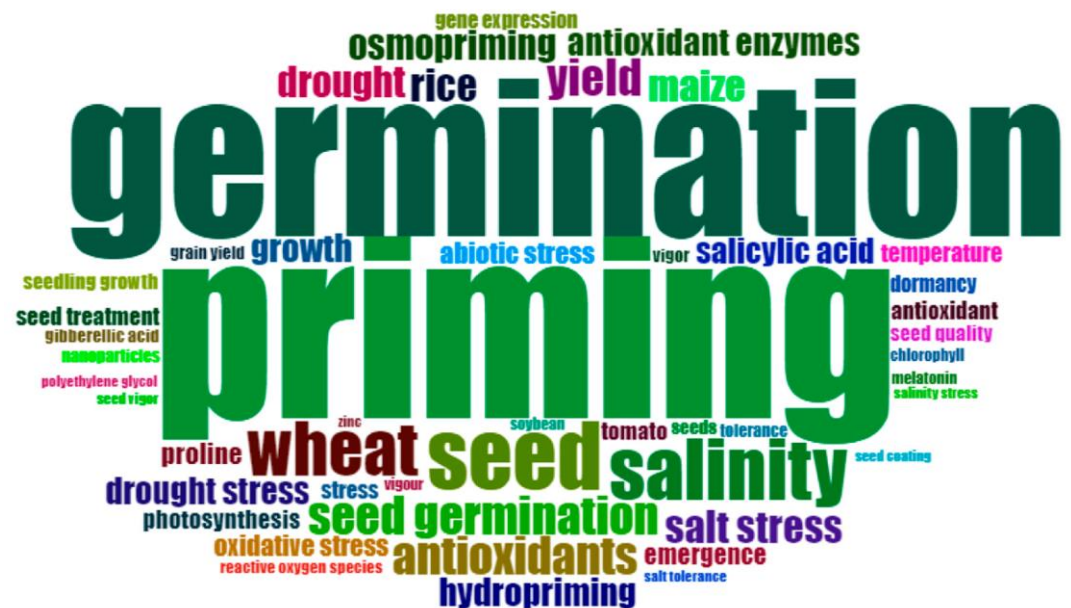
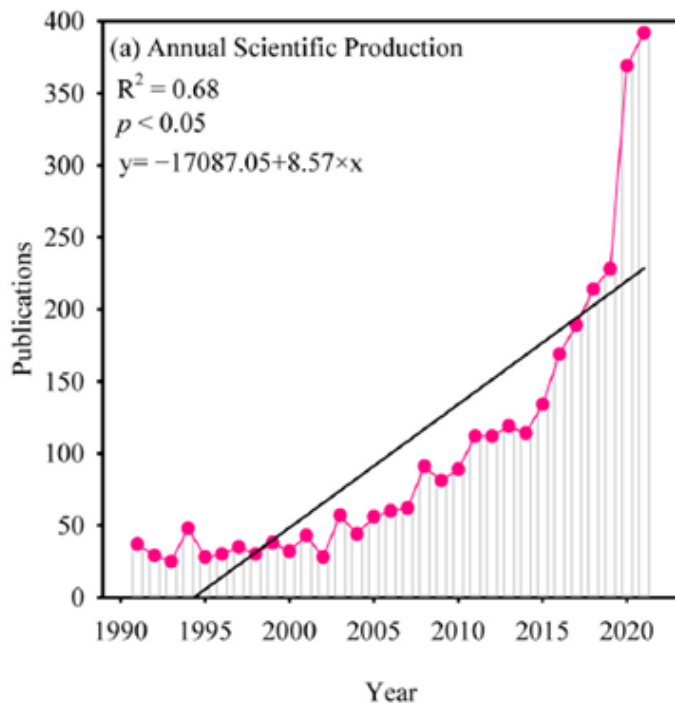
- **The chemical stress system**
- **The priming/overpriming system**
- **The intra- and inter-specific variation system**

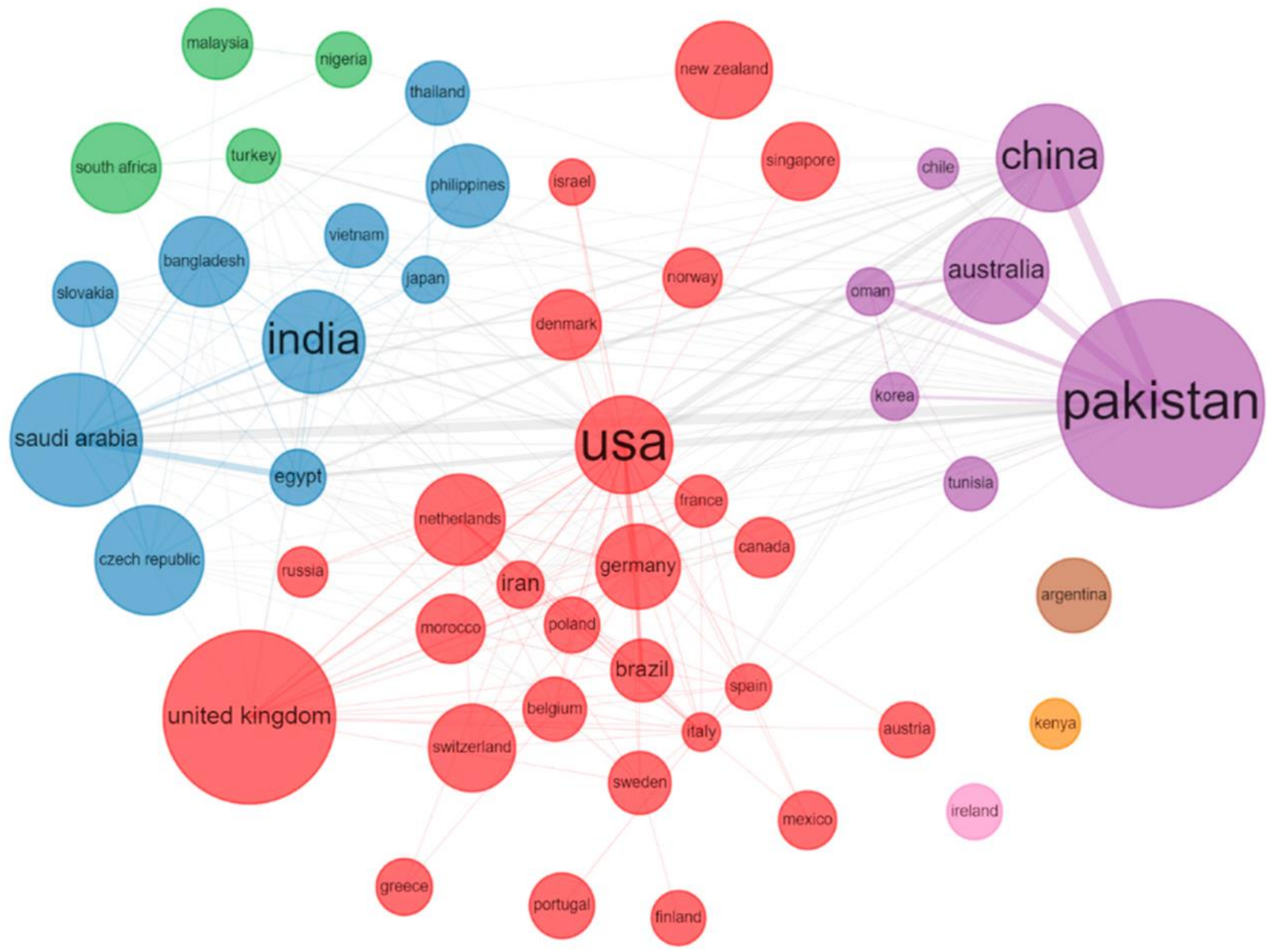
Review

Trends in Seed Priming Research in the Past 30 Years Based on Bibliometric Analysis

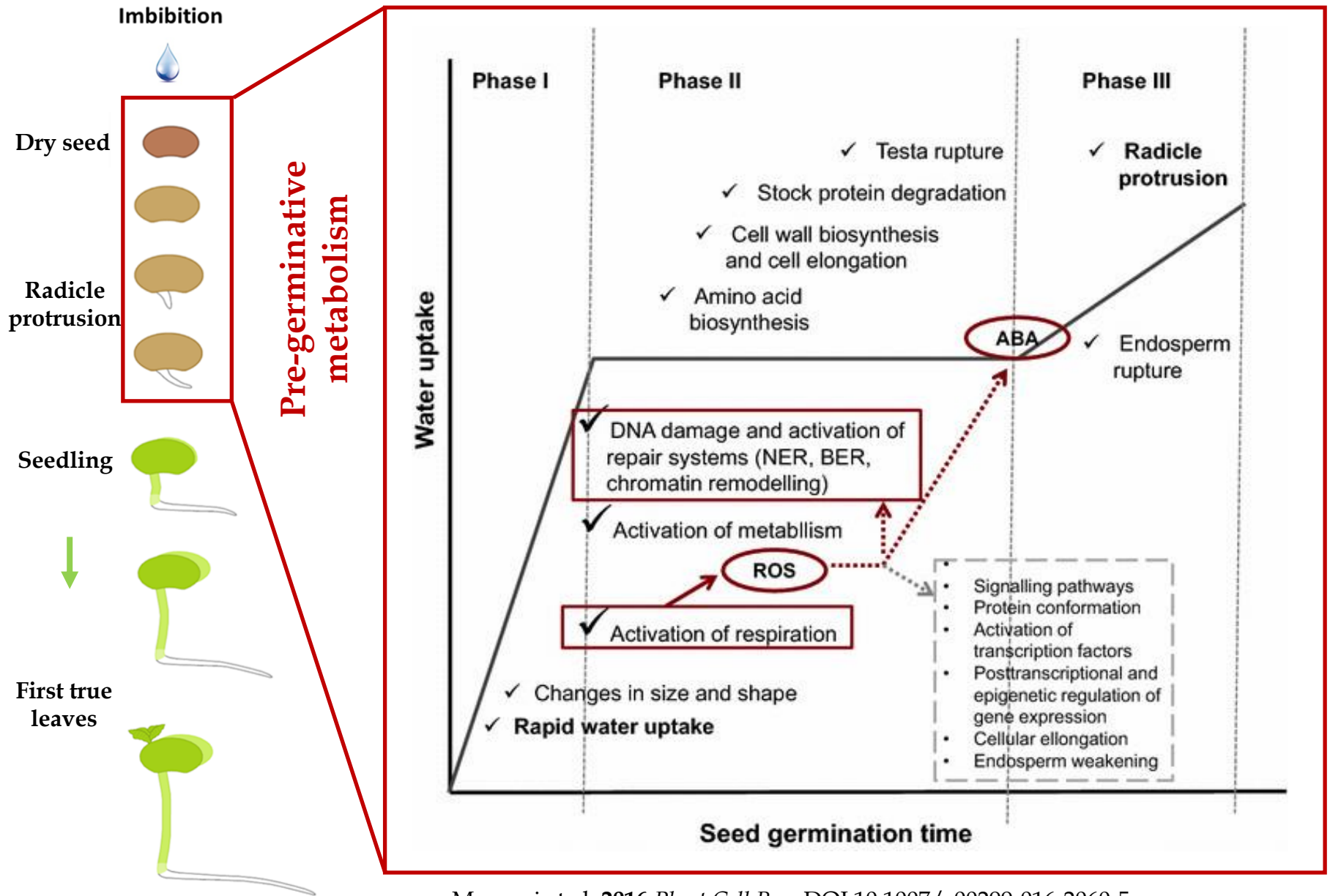
Yu Tian ¹ , Nalin Suranjith Gama-Arachchige ² and Ming Zhao ^{3,*}

Web of Science (WOS) core collection database <https://www.webofscience.com/WOS> accessed on 14 November 2022

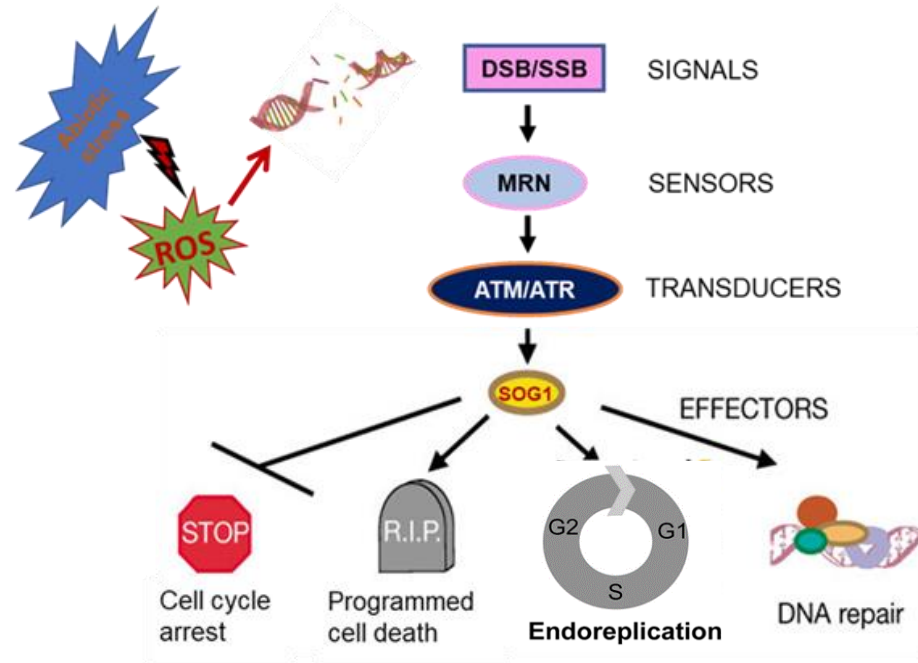




Seed pre-germinative metabolism



DNA Damage Response (DDR)

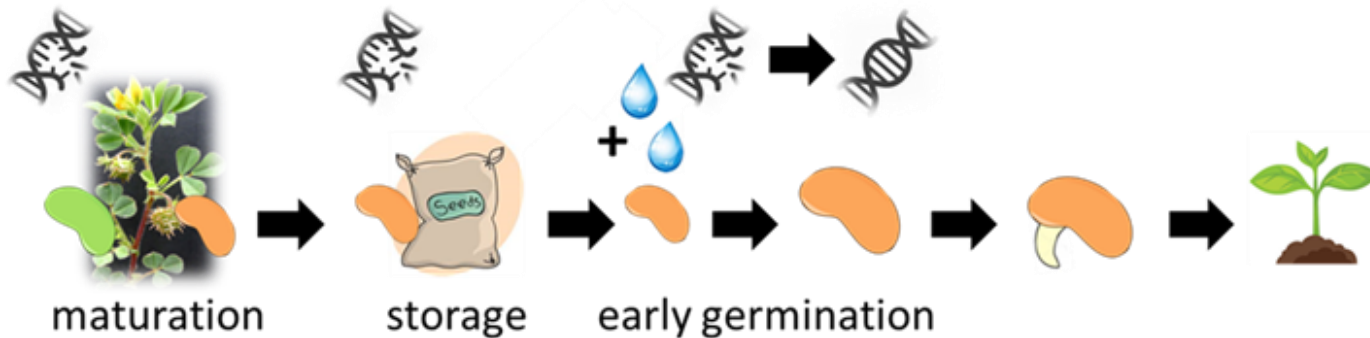


Plant growth stages



What happens to DDR during plant life...?

When do seeds accumulate or repair DNA damage?



DDR and the seed repair response



Seeds and the Art of Genome Maintenance

Wanda M. Waterworth^{1*}, Clifford M. Bray² and Christopher E. West¹

¹ University of Leeds, Leeds, United Kingdom, ² The University of Manchester, Manchester, United Kingdom



Seed DNA damage responses promote germination and growth in *Arabidopsis thaliana*

Wanda M. Waterworth¹, Rosalind Latham¹, Dapeng Wang^{1,2,3,4}, Mona Alshari¹, and Christopher E. West^{1,4*}

Edited by Maarten Koornneef, Max-Planck-Institut für Pflanzenzüchtungsforschung, Cologne, Germany, received February 14, 2022; accepted June 6, 2022

Biochemical Journal (2023) 480 461–470
<https://doi.org/10.1042/BCJ20230006>



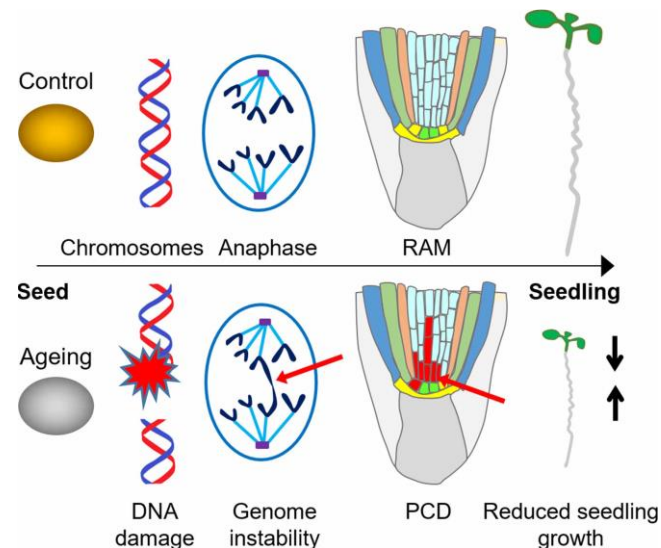
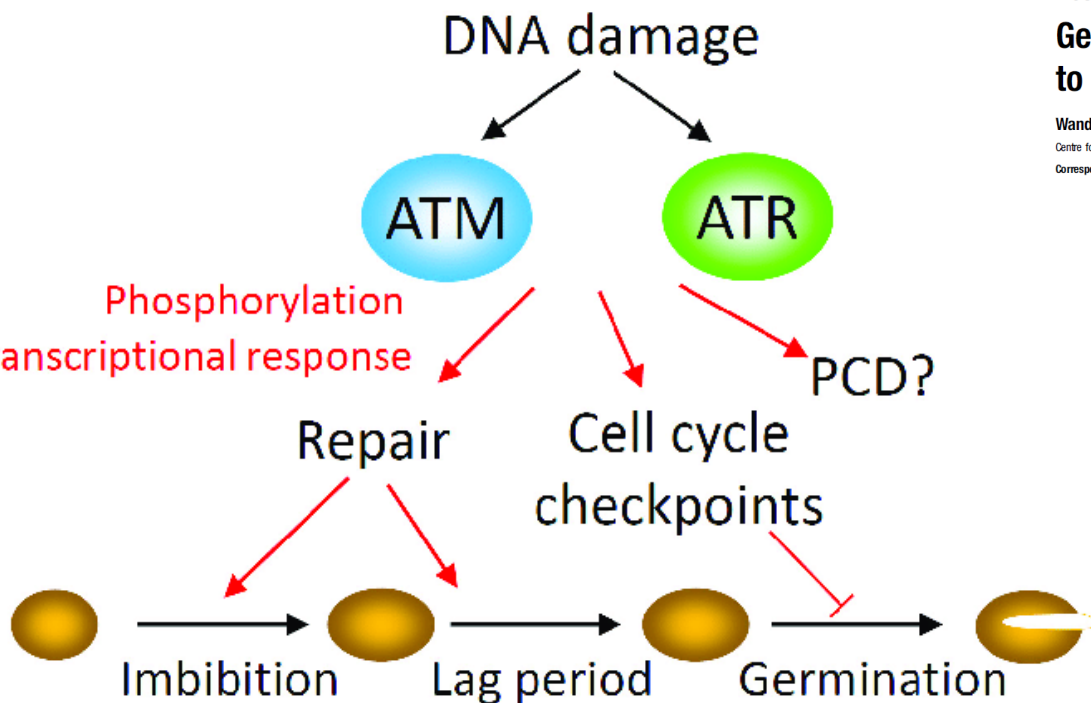
Research Article

Genome damage accumulated in seed ageing leads to plant genome instability and growth inhibition

Wanda M. Waterworth and Christopher E. West

Centre for Plant Sciences, University of Leeds, Woodhouse Lane, Leeds LS2 9JT, U.K.

Correspondence: Christopher E. West (c.e.west@leeds.ac.uk)





...how is DDR related to seed priming?



PRIMING - adaptive strategy where exposure to one stimulus can influence the response to subsequent stimuli - physiological state that enable plants to respond more rapidly to stress

Seed Priming



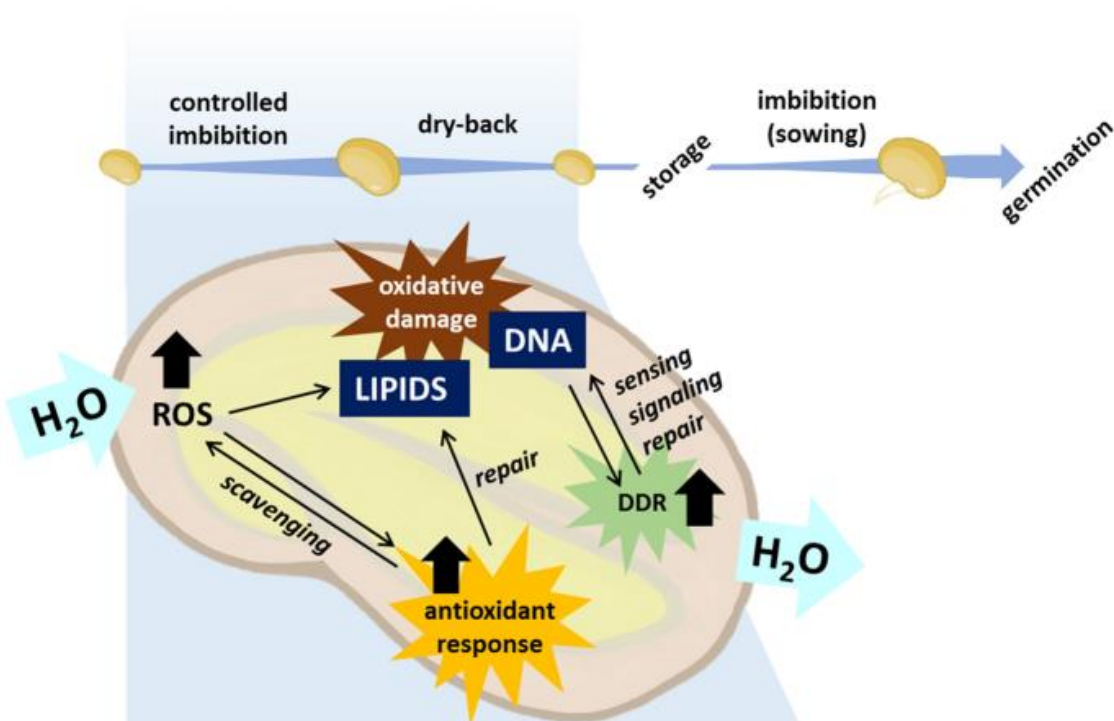
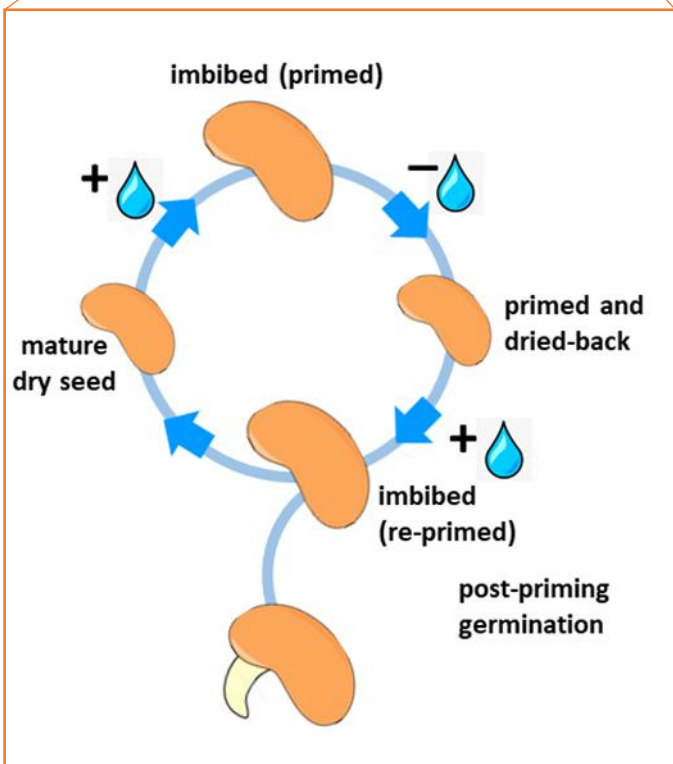
Chemical methods



Physical methods



Biological methods

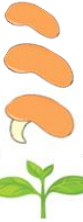


Systems developed to investigate seed priming at the molecular level

1



TIMEPOINTS

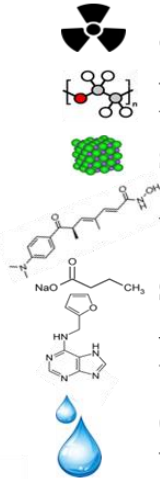


dry seed
imbibition (2h, 8h)
radicle protrusion
seedlings (4,7,14-days)

2



TREATMENTS

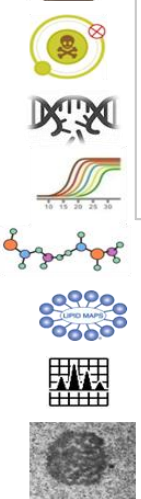


gamma irradiation
polyethylene glycol (PEG)
sodium chloride (NaCl)
trichostatin A (TSA)
sodium butyrate (NaB)
kinetin
desiccation-rehydration
hydropriming

3



ANALYSES



phenotyping
ROS detection
DNA damage/repair
gene expression
metabolomics
lipidomics
biochemical HPLC
ultrastructural TEM

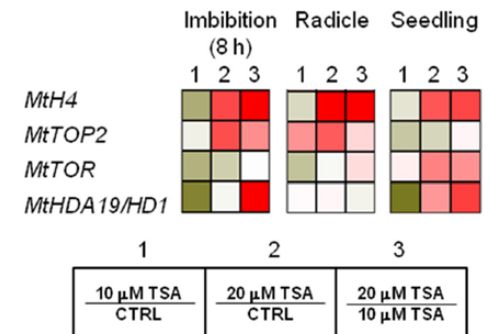
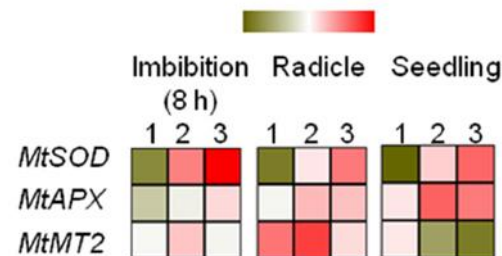
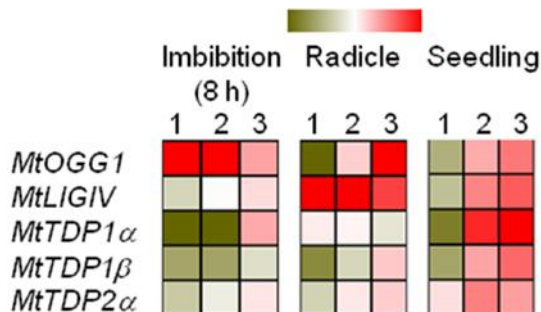
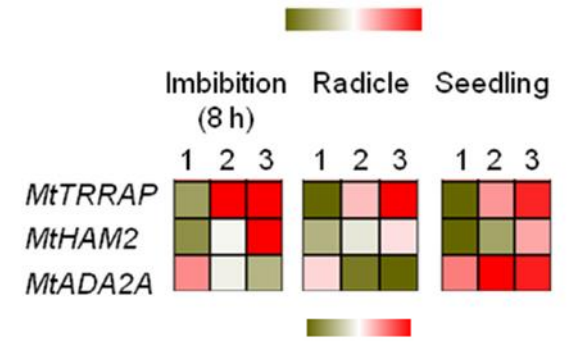
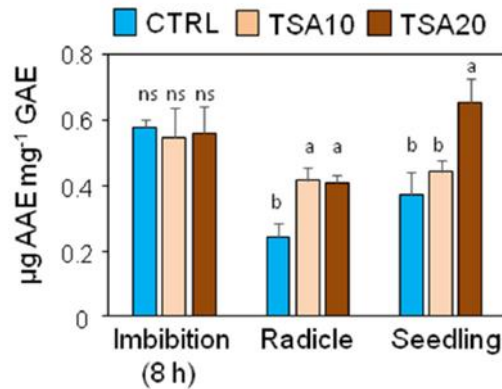
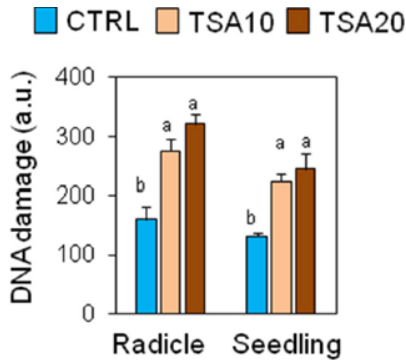
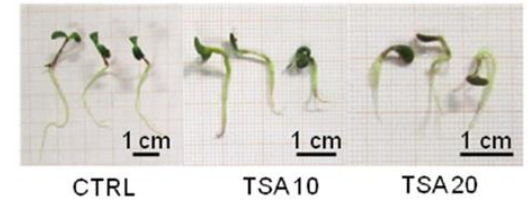
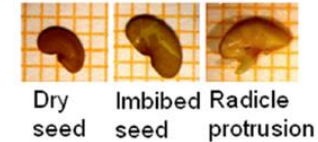
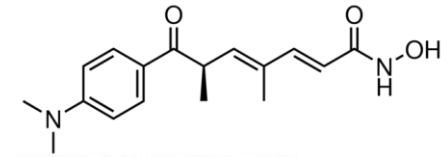
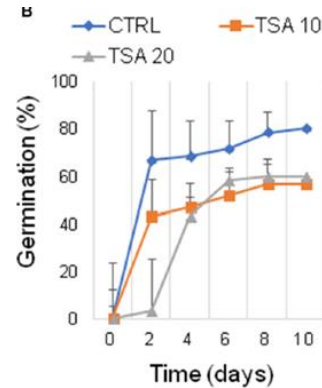
toolkit



The «chemical stress» system

The Seed Repair Response during Germination: Disclosing Correlations between DNA Repair, Antioxidant Response, and Chromatin Remodeling in *Medicago truncatula*

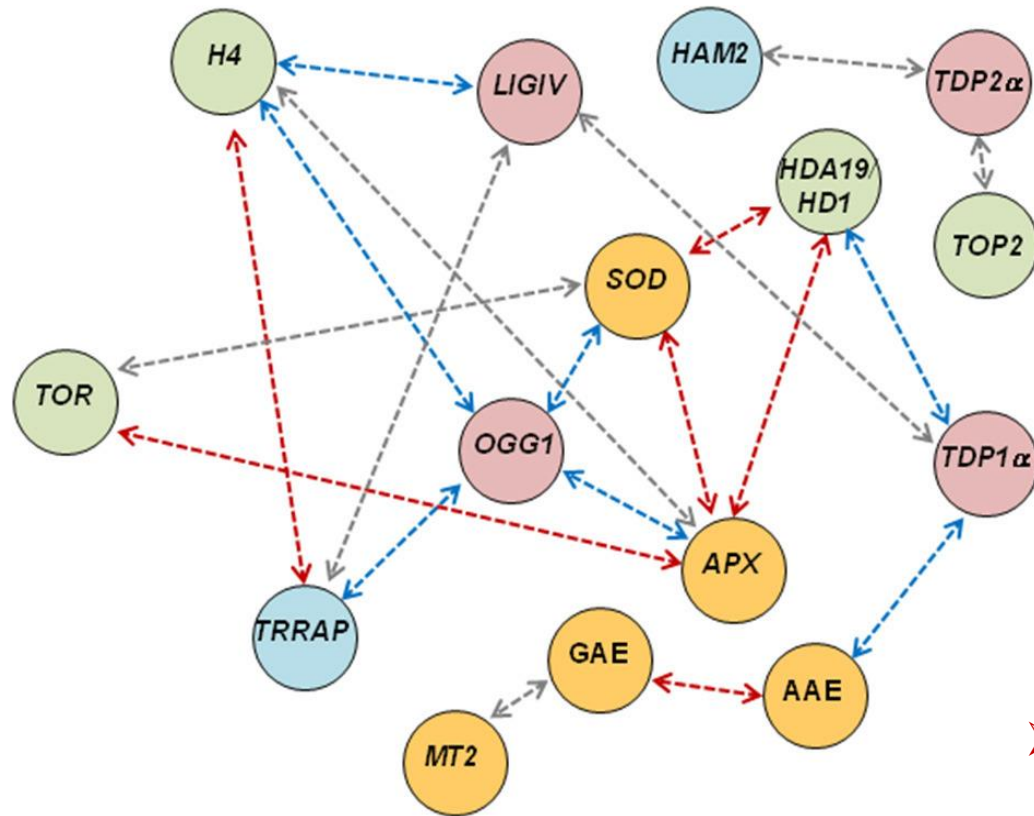
Andrea Pagano¹, Susana de Sousa Araújo², Anca Macovei¹, Paola Leonetti³ and Alma Balestrazzi^{1*}



1	2	3
10 μ M TSA CTRL	20 μ M TSA CTRL	20 μ M TSA 10 μ M TSA

The «chemical stress» system

Schematic representation of the most relevant cases of correlation of biochemical and gene expression variables based on the Pearson correlation coefficients r



● antioxidant response ● DNA damage response
● chromatin remodeling ● proliferation/development

↔ $r^2 \geq 80\%$ ($P \leq 0.05$)
↔ $r^2 75\% \leq R < 80\%$ ($P \leq 0.05$)
↔ $r^2 70\% \leq R < 75\%$ ($P \leq 0.05$)

- Correlation analysis discloses novel putative links between DNA repair, chromatin remodeling, antioxidant response and proliferation markers.
- ❑ *TRRAP - OGG1* - bridge DNA repair and chromatin remodeling in the context of seed germination
- ❑ *OGG1 - APX, SOD* - link between DNA repair and antioxidant response a crucial aspect of seed vigor

➤ TSA blocks cell cycle, induces ROS accumulation and DNA damage, and all these events touch directly or indirectly molecular processes that contribute to the seed stress response

The «chemical stress» system

Plant, Cell & Environment



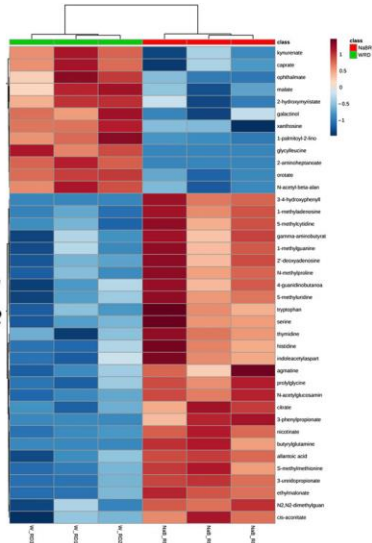
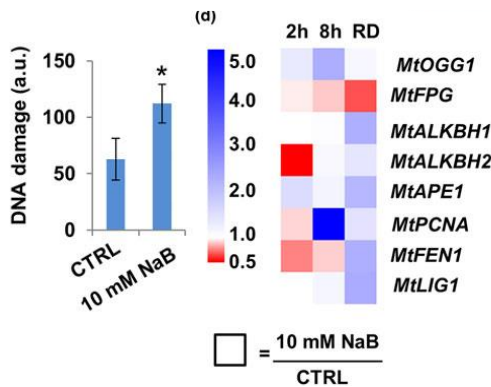
Received: 28 November 2017 | Revised: 3 May 2018 | Accepted: 5 May 2018
DOI: 10.1111/pce.13342

ORIGINAL ARTICLE

WILEY Plant, Cell & Environment

Metabolic and gene expression hallmarks of seed germination uncovered by sodium butyrate in *Medicago truncatula*

Andrea Pagano¹ | Susana de Sousa Araújo² | Anca Macovei¹ | Daniele Dondi³ | Simone Lazzaroni³ | Alma Balestrazzi¹



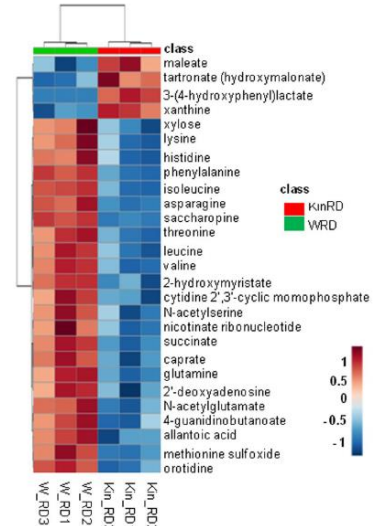
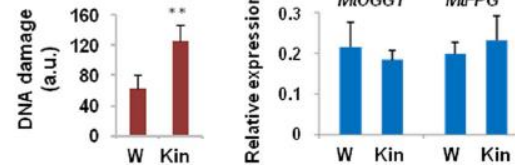
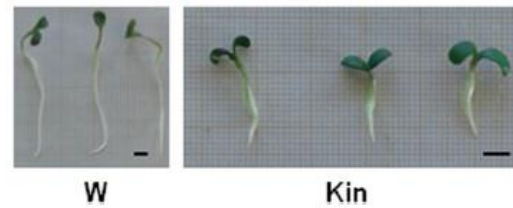
- Polyamine biosynthesis
- Uracil degradation pathway
- Purine and pyrimidine metabolism
- METABOLIC HALLMARKS OF SEED RESPONSE TO GENOTOXIC STRESS

SCIENTIFIC REPORTS

OPEN Metabolic signatures of germination triggered by kinetin in *Medicago truncatula*

Received: 11 March 2019
Accepted: 1 July 2019
Published online: 18 July 2019

Susana Araújo¹, Andrea Pagano², Daniele Dondi³, Simone Lazzaroni³, Eduardo Pinela⁴, Anca Macovei² & Alma Balestrazzi²



- 27 metabolites showed significant changes triggered by kinetin
- inositol, pentakisphosphate, agmatine, digalactosylglycerol, inositol hexakisphosphate, oleoylcholine
- changes linked to fast metabolic depletion associated with a fast germination

The «chemical stress» system

- Identification of "quality"/stress hallmarks during the early phases of seed germination



DDR (e.g. OGG1, TDP1)
Antioxidants (e.g. APX, SOD)

Dry seed



Radicle protrusion



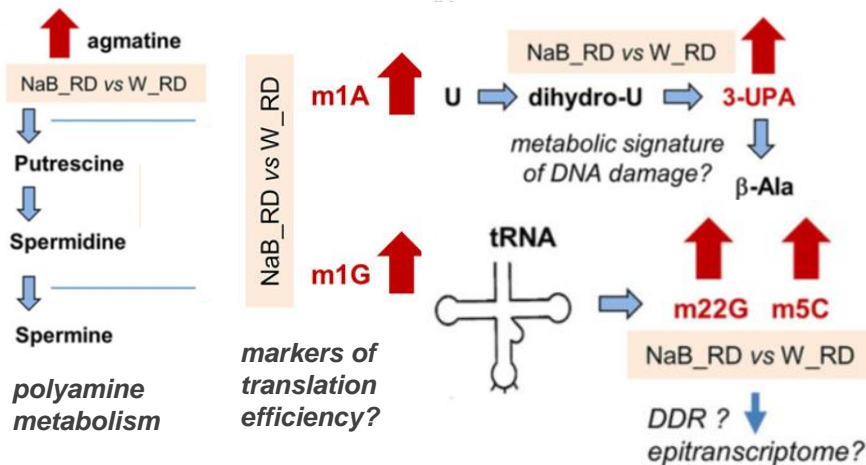
Seedling →



First true leaves



STRESS



The «priming/overpriming» system



Overpriming = loss of desiccation tolerance



agronomy



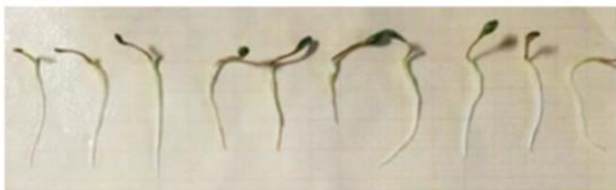
Article

ROS Accumulation as a Hallmark of Dehydration Stress in Primed and Overprimed *Medicago truncatula* Seeds

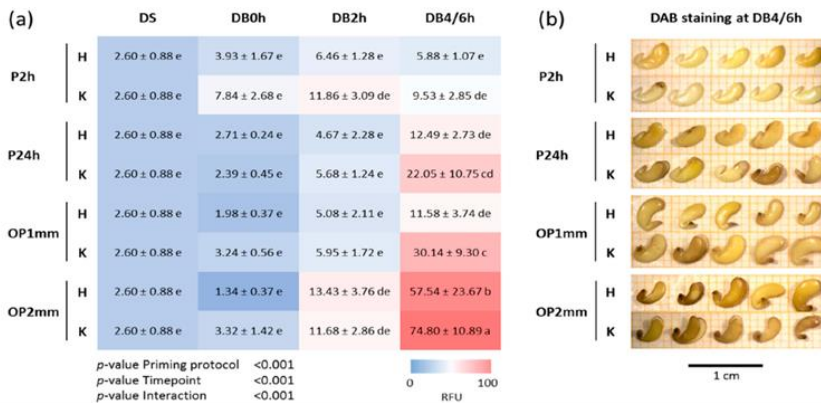
Andrea Pagano ¹, Giulia Folini, Paola Pagano, Federico Sincinelli, Andrea Rossetto, Anca Macovei and Alma Balestrazzi



Hydropriming/ Hormopriming 2 h



Overpriming 24 h



antioxidants

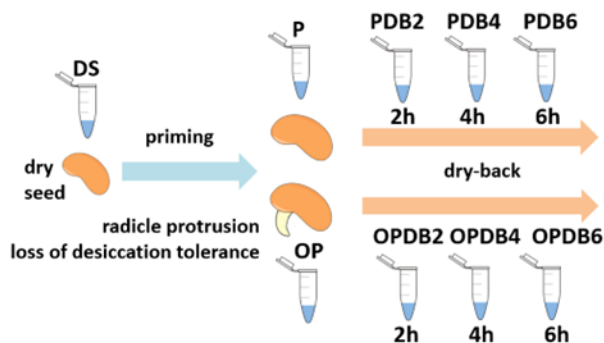


Article

Noninvasive Methods to Detect Reactive Oxygen Species as a Proxy of Seed Quality

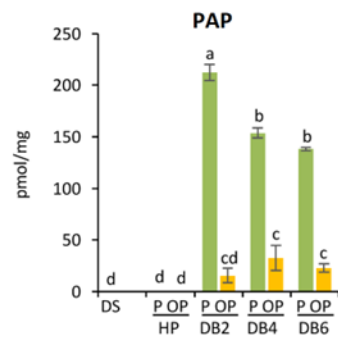
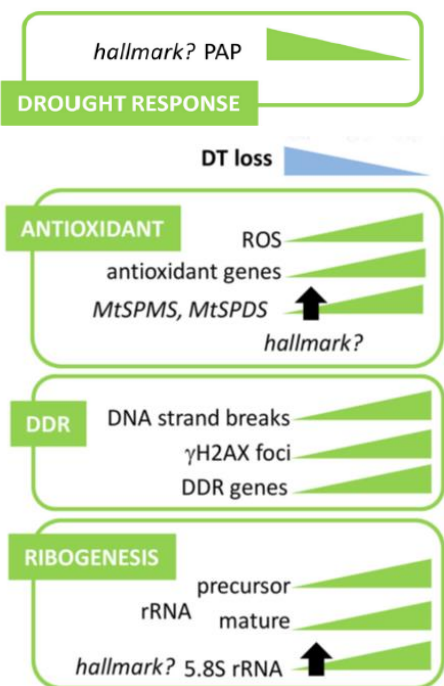
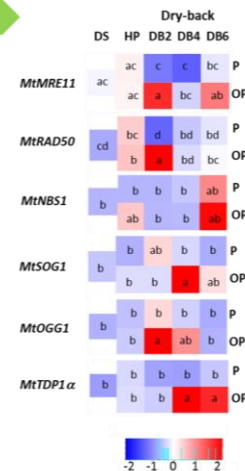
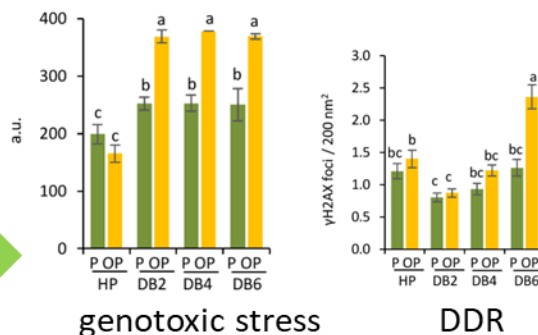
Adriano Griffo ^{1,†}, Nicola Bosco ^{1,†}, Andrea Pagano ¹, Alma Balestrazzi ^{1,2,*} and Anca Macovei ^{1,2,*}

The «priming/overpriming» system

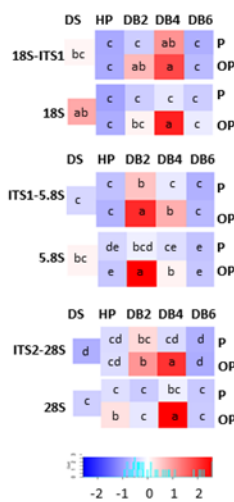
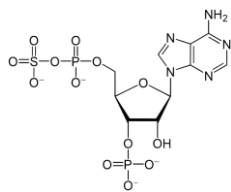


primed
vs.
overprimed

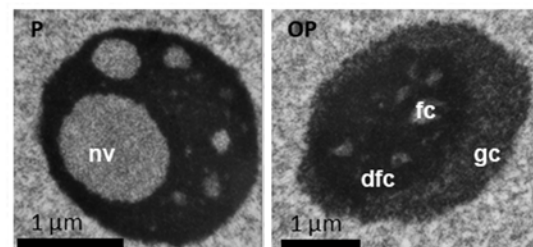
HALLMARKS



3'-phosphoadenosine
5'-phosphate



altered
ribogenesis



nucleolus as stress sensor

Received: 27 December 2021 | Revised: 4 February 2022 | Accepted: 5 February 2022
DOI: 10.1111/pce.14295

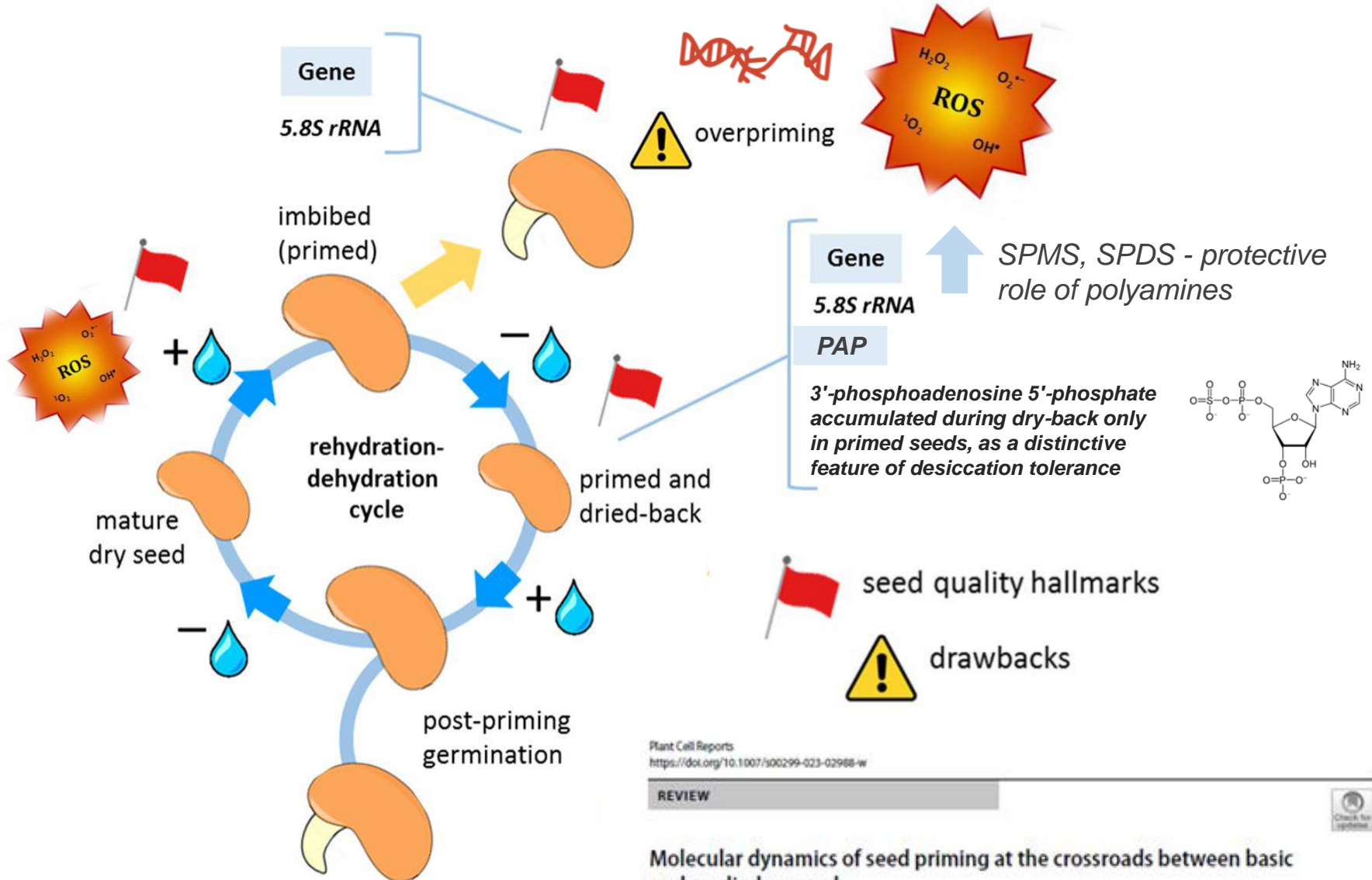
ORIGINAL ARTICLE



Changes in genotoxic stress response, ribogenesis and PAP (3'-phosphoadenosine 5'-phosphate) levels are associated with loss of desiccation tolerance in overprimed *Medicago truncatula* seeds

Andrea Pagano¹ | Lorena Zannino¹ | Paola Pagano¹ | Enrico Doria¹ | Daniele Dondi² | Anca Macovei¹ | Marco Biggiogera¹ | Susana de Sousa Araújo³ | Alma Balestrazzi¹

The «priming/overpriming» system



Plant Cell Reports
<https://doi.org/10.1007/s00299-023-02988-w>

REVIEW

Molecular dynamics of seed priming at the crossroads between basic and applied research

Andrea Pagano¹ · Anca Macovel^{1,2} · Alma Balestrazzi^{1,2}





The «intra- and inter-specific variation» system



✓ Priming protocols – species/genotype/seed lot-dependent

□ Gene expression as indicators of seed quality and priming efficiency


Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy




International Journal of
Molecular Sciences




Research article

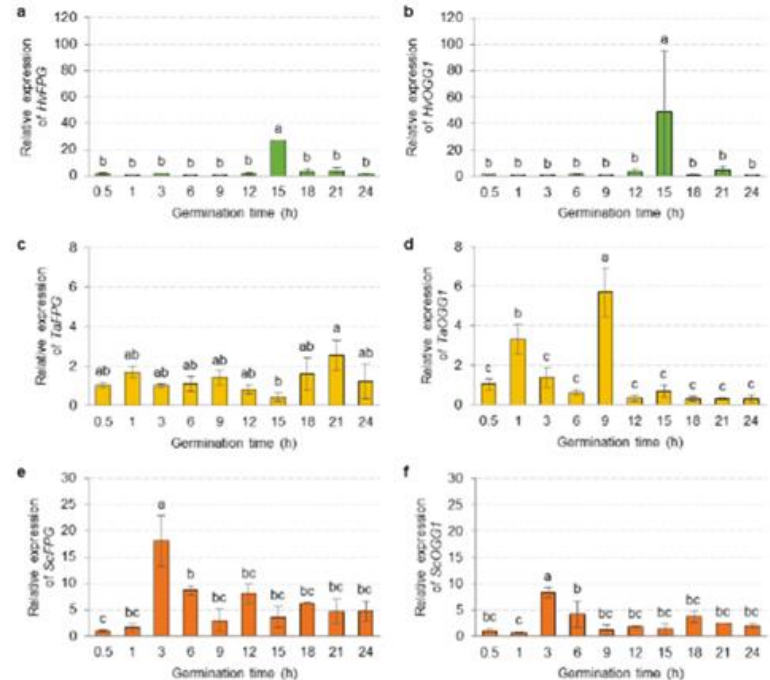
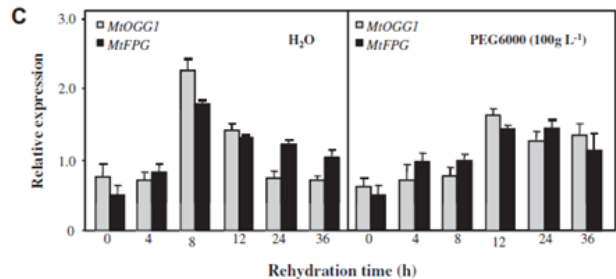
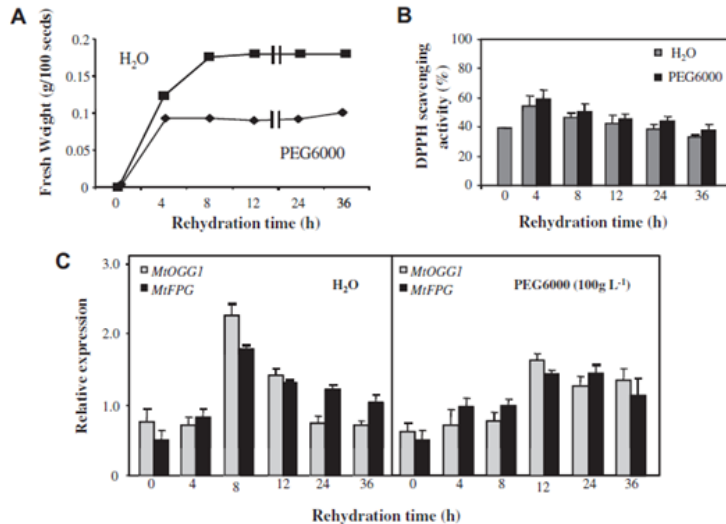
New insights on the barrel medic *MtOGG1* and *MtFPG* functions in relation to oxidative stress response in planta and during seed imbibition

Anca Macovei^a, Alma Balestrazzi^a, Massimo Confalonieri^b, Matteo Faè^a, Daniela Carbonera^{a,*}

Communication

Profiling of Barley, Wheat, and Rye *FPG* and *OGG1* Genes during Grain Germination

Sylvia Kowalik[†] and Jolanta Groszyk^{*†}





The «intra- and inter-specific variation» system



Forti et al. *Horticulture Research* (2020)7:87
<https://doi.org/10.1038/s41438-020-0310-8>

Horticulture Research
www.nature.com/hortres

frontiers
in Plant Science

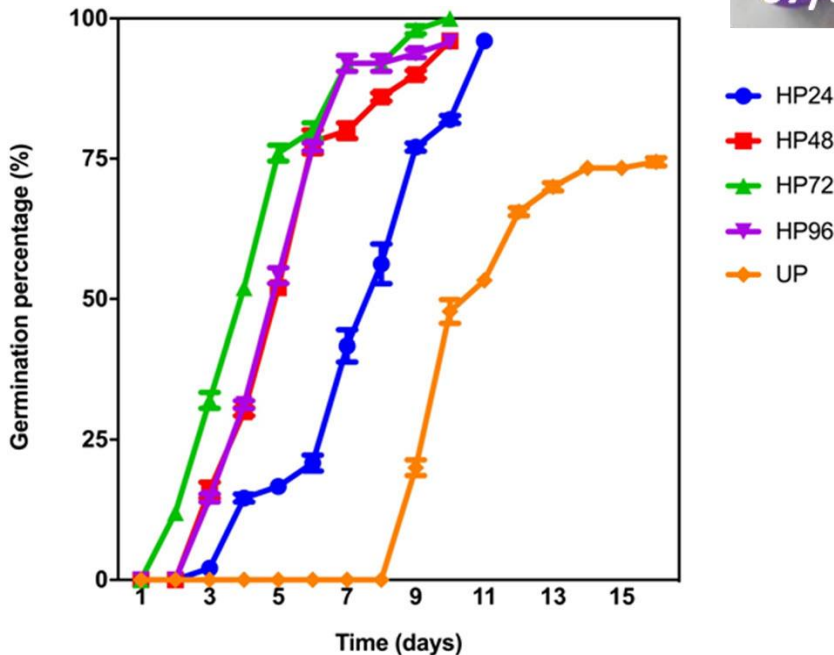
ORIGINAL RESEARCH
published: 25 March 2021
doi: 10.3389/fpls.2021.639336

ARTICLE

Open Access

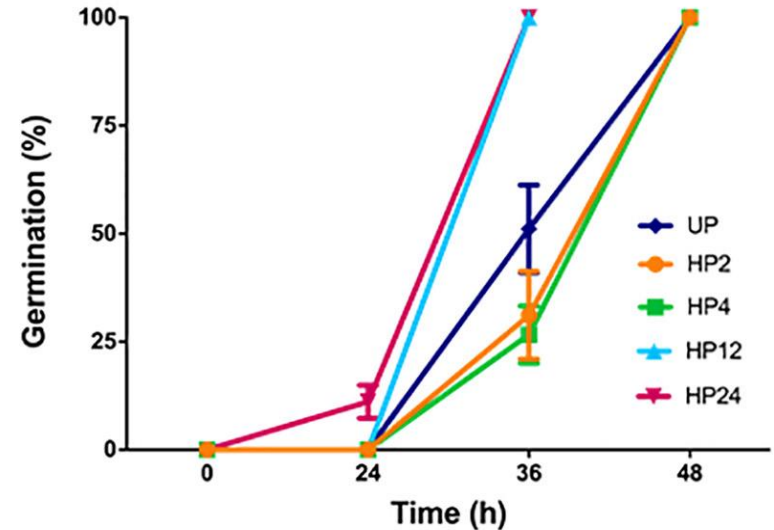
Molecular dynamics of pre-germinative metabolism in primed eggplant (*Solanum melongena* L.) seeds

Chiara Forti¹, Valentino Ottobriano¹, Laura Bassolino^{2,3}, Laura Toppino², Giuseppe Leonardo Rotino², Andrea Pagano^{1,4}, Anca Macovei¹ and Alma Balestrazzi¹



Hydropriming Applied on Fast Germinating *Solanum villosum* Miller Seeds: Impact on Pre-germinative Metabolism

Chiara Forti^{1*}, Valentino Ottobriano¹, Enrico Doria¹, Laura Bassolino^{2,3}, Laura Toppino², Giuseppe Leonardo Rotino², Andrea Pagano¹, Anca Macovei¹ and Alma Balestrazzi^{1*}





The «intra- and inter-specific variation» system

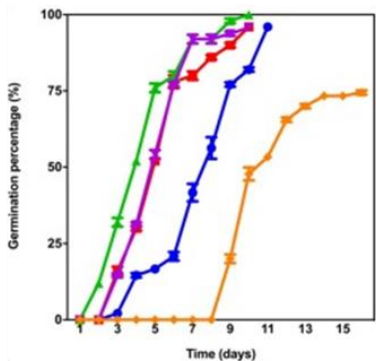


- ✓ 34 *S. melongena* accessions + 9 CWRs from the CREA-GB Germplasm Collection
- ✓ grouped based on germination phenotypes (EARLY, NORMAL, LATE)
- ✓ seed lots collected at different years



HALLMARKS

germination delay

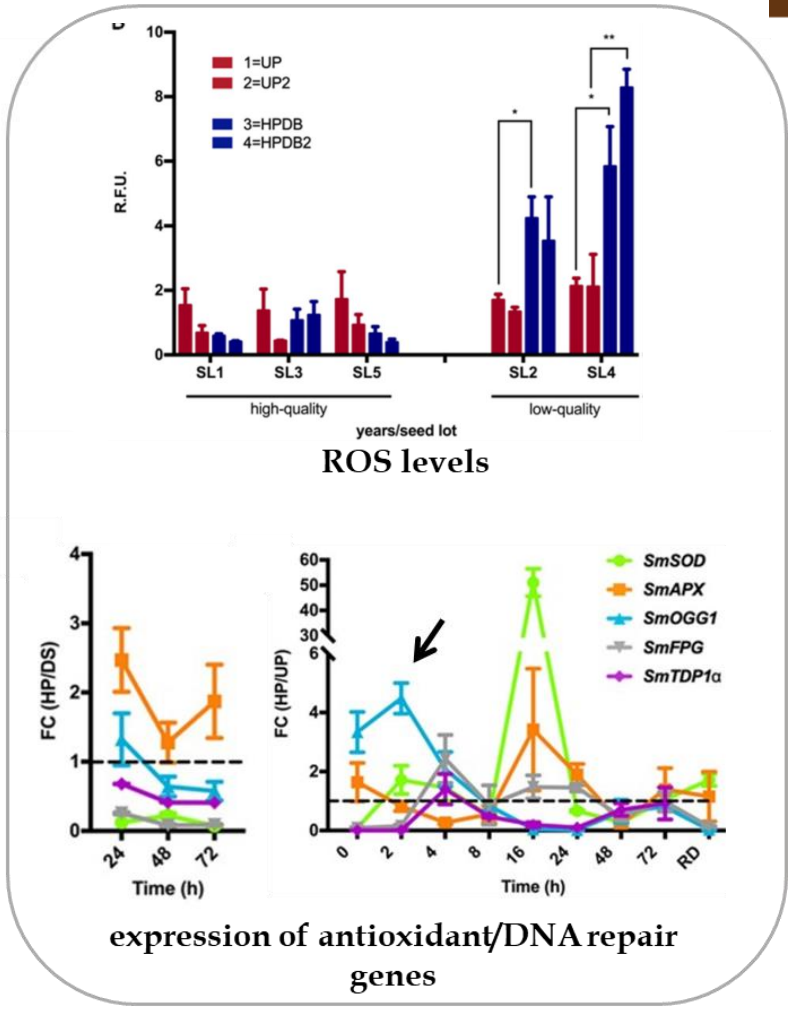


selected treatment
 →
 impact on pre-germinative metabolism

hydropriming accelerates germination of '67/3' seeds

variability and heterogeneity observed when comparing different genotypes and seed lots

... search for hallmarks is delayed





The «intra- and inter-specific variation» system



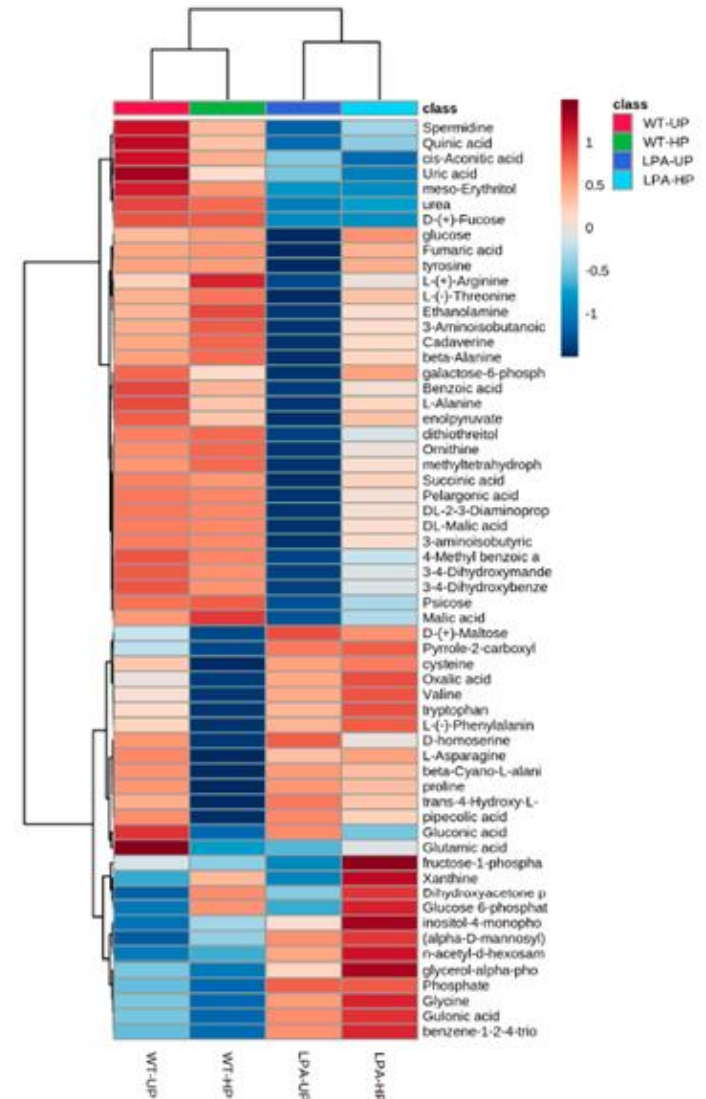
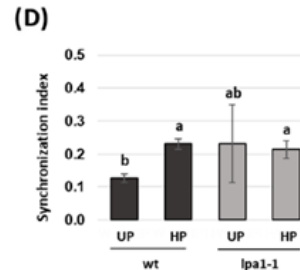
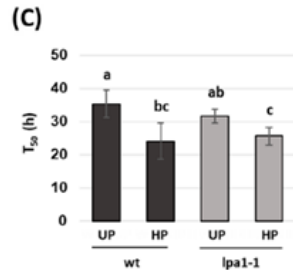
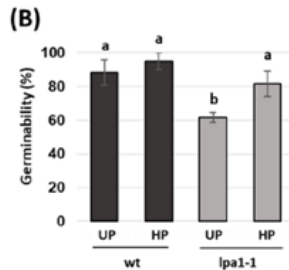
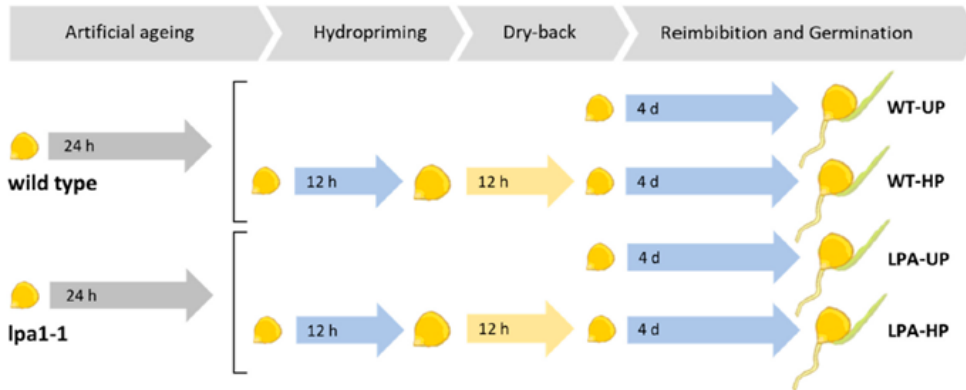
International Journal of
Molecular Sciences



Article

Study of Seed Ageing in *lpa1-1* Maize Mutant and Two Possible Approaches to Restore Seed Germination

Federico Colombo ^{1,t}, Andrea Pagano ^{2,t}, Stefano Sangiorgio ¹, Anca Macovei ², Alma Balestrazzi ², Fabrizio Araniti ¹ and Roberto Pilu ^{1,*}



➤ metabolomic differences among genotypes and priming treatments

CLOSING REMARKS

Basic

Models of the pre-germinative metabolism responding to seed priming and stress conditions



Identification of quality-related traits



Identification of key molecular players

Translational

Proofs-of-concept in different model, crop and wild species



Assays for seed quality evaluation



Tailored seed priming protocols

Applied

Scale-up validation and biomarker-assisted breeding in various agricultural contexts



Climate-ready crops



Food security

Boosting technologies of **orphan legumes** towards resilient farming systems in the Greater Mediterranean Region: from bench to open field

Strengthening capacities and promoting innovation in **plant omics** at University of Sfax

Plant-based biostimulants for seeds in the context of circular economy and sustainability



<https://www.benefit-med.eu/>



NPLANTOMICS



Université de Sfax
TUNISIE

<https://www.inplantomics.org/>



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CENTER



NODES

Nord Ovest Digitale E Sostenibile

PIANO NAZIONALE DI RIPRESA E RESILIENZA

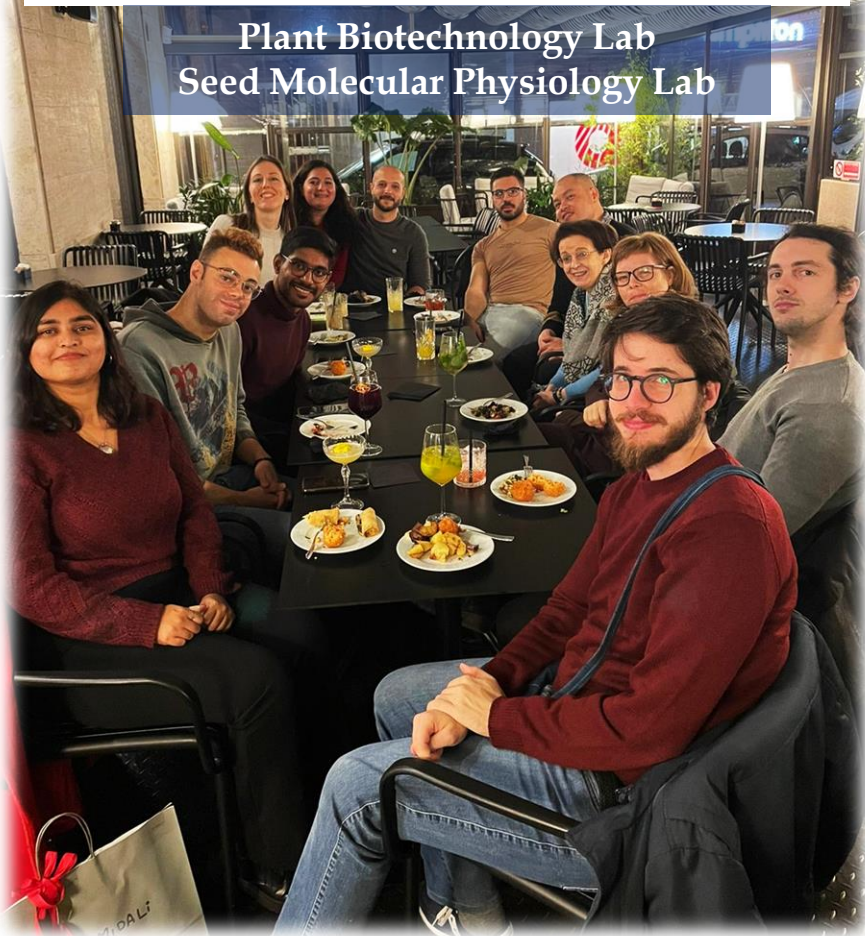
ACKNOWLEDGEMENTS



UNIVERSITÀ DI PAVIA
Dipartimento di
Biologia e Biotecnologie
"Lazzaro Spallanzani"



Plant Biotechnology Lab
Seed Molecular Physiology Lab



Collaborators S. Araújo



G. Rotino

R. Pilu

E. Doria

L. Bassolino

F. Colombo

M. Bioggiogera

L. Toppino

F. Araniti

D. Dondi



UNIVERSITÀ
DEGLI STUDI
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Financial support



Fondazione
CARIPLO



Piano Nazionale
di Ripresa e Resilienza
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Symbiaagrò




TOKITA



Innova-Tech



Hoopman
Group



**THANK YOU FOR YOUR
ATTENTION**

...AND...

**THANK THE INTERNATIONAL
COMMUNITY FOR ALL THE
WORK !!!**