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Integrated acclimation responses of maize root hydraulic architecture to water deficit

Protto Virginia, Bauget Fabrice, Rishmawi Louai, Tournaire-Roux Colette, Boursiac Yann, Cyril Bauland, Nicolas Stéphane D., Charcosset Alain, Nacry Philippe, Maurel Christophe

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Welcome to the Embo workshop
Molecular responses of plants
facing climate change

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Introduction

Climate change profoundly alters plant growth and development, presenting a serious threat to food security in the coming decades. Fortunately, considerable research in the plant sciences community focuses on understanding the physiological processes and underlying molecular mechanisms through which plants can adapt to these changes. This recently resulted in significant progress, and even positions plants as a solution to mitigate greenhouse gas emissions and associated climate change effects.

This EMBO Workshop will convene scientists with a broad, integrative view of plant science in the context of climate change. It will highlight the molecular mechanisms of plant adaptation to major factors associated with climate change (elevated CO₂, temperature, drought and salinity), and identify processes beyond resilience to and memory of climate fluctuations. It will also pinpoint the recent molecular approaches for harnessing plants to mitigate climate change effects, and will highlight examples of adaptation to extreme life conditions to insure food security under ever more constrained environmental conditions.

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Salma Balazadeh, Institute of Biology Leiden, NL
Isabel Bäurle, University of Potsdam, DE
Malcom Bennett, University of Nottingham, UK
Arnold Bloom, University of California at Davis, US
Florian Busch, University of Birmingham, m, UK
Ana I. Caño-Delgado, Centre for Research in Agricultural Genomics, ES
Jorge Casal, Universidad de Buenos Aires, AR
Joanne Chory, Salk Institute, USA
Ivan Couée, University of Rennes, FR
Carolin Delker, Martin Luther University Halle, DE
Jose Dinneny, Stanford University, US
Christine Foyer, University of Birmingham, UK
Rodrigo Gutiérrez, Pontificia Universidad Católica de Chile,
Heribert Hirt, Center for Desert Agriculture, SA
Ndjido Kane, ISRA, SN
Hannes Kollist, University of Tartu, ES
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Hilde Nelissen, VIB Center for Plant Systems Biology, BE
Alejandro Perdomo, Lancaster University, UK
Leandro Quadrana, Institute of Plant Science Paris-Saclay, FR
Julia Questa, Centre for Research in Agricultural Genomics, ES
Keiko Sugimoto, RIKEN Center for Sustainable Resource Science, JP
Vincent Vadez, IRD, FR
Philip Wigge, University of Potsdam, DE

Sponsors



GIR IBCO2



UNIVERSITÉ DE MONTPELLIER



Journal of Experimental Botany



General information

All oral presentations will be held in the auditorium Lamour, located at the heart of L'institut Agro Montpellier campus (see plan in the following pages). Poster sessions will be held in the hall Lamour, close to the auditorium.

Registration desk:

The registration desk will be located in the hall Lamour. The opening hours are:

-Monday, 13rd June: 15:00 – 19:00

-Tuesday 14th June to Friday 17th: 08:00 – 09:00 and during coffee breaks.

Name badges:

Participants are requested to wear name badges at all times during the Workshop hours. These badges will be required for entrance in the L'institut Agro Montpellier Campus and in the hall Lamour (safety controls will be made by security staff), for lunches and for the gala dinner.

Gala Dinner:

The gala dinner is offered to all attendees and registered accompanying persons on Thursday, 16th June, at the 'Voile Bleue', Av. de Carnon, 34280 La Grande-Motte, nearby the sea side. Buses will leave the SupAgro campus at 18:15, and will take back attendees to the city center and to L'institut Agro Montpellier campus.

Bank, shops and services:

You will find all facilities in the middle of Montpellier city center (see map in the following pages) (cash dispensers, currency exchange service, pharmacy, dozen of bars, restaurants and shops).

Tourist information:

The tourist information office (<http://www.montpellier-france.com/>) is located at the end of the 'esplanade Charles de Gaulle' (see map in the following pages).

Police and Emergency:

A police station is located on the 'esplanade Charles de Gaulle', nearby the tourist information office. Medical emergency: Hospital Lapeyronie, 371 avenue du doyen Gaston Giraud. Tramway line 1: Stop Lapeyronie. The general-purpose emergency phone number for all Europe is: 112. In France, you can also use: Medical emergency: 15. Police (or Gendarmerie): 17. Fire emergency: 18

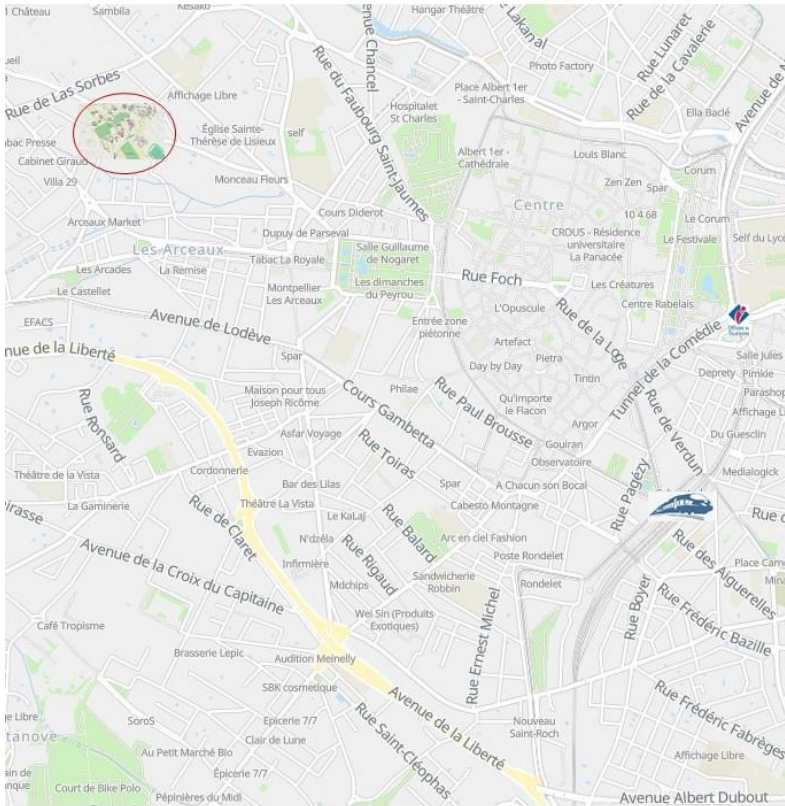
Venue : INRAE/L'institut Agro Montpellier 2 place Pierre Viala, 34060 Montpellier

Bus 6 direction Euromédecine Stop : Pierre Viala

See <http://tam.cartographie.pro/> for a general map of Montpellier public transportation lines



Location of the Conference Centre in Montpellier



Campus map



- ① Amphithéâtre Philippe Lamour
- ② Espace Philippe Lamour
- ③ Amphithéâtres 206 & 208
- ④ Amphithéâtre 2
- ⑤ Brasserie « l'étage » & Salon
- ⑥ Restaurant self-service « La Marmite »
- ⑦ AgroForum & terrasse

Ligne 6 Arrêt Pierre Viala
Direction Centre Ville
Direction Pas du Loup

Detailed Program

Monday 13 June

- 15:00-18:00 Registration opening
17:45-18:00 Opening remarks
18:00-18:40 **Vincent Vadez**, IRD, FR
Introductory lecture. Linking scales to achieve impact
18:40-20:00 Welcoming reception

Tuesday 14 June

Session 1: Effect of climate change on plant growth, development and physiology

Chair: Laurent Laplaze, IRD, FR

- 09:00-09:45 **Malcolm Bennett**, University of Nottingham, UK
Keynote lecture. Discovering how plant roots sense and adapt to dynamic changes in soil water availability
- 09:45-10:15 **Hannes Kollist**, University of Tartu, ES
Stomatal regulation by the environment
- 10:15-10:30 **Ive De Smet**, Ghent University and VIB Center for Plant Systems Biology, Belgium.
A conserved signaling axis integrates conflicting environmental signals to control transpiration in plants
- 10:30-11:00 Coffee break
- 11:00-11:30 **Carolin Delker**, Martin Luther University Halle, DE
Dissection of Arabidopsis thermomorphogenesis
- 11:30-12:00 **Jose Dinneny**, Stanford University, US
The divining root: discovering the genetic mechanisms controlling moisture-regulated branching in grasses
- 12:00-12:15 **Christophe Maurel**, IPSiM, Univ Montpellier, CNRS, INRAE, Institut Agro, Montpellier

- Integrated acclimation responses of maize root hydraulic architecture to water deficit
- 12:15-12:30 **Pranav Pankaj Sahu**, Global Change Research Institute, CAS, Brno, Czech Republic
Contextualizing the adaptation strategies of winter wheat against projected climate change
- 12:30-14:00 Lunch

Session 2: Acclimation and resilience mechanisms of plants under climate fluctuations

Chair: Carolin Delker, University of California at Davis, US

- 14:00-14:30 **Isabel Bäurle**, University of Potsdam, DE
Chromatin regulation of environmental stress memory in Arabidopsis
- 14:30-15:00 **Salma Balazadeh**, Institute of Biology Leiden, NL
Control of plant thermomemory by protein stability and degradation
- 15:00-15:15 **Sotirios Fragkostefanakis**, Department of Cell and Molecular Biology of Plants, Goethe University Frankfurt, D-60438, Germany
Regulation of plant acclimation to heat stress by transcription factors and splicing regulators
- 15:15-15:30 **Tom Laloum**, Instituto Gulbenkian de Ciência, Oeiras, Portugal
Posttranscriptional regulation of aba-mediated stress responses by two arabidopsis sr proteins during early plant development
- 15:30-16:00 Coffee break
- 16:00-16:30 **Christine Foyer**, University of Birmingham, UK
Effects of high CO₂ on the growth and stress tolerance
- 16:30-17:00 **Julia Questa**, Centre for Research in Agricultural Genomics, ES
Epigenetic mechanisms enabling plant growth under stressful environments
- 17:00-17:15 **Gad Miller**, Bar Ilan University, Israel

- Pollen thermotolerance: what can we learn from *Arabidopsis* mutant deficient in ascorbate peroxidase²
- 17:15-17:30 **Soyanni Holness**, Sapienza University of Rome, Department of Biology and Biotechnology 'Charles Darwin', Piazzale Aldo Moro 5, 00185, Rome
The effect of sequential abiotic stresses on physiological and molecular defense responses in *Arabidopsis thaliana*
- 17:30-19:00 **Poster session I (Odd numbers)**

Wednesday 15 June

Session 3: Effect of climate change on plant growth, development and physiology

Chair: Antoine Martin CNRS, FR

- 09:00-09:30 **Arnold Bloom**, University of California at Davis, US
How plants maintain carbon/nitrogen homeostasis as atmospheric CO₂ concentrations rise
- 09:30-10:00 **Laurent Laplaze**, IRD, FR
Targeting root traits to improve tolerance to early drought episodes in pearl millet
- 10:00-10:15 **Anja Krieger-Liszkay**, Université Paris-Saclay, Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, 91198 Gif-sur-Yvette cedex, France
Regulation mechanisms of photosynthetic electron transport affect susceptibility to drought
- 10:15-10:30 **Paloma Cubero-Font**, Institute for Plant Sciences of Montpellier (IPSiM)-CNRS, France
Regulation of leaf transpiration under elevated CO₂ involves plasma and vacuolar membrane ion fluxes.
- 10:30-10:45 **Pablo Affortit**, DIADE, Université de Montpellier, IRD, CIRAD, Montpellier, France
High-throughput phenotyping reveals a link between transpiration efficiency and transpiration restriction

under high evaporative demand and new loci controlling water use-related traits in african rice, *oryza glaberrima* steud.

10:45-11:15

Coffee break

11:15-11:45

Ana I. Caño-Delgado, Centre for Research in Agricultural Genomics, ES

Plant cell-specific strategies to overcome climate stress

11:45-12:00

François Chaumont, Louvain Institute of Biomolecular Science and Technology, UCLouvain, 1348, Louvain-la-Neuve, Belgium

Are aquaporins expressed in stomatal complexes promising targets to enhance stomatal dynamics under water deficit?

12:00-12:15

Ido Nir, Department of Biology, Stanford University, Stanford, CA, USA. Howard Hughes Medical Institute, Stanford University, Stanford, CA, USA.

Diverse mechanisms of adaptive flexibility discovered by multi-species analysis of stomatal development

12:15-14:00

Lunch

Session 4: Improving and engineering plants capacities under future climate

Chair: Ana Cano-Delgado, Centre for Research in Agricultural Genomics, ES

14:00-14:30

Florian Busch, University of Birmingham, m, UK

What limits photosynthesis? Identifying targets for crop improvement.

14:30-15:00

Alejandro Perdomo, Lancaster University, UK

Optimizing Rubisco activation as a mechanism to enhance the carbon fixation in wheat

15:00-15:15

Anna Collin, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Poland

- Barley drought-tolerant mutants in the new clothes – the introgression lines for tilling mutants in elite varieties background exposed to drought stress
- 15:15-15:30 **Aanchal Choudhary**, National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi 110067, India
- Dual blow: how the drought affects plant defense machinery against bacterial pathogens
- 15:30-16:00 Coffee break
- 16:00-16:15 **Aditya Prasad Nayak**, ETH Zurich
- PIF4* promoter editing based approach to engineer thermotolerant plants.
- 16:15-16:30 **Ulrich Lutz**, Max Planck Institute for Biology, Department of Molecular Biology, Tuebingen, Germany
- Revealing cryptic variation in both specific and pleiotropic adaptive phenotypes by deleting *flc* in many different genetic backgrounds
- 16:30-18:15 **Poster session II (Even Numbers)**
- 18:15-19:00 **Joanne Chory**, Salk Institute, USA
- Keynote lecture: Fighting Climate Change with Plants. It takes a global village to solve a global problem

Thursday 16 June

Session 5: Effect of climate change on plant growth, development and physiology

Chair: Isabel Bäurle, University of Potsdam, DE

- 09:00-09:45 **Philip Wigge**, University of Potsdam, DE
- Keynote lecture. Temperature sensing in plants
- 09:45-10:15 **Jorge Casal**, Universidad de Buenos Aires, AR
- Plants integrate the information provided by a complex environment

- 10:15-10:30 **Jean-Philippe Reichheld**, Laboratoire Génome et Développement des Plantes, CNRS, Université Perpignan Via Domitia, F- 66860 Perpignan, France
Glutathione-mediated redox regulation of plant response to high temperature
- 10:30-11:00 Coffee break
- 11:00-11:30 **Veronica Arana**, INTA - CONICET, AR
Deciphering the effect of temperature on circadian clock performance in trees
- 11:30-12:00 **Keiko Sugimoto**, RIKEN Center for Sustainable Resource Science, JP
How do plants feel the damage to protect and rebuild themselves
- 12:00-12:15 **Scott Hayes**, Wageningen University
Warm temperature and mild water deficit interdependently control root elongation
- 12:15-12:30 **Kumud Saini**, Sainsbury Laboratory Cambridge University, England
Warming up to rush to exit: temperature regulation of leaf size control in *Arabidopsis*
- 12:30-14:00 Lunch

Session 6: Genomics and systems approaches to understanding the mechanisms of climate adaptation

Chair: Rodrigo Gutierrez, Pontificia Universidad Católica de Chile

- 14:00-14:30 **Antoine Martin**, CNRS, FR
Genomics of plant nutrition in the context of climate change
- 14:30-15:00 **Hilde Nelissen**, VIB Center for Plant Systems Biology, BE
Spatial and temporal regulation of plant organ growth

- 15:00-15:15 **José M. Alvarez**, Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
Network-based integrative genomics reveals convergent transcriptional connections between drought and nitrogen signals
- 15:15-15:30 **Anne Marie Labandera**, School of Biosciences, University of Birmingham, United Kingdom
Uncovering non-canonical functions for o2- and cold-regulated proteins in plants
- 15:30-16:00 Coffee break
- 16:00-16:30 **Leandro Quadrana**, Institute of Plant Science Paris-Saclay, FR
Jumpstarting evolution: How transposition can facilitate adaptation to rapid environmental changes?
- 16:30-17:00 **Ndjido Kane**, ISRA, SN
Omics approaches underpin the molecular basis of pearl millet responses under drought-prone environments
- 17:00-17:15 **Titouan Bonnot**, Agroécologie, AgroSup Dijon, INRAE, Univ. Bourgogne Franche-Comté, 21000 Dijon, France
Multi-omics network analysis identifies putative regulators of molecular responses to water stress and sulfur deficiency in *Pisum sativum*
- 17:15-17:30 **Koen Geuten**, KU Leuven (University of Leuven), Belgium
Towards a mechanistic model for the cereal vernalisation pathway
- 17:30-17:45 **Eva María Gómez-Álvarez**, Scuola Superiore Sant'Anna
Analysis of a large barley panel for capacity to germinate after a short submergence stress
- 17:45-01:00 Social event, gala dinner and party at “La Voile Bleue”

Friday 17 June

Session 7: Molecular mechanisms of adaptation to extreme environments

Chair: Jorge Casal, Universidad de Buenos Aires, AR

- 09:30-10:00 **Heribert Hirt**, Center for Desert Agriculture, SA
Desert plant microbes for enabling agriculture under extreme environmental conditions
- 10:00-10:30 **Ivan Couée**, University of Rennes, FR
Sensing and signalling at the extreme: abiotic stress signalling mechanisms in extremophile plants
- 10:30-10:45 **Jean Alric**, Aix Marseille Univ, CEA, CNRS, BIAM, UMR 7265, Photosynthesis and Environment, F-13108 Saint Paul-Lez-Durance, France
Aquatic photosynthesis under extreme CO₂ conditions
- 10:45-11:15 Coffee break
- 11:15-11:45 **Rodrigo Gutiérrez**, Pontificia Universidad Católica de Chile,
Plant life at the extreme in the Atacama Desert
Phylogenomics and systems biology approaches reveal conserved adaptive processes in Atacama Desert plants
- 11:45-12:00 **Jean Colcombet**, INRAE
Mkk3 module integrates stresses and nutrition signals in *Arabidopsis*
- 12:00-12:15 **Elodie Rey**, Biological and Environmental Sciences and Engineering Division (BESE) King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia
Uncovering the wild genetic diversity in *Chenopodium* spp. For improvement of heat stress tolerance in quinoa.
- 12:15-12:30: Concluding remarks
- 12:30-14:00: Lunch & departure

Oral Presentations

Introductory lecture

LINKING SCALES TO ACHIEVE IMPACT

Vincent Vadez

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Water stress is the number one constraint limiting agriculture production around the world. Climate change will only make it worse, affecting precipitation quantities / patterns, raising temperature, increasing plant water demand, jeopardizing crops but also groundwater resources that irrigate these crops. While agriculture is also the largest water user, developing societies increase their non-agricultural water demand. Therefore, agriculture needs to become more water efficient. Improving crops is only a part of this goal that requires a holistic approach to water management / use. This talk aims at providing a broad perspective on climate/water issues, from plants to landscape. I'll start with results on plant traits that contribute to crop adaptation to water limitation and climate change. One of these traits is the capacity of certain plant genotypes across species to restrict transpiration when vapor pressure deficit (VPD) is high, i.e. when fixing CO₂ bares a high water cost. Plant and/or root hydraulics are involved in this trait. Aquaporin genes may play a role in the cell and molecular mechanisms regulating this trait. This trait increases water use efficiency, but not always, shifts water use during the crop cycle to support grain filling, and increases yield. I'll then show how crop simulations help us predict the performance of such traits across time and geographical scales. By telling us when/where a genetic trait is worthy of investment, modelling becomes a very powerful tool to guide breeding/genetic/agronomy decisions. Beyond drought tolerant crops, water management at farm and landscape level is also necessary. Cropping, irrigation, water allocation decisions have impacts on the water resource, at a scale and timeframe beyond the decision taker. Connecting models (crop, farm, hydrology, economic) working at different scales could help make holistic and inclusive decisions about water use, to then optimize system resilience at a landscape scale.

Session 1: Effect of climate change on plant growth, development and physiology

KEYNOTE LECTURE:

AN OPEN AND SHUT CASE: DISCOVERING HOW TRANSIENT WATER STRESS MODIFIES ROOT DEVELOPMENT

Poonam Mehra* and **Malcolm J. Bennett**

Plant & Crops Sciences, School of Biosciences, University of Nottingham, LE12 5RD, UK.

Email : Malcolm.Bennett@nottingham.ac.uk

Water stress is an increasing problem in agriculture given the impact of climate change. Discovering how plant abiotic stress signals like abscisic acid (ABA) control adaptive responses to fluctuating water stress is vital for futureproofing crops. ABA induced changes to root architecture play a vital role alleviating transient water stress. Xerobranching provides a model response to study ABA-mediated root adaptive mechanisms to fluctuating soil moisture (Orman et al, 2018, *Current Biology*). Xerobranching represses lateral root formation when roots lose contact with water. A Xerobranching stimulus triggers the transient release of abscisic acid (ABA) from root phloem tissues. This important water stress signal then moves radially, causing plasmodesmata to close in outer root tissues. Closing these inter-cellular pores disrupts the inward symplastic movement of the hormone signal auxin, blocking lateral root branching. Strikingly, once root tips regain contact with moisture, ABA response rapidly attenuates. Xerobranching reveals how dynamic hormone responses enable roots to adapt to heterogeneous soil moisture conditions and reveal molecular and cellular targets to re-engineering crops to become more climate resilient.

**Special thanks to EMBO for awarding a long-term Fellowship to PM*

STOMATAL REGULATION BY THE ENVIRONMENT

Hannes Kollist

Institute of Technology, University of Tartu, Tartu, 50411, Estonia

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Guard cells can sense changes inside the plant as well as in the surrounding environment. This is controlled by signaling systems that adjust stomatal aperture and by that plant gas exchange. When the CO₂ concentration increases, stomata close partially. This reduces water loss and could improve the water economy but also increase leaf temperature and suppress plant growth. Plant hormone abscisic acid (ABA) is involved in the regulation of stomatal closure during drought but it also has a role in VPD- (vapor pressure deficit) and CO₂-induced stomatal regulation. To breed water-saving crops for a future world with elevated CO₂ and more frequent drought episodes we need to know molecular switches in response to ABA/drought and CO₂. I will present our results in studying mechanisms of stomatal movements in response to changes in the environment. The function of protein kinases MPKs, HT1, OST1 and receptor protein GHR1 in the regulation of guard cell anion channels and subsequently on stomatal movements will be presented.

A CONSERVED SIGNALING AXIS INTEGRATES CONFLICTING ENVIRONMENTAL SIGNALS TO CONTROL TRANSPIRATION IN PLANTS

Xiangyu Xu¹, Myrthe Praat², Gaston A. Pizzio³, Jiang Zhang², Brigitte Van De Cotte¹, Kris Gevaert⁴, Pedro L. Rodriguez³, Martijn van Zanten², Lam Dai Vu^{1,4}, **Ive De Smet**¹

¹Ghent University and VIB Center for Plant Systems Biology, Belgium

²Utrecht University, The Netherlands

³Universidad Politécnica de Valencia, Spain

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Plants are exposed to environmental stresses, such as high temperature and drought, and continuously respond to changing conditions to prevent damage and maintain optimal performance. In this context, stomata contribute to abiotic stress relief and avoidance by regulating the rate of gas and water vapor exchange with the environment. Leaf temperature acts as a read-out of changes in ABA-mediated transpiration and leaf water content. However, how the high temperature signal is perceived and transmitted to regulate stomatal aperture is poorly understood. In contrast, under drought – which results in dehydration – closing stomata limits water loss. Since both drought and heat stresses often coincide, this leads to a regulatory conflict as the individual signals trigger opposite stomatal responses. To resolve this conflict, there is likely an overlapping signaling module providing a tight regulation of stomatal aperture during the coinciding stresses, but we know very little on how plants integrate drought and temperature signals. Here, I will propose a model including a conserved signaling axis that integrates conflicting environmental signals to control transpiration in plants.

DISSECTING THE ORGAN-SPECIFIC MECHANISMS THAT REGULATE PLANT THERMOMORPHOGENESIS

Carolin Delker

Institute of Agricultural and Nutritional Sciences, Martin Luther University Halle-Wittenberg, Betty-Heimann-Str. 5, 06120 Halle (Saale), Germany

Email: carolin.delker@landw.uni-halle.de

Warm ambient temperatures can have profound effects on various aspects of plant growth and development. Morphological acclimation responses to elevated temperatures are collectively termed thermomorphogenesis and are characterized by the induction of elongation growth in hypocotyls, stems, petioles and roots, while simultaneously reducing leaf blade size. On a molecular level, shoot thermomorphogenesis is regulated by the photosensor phyB, PIF transcription factors, and numerous other components that integrate light and temperature cues. Temperature changes are sensed in the leaves which generates a mobile auxin signal that promotes elongation of petioles and triggers brassinosteroid-mediated hypocotyl elongation. In contrast, molecular components involved in the regulation of root thermomorphogenesis are still relatively scarce. Interestingly, most regulators of shoot temperature responses (e.g. PIFs) seem to be of little to no importance in mediating root temperature responses. Rather, root temperature responses involve components and mechanism that are quite distinct from shoot thermomorphogenesis regulation.

THE DIVINING ROOT: DISCOVERING THE GENETIC MECHANISMS CONTROLLING MOISTURE-REGULATED BRANCHING IN GRASSES

José R. Dinneny

Department of Biology, Stanford University, Stanford, CA 94305, USA

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Water represents the most limiting resource for plant growth on earth and plant root systems have evolved mechanisms to search for and capture this resource from their soil environments. In my talk I will illustrate two of these mechanisms that impact the architecture of grass roots, which are dominated by shoot borne roots, termed crown roots, and the secondary branches of roots, termed lateral roots. Crown root development occurs near the soil surface and is highly sensitive to the local availability of water in this region. In response to a watering event, crown root initiation is rapidly induced and leads to a flush of new root growth. When water becomes limiting, crown root growth is suppressed at the post-emergence stage, which allows plants to preserve water in the soil. We have recently identified a novel locus termed *CROWN ROOT DEFECTIVE (CRD)*, which promotes crown root development under well-watered conditions in *Setaria viridis*. Moisture can also pattern root branching at the micron scale through a process we termed hydropatterning, which allows roots to detect the spatial pattern of water across the circumferential axis of the root. In maize inbreds, extensive phenotypic variation exists for hydropatterning and we have used GWAS and TWAS to identify genetic loci contributing to this variation. Phenotypic variation in hydropatterning is a significant predictor of root system depth in field grown maize suggesting that characterizing the genetic basis for this variation will facilitate the breeding of root system traits.

INTEGRATED ACCLIMATION RESPONSES OF MAIZE ROOT HYDRAULIC ARCHITECTURE TO WATER DEFICIT

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Understanding and controlling the mechanisms that underlie plant responses to drought has become essential for agriculture in the context of global change. Here, we used maize as a model for studying the physiological and genetic bases of root responses to drought. Three major functions shaping root hydraulic architecture were investigated: hydraulics, growth and signaling.

Root system architecture (RSA) and root hydraulic conductivity (L_{p_r}) were analysed in plants subjected to water deficit of different intensities as induced by various polyethylene glycol (PEG) concentrations. Detailed studies on primary and seminal roots revealed distinct anatomical, architectural and hydraulic response patterns depending on water deficit intensity and root type. In particular, a functional/structural model of primary or seminal roots was developed and used in an inverse modelling approach to concomitantly determine their axial and radial hydraulic properties, and their dependence on water deficit. A split root system was used to dissect the effects of heterogeneous water availability revealing systemic effects of water deficit on root growth. The significance of these responses with respect to plant acclimation to drought will be discussed. In parallel, a genetic dissection of maize root hydraulics (L_{p_r}) was carried out using Genome Wide Association (GWA) in a diversity panel of 316 dent lines. Associated SNPs and underlying candidate genes are currently under genetic validation using biparental recombinant populations and knock-out mutants obtained by transposon insertion or CRISPR-Cas9. In the future, these contrasting maize genotypes will be used to analyse the impacts of root hydraulic architecture on plant growth under drought.

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CONTEXTUALIZING THE ADAPTATION STRATEGIES OF WINTER WHEAT AGAINST PROJECTED CLIMATE CHANGE

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Examining the direct and indirect effects of climate change is complicated because of the potential of multiple interactions between underlying factors. Such combinations could trigger explicit responses which may differ from the individually applied climate variable. In our study, the impact of eight different climate regimes comprising elevated temperature, drought and CO₂ levels (individually and in combinations) as predicted for the year 2100 were examined to decipher adaptation mechanism of four European winter bread wheat cultivars. The climatic variables critical to vegetative and reproductive stages were identified by evaluating their agronomic fitness, physiological alteration as well as transcriptional changes. Individually, each factor affects morpho-physiological traits and gene expression; however, their combinations lead to a unique response. Alterations in the critical phenotypic traits such as timing of the flowering time, plant height as well as spike/seed morphology was observed. We further demonstrated that although not all cultivars have the capacity to take advantage of increasing atmospheric CO₂ concentration, the CO₂-responsive cultivar utilizes this for higher biomass production as well as to minimize the effect of drought by improving its water use efficiency. The interactive effect of climate variable caused unique shifts in transcriptional profile. Noticeably, we found that CO₂ concentration and temperature have major effect on the gene expression than drought. Signature changes in the transcriptional pattern of genes, leading to specific acclimation responses, were mainly conferred by the antagonistic response mode of individual climate variables under the multifactor interactions. These results shed light on the acclimation mechanisms of plants under multifactorial combinations and are expected to progress towards a more effective strategy to counterbalance the negative impacts of changing climate.

Session 2: Acclimation and resilience mechanisms of plants under climate fluctuations

CHROMATIN REGULATION OF ENVIRONMENTAL STRESS MEMORY IN *ARABIDOPSIS*

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In nature, plants often encounter chronic or recurring stressful conditions. An increasing number of observations suggest that plants can be primed by exposure to stress, thereby activating a stress memory that enables a more efficient response upon a recurring stress incident. My lab studies heat stress memory in plants as a model case for environmental stress memory. Seedlings acquire thermotolerance through a heat treatment at sublethal temperatures (priming heat stress) that enables them to survive an otherwise lethal heat stress. This thermotolerance is actively maintained for several days as indicated by the existence of mutants which are able to establish thermotolerance, but fail to maintain it. Heat stress induces sustained histone methylation at heat stress memory-related loci that outlasts the transcriptional activity of these loci and marks them as recently transcriptionally active.

In different forward genetic screens we have identified both chromatin modifiers and transcription factors that work together to promote transcriptional memory. I will present our latest findings that indicate complex interaction between different levels of regulation to sustain transcriptional memory.

CONTROL OF PLANT THERMOMEMORY BY PROTEIN STABILITY AND DEGRADATION

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Global warming due to climate change adversely affects crop yield, jeopardizing food supply for a growing world population. Breeding stress-resilient cultivars is, therefore, an urgent need. An exciting, but poorly understood phenomenon is ‘thermomemory’ whereby plants ‘remember’ a high temperature from the past to robustly withstand a later – and even more extreme – heatwave. During the memory period, several but not all molecular and biochemical heat stress (HS)-induced changes are maintained which prepares, or ‘primes’, the plant to respond more effectively to future HS events. Recent research from my lab has discovered the importance of protein stability control as a key regulator of thermomemory. During my talk, I will present our recent findings and highlight open questions for future research.

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REGULATION OF PLANT ACCLIMATION TO HEAT STRESS BY TRANSCRIPTION FACTORS AND SPLICING REGULATORS

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Plants are often exposed to high temperatures which cause heat stress. The protection of macromolecules and cellular structures from irreversible damages and maintenance of protein homeostasis are essential for survival and recovery from stress. The transcriptional upregulation of many genes, including the essential for thermotolerance heat shock proteins (HSPs) is central for heat stress response. The induction of the majority of these genes is dependent on activity of the heat stress transcription factors (HSF). HsfA2 and HsfA7 play a central role in acclimation of tomato plants to high temperatures. In addition to transcription, pre-mRNA splicing is another important level of regulation of heat stress response, as many genes are alternatively spliced under heat conditions. We have identified splicing regulators belonging to the Serine/Arginine-rich protein family that mediate temperature-sensitive alternative splicing thereby playing along with HSFs a core role on transcriptome landscape in tomato plants. We show that transcription and pre-mRNA splicing are directly linked as HSF and SR genes regulate each other, suggesting the existence of feedback mechanisms directly linking RNA synthesis and processing to coordinate the molecular response to environmental changes.

POSTTRANSCRIPTIONAL REGULATION OF ABA-MEDIATED STRESS RESPONSES BY TWO *ARABIDOPSIS* SR PROTEINS DURING EARLY PLANT DEVELOPMENT

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SR (serine/arginine-rich) proteins are conserved RNA-binding proteins best known as key regulators of splicing that have also been implicated in other steps of gene expression. Despite mounting evidence for their role in plant development and stress responses, the molecular pathways underlying SR protein regulation of these processes remain elusive.

Using gene expression analyses and reverse genetics approaches, we show that two *Arabidopsis* SR proteins act as negative regulators of ABA-mediated stress responses during seed germination and early seedling development. Loss of function of either of these SR proteins results in elevated expression of ABA-responsive genes and genes repressed during the germination process. Moreover, the corresponding knockout mutants display hypersensitivity to ABA and osmotic stress during seed germination and postgermination growth. Using pharmacological and epistatic analyses, we confirm that the proper control of osmotic stress responses by *Arabidopsis* SR proteins is fully dependent on the ABA pathway.

Our results reveal new key players in ABA-mediated control of early development and stress response. To gain mechanistic insight into the mode of action of SR proteins during early plant development, the endogenous transcripts targeted by these splicing factors to achieve plant stress tolerance are being identified using a combination of RNA immunoprecipitation methods and large-scale RNA-seq analyses in the SR protein mutant plants. Results stemming from this work could pave the way for novel strategies to improve plant productivity under challenging environmental conditions, such as drought or high salinity.

EFFECTS OF HIGH CO₂ ON THE GROWTH AND STRESS TOLERANCE

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High atmospheric CO₂ concentrations (eCO₂) have positive effects on the productivity and yield of through effects on photosynthesis (Ainsworth & Long, 2021 *Global Change Biology* **27**, 27–49.). However, eCO₂ is likely to alter the redox balance and signalling of plant cells in ways that alter biotic and abiotic stress tolerance. In this talk, I will firstly address how growth under eCO₂ is predicted to alter cellular redox processes and associated signalling and whether plants perceive eCO₂ as a stress in itself. Thereafter, I will discuss recent results from studies on the effects of (eCO₂) on pea development and susceptibility to the pea aphid (*Acyrtosiphon pisum*). We studied aphid fecundity in wild type peas and mutants defective in either strigolactone (SL) synthesis or signalling. Plants were grown in air or under eCO₂. Growth under eCO₂ significantly increased the height and the branching of the wild type shoots. The mutant shoots had significantly higher fresh weight/dry weight ratios than the wild type under both growth conditions. No direct of eCO₂ on aphid fecundity was observed but aphid numbers were increased in the SL mutants under both ambient and eCO₂ conditions. SL-dependent regulation of aphid fecundity is related to effects on shoot phytohormone levels, which were modified in the SL mutants.

EPIGENETIC MECHANISMS ENABLING PLANT GROWTH UNDER STRESSFUL ENVIRONMENTS

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Plants are naturally adapted to tolerate and survive seasonal changes in environmental conditions. In fact, plants rely on perception of these variable signals to reprogram their genetic networks thereby driving developmental transitions. In this context, chromatin regulation modulates the transcriptome in response to exogenous signals both rapidly and in the long-term. Climate change is predicted to increase the frequency of extreme, unseasonable weather. How the chromatin machinery will respond to unfavourable environments remains to be fully assessed. Increasing evidence is revealing an active role of the non-coding genome in plant responses to extreme environments. In line with this, our previous work demonstrated that non-coding cis variation controls the floral transition in *Arabidopsis thaliana* plants adapted to extreme winters. In addition, numerous long non-coding RNAs (lncRNAs) are specifically triggered under stressful conditions such as freezing, high salinity and drought. Here, I will present our current efforts to reveal how epigenetic regulators, including VAL B3 domain proteins and lncRNAs, modulate plant growth under stressful environments. We have adapted new tools to monitor the *Arabidopsis* nascent transcriptome and chromatin dynamics in plants undergoing germination and early seedling establishment. Our work is revealing novel non-coding regulatory modules with potential roles in regulation of plant growth under suboptimal environmental conditions.

POLLEN THERMOTOLERANCE: WHAT CAN WE LEARN FROM *ARABIDOPSIS* MUTANT DEFICIENT IN ASCORBATE PEROXIDASE 2

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To ensure reproductive success, flowering plants produce an excess of pollen to fertilize a limited number of pollen grains. Yet, high temperatures, even for one hot day, can cause male sterility in many plants, causing reduced crop yield. We showed previously that *Arabidopsis* plants deficient in the H₂O₂ reducing enzyme ascorbate peroxidase 2 (APX2) have increased heat stress sensitivity during the vegetative stages but increased tolerance during the reproductive phase. Our findings show that the enhanced reproductive resilience of *apx2* results from increased thermotolerance in pollen, which is associated with improved ability to maintain their ROS and redox poise. Transcriptomics comparison between *apx2* and WT pollen and leaves identified nearly 1,400 *apx2* pollen-specific transcriptional changes indicating extensive reprogramming that include activation of the flavonols metabolism and auxin signaling pathways. We further show that these two antagonistic pathways are altered in the *apx2* mutant during high temperatures, supporting their involvement in thermotolerance. In addition, *Arabidopsis* and tomato plants overproducing flavonols in pollen gain increased reproductive resilience under high temperatures. Our findings indicate that regulating redox, auxin, and flavonols pathways impact thermotolerance in pollen and provide a framework for developing new crop varieties that would be more suitable for agriculture under warmer temperatures.

THE EFFECT OF SEQUENTIAL ABIOTIC STRESSES ON PHYSIOLOGICAL AND MOLECULAR DEFENSE RESPONSES IN *ARABIDOPSIS THALIANA*

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Single abiotic stress treatments with drought or light can trigger a molecular response that can last from several days to weeks and can help plants to deal with reoccurring stresses. However, the underlying mechanisms that help plants to cope with subsequent stresses are often not clear. We are interested in investigating the potential priming effects of high light (HL) stress in plants subjected to a subsequent drought (D) stress. *Arabidopsis* plants were either subjected to a short-term HL stress, a progressive D stress and to the two stresses administered sequentially (HL+D). The stress responses were assessed at the physiological and molecular level. Operating efficiency of PSII was not affected by the single stress treatments nor after sequential stress treatments suggesting there might not be a priming memory on this process. Carbon assimilation, stomatal conductance and leaf water potential were affected after stress treatments however there was no differences between D and HL+D treatments suggesting that a preliminary HL treatment does not have an impact on how these mechanisms react to the subsequent D stress. At the molecular level, we found that the stress marker genes *RD29A* (D), *ELIP1*(HL), as well as *NCED3* and *ABA1* (HL+D) increased upon stress induction. Additionally, HL seems to mediate a gene priming response under D as *RD29A*, *ABA1* and *NCED3* increased in HL+D treated plants compared to the single D treated plants. Our results provide an initial characterization of how HL can influence the plant response to a subsequent D stress.

Keywords: Abiotic stress, drought, high light, priming, *Arabidopsis*

Session 3: Effect of climate change on plant growth, development and physiology

HOW PLANTS MAINTAIN CARBON/NITROGEN HOMEOSTASIS AS ATMOSPHERIC CO₂ CONCENTRATIONS RISE

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Studies on plant responses to elevated CO₂ atmospheres generally expose plants to concentrations that are at least 40% above the current ambient level of 418 ppm CO₂. In the short-term, such conditions stimulate carbon fixation and inhibit photorespiration, thereby accelerating organic carbon accumulation and primary productivity. In the long-term, however, inhibition of photorespiration impedes the conversion of nitrate nitrogen into protein in leaves, upsets plant carbon to nitrogen balance, and decreases food quality. Nitrogen fertilization cannot fully compensate for these long-term changes. Yet plants subjected to less severe CO₂ enrichments—such as the 20% increase that plants have experienced over the last 35 years—exhibit little change in protein concentrations and food quality. We focus on three mechanisms through which plants maintain carbon/nitrogen homeostasis during CO₂ fluctuations that they encounter on a daily, seasonal, and decadal basis. (1) Plants rely more heavily on ammonium than nitrate as a nitrogen source. (2) They shift to some extent assimilation of nitrate from the shoots to the roots. (3) They increase the ratio of manganese to magnesium in chloroplasts, stimulating Rubisco oxygenation and inhibiting Rubisco carboxylation to maintain the balance between photorespiration and carbon fixation and thereby the balance between organic nitrogen and organic carbon. We will present evidence for a new biochemical pathway that integrates C₃ carbon fixation, photorespiration, and the pentose phosphate shunt.

TARGETING ROOT TRAITS TO IMPROVE TOLERANCE TO EARLY DROUGHT EPISODES IN PEARL MILLET (*Pennisetum glaucum* L.)

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In West Africa, cereals production per capita is declining due to the fast growth in population outpacing the increase in food production. Moreover, several models predict that global changes will reduce cereals yield in this region. In order to achieve future food security, it is therefore necessary to improve productivity and resilience through the combined development of adapted varieties and agricultural practices. Pearl millet is a key cereal for food security in sub-Saharan Africa. It is mostly grown in areas with limited agronomic potential characterized by low rainfall and low-fertility soils. Moreover, it lags behind other crops in its genetic development and its average yields remain low. Root traits represent potential new targets for breeding new pearl millet varieties more resilient to abiotic stresses and more adapted to future climate scenarios. For instance, we found that early root growth is important for crop establishment and adaptation to early drought stress in Sahelian environments. Here, I will describe the identification and characterisation of a QTL controlling primary root growth in pearl millet through modulation of cell elongation in the root apical meristem.

REGULATION MECHANISMS OF PHOTOSYNTHETIC ELECTRON TRANSPORT AFFECT SUSCEPTIBILITY TO DROUGHT

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Drought induces stomata closure and lowers the CO₂ concentration in the mesophyll, limiting CO₂ assimilation and favouring photorespiration. The photosynthetic apparatus is protected under drought conditions by a number of downregulation mechanisms like photosynthetic control, state transitions and activation of cyclic electron transport leading to the generation of a high proton gradient across the thylakoid membrane. In addition, photorespiration may exert a feedback control on photosystem II via the binding of a photorespiratory metabolite at the non-heme iron at the acceptor side of photosystem II (PSII) [1,2]. According to our hypothesis an exchange of bicarbonate at the non-heme iron by a photorespiratory metabolite such as glycolate changes the energetics of the quinone acceptors lowering the yield of singlet oxygen production. Gas exchange measurements coupled with measurements of fluorescence decay indicate that electron transfer between the quinone acceptors is slowed down under photorespiratory conditions.

Depending on light quality and intensity, a part of the light harvesting complex at PSII becomes phosphorylated and migrates to PSI to balance the excitation between the two photosystems, a process called state transition. We have observed that *Arabidopsis* mutants unable to perform state transitions are more resistant to drought. We determined chlorophyll fluorescence parameters, stomata opening, and root growth. Root growth is affected in these mutants. Differences between wild type and state transition mutants in respect to growth of the primary root are abolished in the presence of the PSII inhibitor DCMU and the cytochrome b6f inhibitor DBMIB, with DCMU inhibiting and DBMIB stimulating root elongation. We propose that a signalling molecule deriving from the redox state of the plastoquinone pool controls primary root elongation.

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REGULATION OF LEAF TRANSPIRATION UNDER ELEVATED CO₂ INVOLVES PLASMA AND VACUOLAR MEMBRANE ION FLUXES.

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The current climate change associated to elevated CO₂ is directly impacting on plant development. Environmental CO₂ directly acts on leaf transpiration, regulating stomatal aperture. Stomata are pores on the surface of land plants, delimited by two guard cells, and regulate gas exchanges. Stomatal aperture is key for determining CO₂ uptake and, simultaneously, water loss by transpiration. Therefore, it is finely modulated by environmental factors. To regulate the stomatal aperture, guard cells undergo a regulated swelling and shrinking mechanism that depends on ion fluxes across the plasma membrane (PM) and the vacuolar membrane (VM). The transport systems residing in these membranes and mediating such ion fluxes need coordination. Ion channels of the ALMT family localized in the plasma and the vacuolar membranes are important actors of the guard cell responses. Specifically, both the VM channel ALMT4 and the PM channel ALMT12 are involved in stomatal closure. We generated double knock-out lines *almt4-almt12* and we measured the stomatal conductance and net photosynthesis rate upon application of ABA and high CO₂ concentration. Our data show that ALMT12 function dominates the induction of stomatal closure after ABA treatment. Notably, we found that in response to increased CO₂, ALMT4 and ALMT12 are likely to work in synergy to drive stomatal closure. Our findings highlight the importance of the interplay between the PM and the VM ion channels in stomatal responses

HIGH-THROUGHPUT PHENOTYPING REVEALS A LINK BETWEEN TRANSPIRATION EFFICIENCY AND TRANSPIRATION RESTRICTION UNDER HIGH EVAPORATIVE DEMAND AND NEW LOCI CONTROLLING WATER USE-RELATED TRAITS IN AFRICAN RICE, *ORYZA GLABERRIMA* STEUD.

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Because water availability is the most important environmental factor limiting crop production, improving water use efficiency, the amount of carbon fixed per water used, is a major target for crop improvement. In rice, factors controlling transpiration efficiency, i.e. the derivation of water use efficiency at the whole-plant scale, and its putative component trait transpiration restriction under high evaporative demand, remain poorly known. These traits were measured in a panel of 147 African rice *Oryza glaberrima* genotypes, known as potential sources of tolerance genes to biotic and abiotic stresses. Our results revealed that higher transpiration efficiency is associated with transpiration restriction in African rice. Detailed measurements in a subset of highly contrasted genotypes confirmed these associations and suggested that root/shoot partitioning is an important component of transpiration restriction. Root phenotyping was performed on the same panel to assess the contribution of root architecture to transpiration related traits. Moreover, genetic bases of transpiration efficiency and transpiration restriction were analyzed by Genome Wide Association Studies and revealed new potential molecular mechanisms controlling these traits.

DECIPHERING THE ROLE OF BRASSINOSTEROIDS IN PLANT ADAPTATION TO CLIMATE CHANGE

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Despite the massive amount of information gathered around the functions and mechanisms of Brassinosteroids (BRs) in plants, an important limitation persists in our knowledge of this signaling pathway: almost all we know comes from observations on the BRI1 receptor pathway, that is essential for growth and development, and for which mutants are highly pleiotropic and typically dwarf. Since the discovery of BRI1-like receptors (BRL1/3), we still do not really grasp what are their fundamental functions in plants. Twenty years of research have resumed the analysis of BRLs as redundant BRI1 receptors with a marginal vascular expression and lack of apparent mutant phenotypes. Strikingly, our research takes a novel perspective to explore the function of BRLs in *Arabidopsis*, to understand the inner working of this pathway. In light of our recent findings showing that overexpression of BRL3-receptors confers drought resistance, we have now deciphered the BRL3 pathway in *Arabidopsis*, including novel components in the pathway essential to plant adaption to climate change. Our new data change the paradigm for our present understanding of BR signaling in plants and open new possibilities for producing climate resilient crops. The latest results of our research work will be presented at this EMBO workshop.

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ARE AQUAPORINS EXPRESSED IN STOMATAL COMPLEXES PROMISING TARGETS TO ENHANCE STOMATAL DYNAMICS UNDER WATER DEFICIT?

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Water availability is a major limiting factor for plant growth. Therefore, to preserve their internal water content, plants close their stomata. Nonetheless, reduction of water loss by stomatal closure occurs at the expense of CO₂ assimilation. Despite the fact that the dynamic of the stomatal movement relies on water fluxes into and out of the guard cells controlled by many factors, such as light, CO₂, ABA, few information is available regarding the role of aquaporins, channels facilitating the membrane diffusion of water and small solutes such as H₂O₂ and CO₂. This is particularly true for the graminoid stomata of grasses, including maize, in which the stomata consist of two dumbbell-shaped guard cells flanked by two subsidiary cells. Our laboratory aims at elucidating the physiological function of aquaporins belonging to the plasma membrane intrinsic protein (PIP) subfamily. We generated different maize lines deregulated in the expression of specific *PIP* genes, and we are investigating the stomatal behavior in both intact plants and epidermal strips under various conditions. Differences in gas exchange, stomatal development, and stomatal movement were recorded, suggesting that aquaporins play important roles in these complexes. For instance, we showed that stomata from maize PIP2;5 or PIP1;6 overexpressing lines closed faster upon water stress or ABA treatment, indicating that PIP aquaporins might constitute interesting targets to enhance stomatal dynamics and improve crop water use efficiency in fluctuating environment.

DIVERSE MECHANISMS OF ADAPTIVE FLEXIBILITY DISCOVERED BY MULTI-SPECIES ANALYSIS OF STOMATAL DEVELOPMENT

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Stomata are pores in the plant epidermis that control gas exchange between the plant and atmosphere. In *Arabidopsis*, stomatal development requires the bHLH transcription factor SPEECHLESS (AtSPCH) and perception of signals from adjacent cells, from other parts of the plant and from the environment. SPCH is thought to be a critical target for environmental inputs into development. Despite the power of *Arabidopsis* as a model for stomatal development, we found crop plants like tomatoes often lean on different cellular and genetic strategies to achieve optimal stomatal distributions. By making genetically encoded reporters of the stomatal lineage, and long-term confocal microscopy we tracked the developing epidermis of M82 (WT) and mutant tomato seedlings. We found that, like in *Arabidopsis*, tomato undergoes a series of asymmetric and symmetric cell divisions to produce stomata. However, we found one type of asymmetric division (ACD) was missing in the tomato epidermis, and other ACDs could be used to generate non-stomatal cells. These data suggest differences in ACD strategies that control stomatal production between plant species. Since SPCH serves as the major integrator of environmental information in stomatal development, we targeted the tomato SPCH promoter for CRISPR-based mutagenesis. By screening lines in response to light and temperature, we found putative SPCH cis-regulatory elements that indicate complexity in the regulation of developmental flexibility. Taken together these results further our understanding of the species-specific cellular and genetic pathways plants use to adapt to their environment.

Session 4: Improving and engineering plants capacities under future climate

WHAT LIMITS PHOTOSYNTHESIS? IDENTIFYING TARGETS FOR CROP IMPROVEMENT

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Photosynthesis is the process that fixes atmospheric CO₂ into organic carbon and as such the mechanism that underpins plant biomass accumulation and ultimately crop yield. A generation of scientists has therefore tried to improve photosynthesis in an attempt to increase crop yield and improve food security. The strategies employed have been diverse, owed to the complexity of the photosynthetic processes, and are ranging from improving catalytic properties of individual enzymes to introducing photorespiratory bypasses or whole photosynthetic pathways. The underlying assumption is that the targeted process is limiting carbon uptake and that the modification will remove that limitation. In reality, the environment a plant grows in very much determines the individual process that limits photosynthesis under this condition and thus a crop improvement strategy that may prove beneficial under one environment could be futile in another environment. Measurements of gas exchange and chlorophyll fluorescence parameters and how they respond to small changes in environmental condition allow to pinpoint the biochemical process that limits photosynthesis under a given environment. Here I will discuss how this approach can be used to assess the environmental envelope under which a crop improvement strategy may be expected to be beneficial under current environments and how this might be affected under future climate change conditions.

OPTIMIZING RUBISCO ACTIVATION AS A MECHANISM TO ENHANCE CARBON FIXATION IN WHEAT

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CO₂ assimilation during photosynthesis is the primary determinant of plant biomass production. Rubisco plays a key role in CO₂ assimilation, but is susceptible to inhibition by unproductive binding of sugar-phosphates that lock active sites in a closed conformation. Rubisco activase (Rca) remodels the conformation of Rubisco by removing inhibitors from active sites and restoring its catalytic competence. Rca activity and its consequent effect on Rubisco activation are modulated by changes in the chloroplast environment induced by fluctuations in light levels that reach the leaf, including redox status and ADP/ATP ratios, and by high temperature due to Rca being sensitive to elevated temperatures. The wheat, *Triticum aestivum*, genome encodes for three Rca protein isoforms: 1 β , 2 β and 2 α . The regulatory properties of these isoforms differ in their Rubisco re-activation and ATP hydrolysis sensitivity to physiological ADP/ATP ratios, as well as its thermostability and relative abundance which could be exploited to improve the resilience of wheat to global warming and light fluctuations under field conditions. Therefore, understanding the regulation of Rca gene expression and its relative isoform composition will assist further research to improve the regulation of Rubisco, with the ultimate aim of increasing carboxylation and crop yield.

BARLEY DROUGHT-TOLERANT MUTANTS IN THE NEW CLOTHES – THE INTROGRESSION LINES FOR TILLING MUTANTS IN ELITE VARIETIES BACKGROUND EXPOSED TO DROUGHT STRESS

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Drought is one of the main causes of crop production loss. Therefore, it is urgent to obtain new crop varieties with elevated level of drought tolerance. Abscisic acid (ABA) is considered as a crucial phytohormone regulating plant response to drought. In the presence of stress, ABA regulates expression of stress-responsive genes, stomatal closure and photosynthesis performance. Previously, we identified barley ABA-related TILLING mutants using *Hor*TILLUS population developed *via* chemical mutagenesis of barley cultivar ‘Sebastian’ in our lab. Point mutations found in *ABA INSENSITIVE 5 (HvABI5)*, *CAP BINDING PROTEIN 20 (HvCBP20)* and *ENHANCED RESPONSE TO ABA 1 (HvERA1)* genes were named *hvabi5.d*, *hvcbp20.ab* and *hver1.b*, respectively, and resulted in single amino acids substitutions in the conserved fragments of encoded proteins. All mutants showed disturbed response to ABA and better performance under drought when compared to parent cultivar. All these alleles were introduced into elite cultivars ‘Planet’, ‘Barke’ and ‘Golden Promise’ via backcrossing followed by double haploid (DH) creation. Obtained lines were subjected to drought stress at seedling stage. All of them showed better water relations than their parent cultivars under drought. Additionally, we investigated stomatal conductance, photosynthesis efficiency and flavonoid accumulation. We observed differences in analysed parameters between studied DH lines and parent cultivars in response to drought. Our findings have potential to be helpful to develop new barley elite cultivars with higher level of tolerance to drought stress.

This work was supported by funding from ERA-NET Cofund activity SusCrop (Sustainable Crop Production), under the Joint Programming Initiative on Agriculture, Food Security and Climate Change: FACCE-JPI, National Centre for Research and Development Poland for the project [SUSCROP/I/BARISTA/02/2019] BARISTA (Advanced tools for breeding BARley for Intensive and SusTainable Agriculture under climate change scenarios).

DUAL BLOW: HOW THE DROUGHT AFFECTS PLANT DEFENSE MACHINERY AGAINST BACTERIAL PATHOGENS

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The dynamics of seemingly bi-directional interaction between plants and bacterial pathogens is frequently influenced by the drought in the field. However, our comprehension of how the drought and defence signaling pathways interact in order for plants to orchestrate an optimal response is limited. We observed that moderate drought stress enhances the susceptibility of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* pv. *tomato* DC3000. Using a combination of transcriptomic and genetic tools, we found antagonistic interaction of drought and defense-response pathways in plants, predominantly involved in salicylic acid (SA) and abscisic acid (ABA) signalling networks. Importantly, under combined stress, drought was observed to downregulate the induction of *CALMODULIN-BINDING PROTEIN 6og* (*CBP6og*) and *SYSTEMIC ACQUIRED RESISTANCE DEFICIENT 1* (*SARD1*), two transcription factors crucial for SA production upon bacterial infection in an ABA-dependent manner. We also identified an important role of *NPR1-LIKE PROTEIN 3* and *4* (*NPR3/4*) transcriptional repressors in the drought-mediated negative regulation of *CBP6og/SARD1* expression. Overall, we found that the *CBP6og-SARD1* module act as critical nodes for the crosstalk between drought and bacterial stress signalling under combined stress in plants. Drought response pathway targets the *CBP6og-SARD1* module to repress the downstream SA machinery and dampens the plant immunity against bacterial pathogens under combined stress.

PIF4 PROMOTER EDITING BASED APPROACH TO ENGINEER THERMOTOLERANT PLANTS.

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As plants face climate change, it's important to engineer newer plant varieties that can maintain yield and productivity at higher ambient temperatures. PIF4 is known to be a hub for ambient temperature signaling in plants and control thermomorphogenesis by activating auxin biosynthesis genes at gene expression levels. It is known that *PIF4* gene expression at higher ambient temperature is activated through BZR1 by binding to the G-Box in the PIF4 promoter. Inactivating the excess PIF4 gene expression at higher ambient temperature could be a minimally invasive, highly effective strategy for engineering temperature resilience in plants. We designed *Arabidopsis thaliana* plants where we edited PIF4 promoter G-box to generate temperature resilience. In these promoter edited plants we observed different thermomorphogenesis related developmental traits and gene expression changes. Results suggest that PIF4 promoter editing could be an effective strategy for engineering thermotolerance in crop plants.

REVEALING CRYPTIC VARIATION IN BOTH SPECIFIC AND PLEIOTROPIC ADAPTIVE PHENOTYPES BY DELETING *FLC* IN MANY DIFFERENT GENETIC BACKGROUNDS

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It is well known that genes do not act in isolation, yet conventional mutant analyses are still largely restricted to individual genetic backgrounds. Taking advantage of progress in genome editing, we have conducted an extensive analysis of background-dependent specific and pleiotropic phenotypic effects of the central flowering regulator *FLOWERING LOCUS C (FLC)*. We generated knock-out and knock-down alleles of *FLC* in a total of 61 accessions of *A. thaliana* and measured effects on flowering, vegetative traits and transcriptomes. Onset of flowering was reduced by up to 83% and some of our genome-edited knockout lines flowered already at 14 days after germination. This was surprising, because accessions with natural *FLC* knockouts are common, but none of them flower that early. Genetic mapping revealed that most of the extremely early flowering was explained by natural variation at the *FLC* target *FT*, indicating either that certain combination of *FLC* and *FT* alleles do not survive in the wild, or have been missed by collection efforts. When analyzing growth trajectories during the early vegetative phase, contrasting, background-specific effects of *FLC* mutations were detected. Mutants representing nine (15%) genomic backgrounds accumulated less biomass than their parents, and eight (13%) more biomass. Transcriptome comparisons revealed that only very few of the differentially expressed genes, which were predicted to act in a wide range of developmental pathways, were shared across accessions. Pleiotropic effects are a major limitation in breeding, and simultaneous knockouts of the same gene in many different background provide a new avenue for understanding the true extent of genetic networks in which a focal gene participates.

**KEYNOTE LECTURE:
FIGHTING CLIMATE CHANGE WITH PLANTS. IT
TAKES A GLOBAL VILLAGE TO SOLVE A GLOBAL
PROBLEM**

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The world is facing a global sustainability crisis. The population stands today at >7.5B and is expected to increase to 11B by 2100. The increasing demand for food, fuel, land, and other natural resources is creating consequences that exceed the planet's capacity to absorb change and maintain stability. At the current pace of change, human life as we know it may not be sustainable on earth in a little over 100 years. Feeling the urgent need to be a part of the solution, Salk Institute plant scientists have developed a plan to bend the upward trending curve back down to a range where the planet's natural systems of maintaining balance can cope. The idea is to improve plants' natural ability to capture and store carbon stably in the ground. I will tell you about our plans to alter 3 traits in plant roots that we hope will allow us to draw down atmospheric CO₂ at a global level in 10-15 years. Recent advances in mechanistic plant biology, genomics, and precision agriculture/breeding suggest that this goal is within our reach. By improving plants' natural ability to deposit carbon in the soil in a form that does not easily decompose, we can sequester enough CO₂ to make a significant contribution to the global effort being pursued on many fronts to become carbon neutral or net negative.

Session 5: Effect of climate change on plant growth, development and physiology

TEMPERATURE SENSING IN PLANTS

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Plants have adapted to a wide variety of climates, from hot springs and the tropics to the polar regions. Temperatures also fluctuate significantly both over the diurnal cycle as well as seasonally. To adapt, plants have evolved an array of stress and developmental responses to both high and low temperature. We are interested in understanding how plants sense temperature and integrate this information into cellular decision-making. Knowledge of temperature perception mechanisms may accelerate the breeding of plants with improved heat stress characteristics, which is particularly relevant during a period of global heating. Previously, we have found that thermomorphogenesis is controlled by multiple sensors, including the temperature sensing phytochromeB (1), the thermosensory molecule EARLY FLOWERING3 (ELF3) (2, 3) as well as a temperature responsive hairpin in the 5' UTR of *PHYTOCHROME INTERACTING FACTOR7* (*PIF7*) (4) that modulates translation efficiency. Additionally, we have found new thermosensory pathways integrating temperature stress signals. These will be discussed in the context of climate change and the threat of more severe temperature extremes.

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PLANTS INTEGRATE THE INFORMATION PROVIDED BY A COMPLEX ENVIRONMENT

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Due to climate change, the vegetation is becoming exposed to environmental conditions that differ from those experienced during selection. Global warming modifies not only temperature patterns but also the availability of other resources, such as water. On top of that, there is a general trend to increase the population densities of agricultural crops to elevate potential yield, but this practice enhances the degree of mutual shading among plants. In this scenario, plants are facing complex environmental changes. Therefore, understanding whether fluctuations in one aspect of the environment disrupt the molecular mechanisms involved in sensing and transduction of other cues is crucial. We will describe the dynamics and function of a transcriptional network that controls cell growth in seedlings of *Arabidopsis thaliana* exposed to combined cues from the environment, including shade, warmth and water availability. PHYTOCHROME INTERACTING FACTORS (PIFs), EARLY FLOWERING 3 (ELF3), BR-INSENSITIVE1-EMS-SUPPRESSOR 1 (BES1) and ELONGATED HYPOCOTYL 5 (HY5) are key transcription factors in this network. DELLA proteins are among the associated negative transcriptional regulators and CONSTITUTIVELY PHOTOMORPHOGENIC 1 (COP1) is a major component of the substrate recognition module of the E3 ligase complex that targets to degradation these regulators. The sensors that modify the status of the network include phytochromes and cryptochromes and ELF3 and PIF7 themselves. We will show that the dynamics and function of the components of the network depends on the cellular context provided by the organ and that the network integrates information from different environmental cues.

GLUTATHIONE-MEDIATED REDOX REGULATION OF PLANT RESPONSE TO HIGH TEMPERATURE

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In the context of climate change, global rise of temperature as well as intense heat waves affect plant development and productivity. Among the molecular perturbations that high temperature induce in living cells is the accumulation of reactive oxygen species (ROS), which can damage macromolecules of the cell and perturb the cellular redox state. To cope with deleterious effects of ROS, plant, as other organisms, have developed strategies to scavenge ROS and to regulate their redox state. Among those, glutathione play a major role in maintaining the cellular redox state and the function of key antioxidant enzymes like peroxidases. Here, we investigated the contribution of the redox systems in plant adaptation to high temperature. We studied two different high temperature regimes: a rise of ambient temperature to 27°C inducing a plant developmental adaptation program called thermomorphogenesis, and a 37°C treatment mimicking intense heat wave and affecting plant viability. Using the genetically encoded redox marker roGFP, we show that high temperature regimes lead to cytoplasm and nuclear oxidation and impact profoundly the glutathione pool rather than the glutathione redox state. Moreover, plants are able to restore the glutathione pool within a few hours, which likely contribute to plant adaptation to high temperature. In contrast, conditional glutathione deficient mutants fail to adapt to intense heat waves or to induce thermomorphogenesis, suggesting that glutathione is involved in both of these heat adaptation mechanisms. We also evaluate by RNAseq analyses, how plant change its genome expression signature upon heat stress and identified a marked genome expression deviation in the glutathione deficient mutant which might contribute to their sensitivity to high temperature. Thus, we define glutathione as a major actor in the adaptation of plant to contrasting high temperature regimes.

DECIPHERING THE EFFECT OF TEMPERATURE ON CIRCADIAN CLOCK PERFORMANCE IN TREES

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Circadian clock increases organisms' fitness by providing a mechanism to anticipate events such as sunrise and adjust their transcriptional programs (Webb et al. 2019, Nature Communications 10:550). Due to its ability to maintain 24h-rhythms over a wide range of temperatures, circadian clocks have been proposed to contribute to thermal adaptation and plasticity in plants (Resco et al. 2009, Ecology letters 12:583). However, consequences of clock performance on plant behavior in natural ecosystems are scarcely known. This constitutes relevant information to understand and predict effect of climate change on plant behavior. In this talk I will show the way in which we use indoor experiments in combination with trials in a natural laboratory in the mountain to explore the influence of temperature on the performance of circadian clocks in trees. I will show genomic, biochemical and eco-physiological experiments that provides evidences in favor that differences between species in the influence of temperature on the performance of their circadian clocks contribute to physiological adaptation of trees to the local thermic environment. Our experimental system is located in the southernmost woody ecosystem of the world: the sub-Antarctic temperate forests of Patagonia where two species of the genera *Nothofagus* which belongs to the order Fagales, are sharply distributed along altitudinal gradients, inhabiting non-overlapping thermal niches.

HOW DO PLANTS FEEL THE DAMAGE TO PROTECT AND REBUILD THEMSELVES

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Plants display remarkable developmental plasticity and regenerate new organs after injury. It is now well established that local signals produced by wounding trigger organ regeneration but molecular mechanisms underlying this control are still poorly understood¹. We investigate how wound stress activates new transcriptional programs to initiate cell fate reprogramming and how chromatin-based mechanisms modulate these transcriptional changes. A group of AP2/ERF transcription factors named WOUND INDUCED DEDIFFERENTIATION1-4 (WIND1-4) act as central regulators of wound-induced cellular reprogramming, and our recent work demonstrated that WINDs play more diverse physiological roles to protect and rebuild plant tissues after injury². In this talk I will discuss our latest findings on how wounding signals activate the WIND-mediated transcriptional pathways and how WINDs in turn promote reprogramming.

¹ Ikeuchi et al. (2019) Molecular mechanisms of plant regeneration. *Annu Rev Plant Biol* 70:3.1-3.30.

² Iwase et al. (2021) WIND transcription factors orchestrate wound-induced callus formation, vascular reconnection and defense response in *Arabidopsis*. *New Phytol* 232:734-752.

WARM TEMPERATURE AND MILD WATER DEFICIT INTERDEPENDENTLY CONTROL ROOT ELONGATION

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Warm ambient temperatures strongly increase elongation of the shoot and our understanding of this process has improved dramatically over recent years. Warm temperatures also promote elongation of the root, but this process is comparatively understudied. We have only limited knowledge of the molecular pathways that underlie root thermomorphogenesis and it is not known how warm temperature interacts with other environmental factors. Here I show that root thermomorphogenesis is tightly interlinked with mild water deficit signaling. Plants grown at warm temperatures on standard media showed only limited temperature-induced root elongation. Adding sorbitol to the media to mimic mild water deficit, greatly enhanced temperature effects on the root. Initial investigations into the signaling pathways that underly this phenotype suggest the involvement of brassinosteroid and abscisic acid signalling components. The interdependency of temperature and mild water deficit signals provides hints towards the molecular mechanisms that regulate root thermomorphogenesis, and starts to place temperature-induced root elongation within a physiological context.

WARMING UP TO RUSH TO EXIT: TEMPERATURE REGULATION OF LEAF SIZE CONTROL IN *ARABIDOPSIS*

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Temperature affects plant growth and development. An increase in global temperature has been shown to affect plant phenology including the timing of vegetative and reproductive growth¹. We are interested in studying the effect of temperature on organ size regulation, particularly of *Arabidopsis thaliana* leaves. High ambient temperatures suppress *Arabidopsis* rosette leaf area, in contrast to elongation of stem and petiole. While the mechanism underlying temperature-induced elongation responses are extensively studied, the genetic basis of temperature-regulation of leaf size is largely unknown. We show that warm temperature inhibits cell proliferation in the mature leaves resulting in fewer cells in the mature leaf compared to the control condition. Detailed time-course cellular imaging and cell-lineage tracking suggested a regulation of organ size control via a differential control of timing and progression of the cell division and expansion processes. Cellular phenotyping, genetic, and biochemical analyses established the key roles of PIF4 and TCP4 transcription factors in the suppression of *Arabidopsis* leaf area under high temperature by a reduction in cell number².

1. Scheffers, B. R. et al. The broad footprint of climate change from genes to biomes to people. *Science* 354, (2016).
2. Saini, K. et al. High temperature limits leaf size via direct control of cell cycle by coordinated functions of PIF4 and TCP4. *BioRxiv* (2022).

Session 6: Genomics and systems approaches to understanding the mechanisms of climate adaptation

GENOMICS OF PLANT NUTRITION IN THE CONTEXT OF CLIMATE CHANGE

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Atmospheric CO₂ concentration may rise up to 1000 ppm before the end of the century. In C₃ plants, growth under elevated CO₂ (eCO₂) results in a decrease of plant nitrogen (N) content on average by 15%, leading to a serious threat to come for food security and human health. Several hypotheses have been proposed to explain the decline of N content under eCO₂, including a direct effect on physiological processes associated to N uptake and assimilation. However, very few regulatory and signaling events involved in the negative effect of eCO₂ on N uptake and assimilation have been identified. Here, I will present our current work describing the physiological and molecular aspects of the negative effect of eCO₂ on plant mineral nutrition, and especially on root nitrate transport and assimilation. By inferring gene regulatory networks, and by exploiting genetic diversity, we investigate how eCO₂ alters major signaling pathways for plant mineral nutrition, in order to propose solutions to pave the way for climate-ready crops.

SPATIAL AND TEMPORAL REGULATION OF PLANT ORGAN GROWTH

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Plant organ growth is highly coordinated in time and space. The growth of different organs is highly correlated, but also within the growing maize leaf, and even in the different cell types, the growth processes undergo temporal and spatial regulation. In the different organs, drought impacts the growth processes by lowering the growth rate while prolonging the duration of growth. This prolonged window of growth enables plants to quickly resume growth when water becomes available again. We have identified several players that affect the duration of growth and by using single cell RNA sequencing, we recently identified a novel player. Using spatial transcriptomics, we study how the expression of those genes is organized in the maize shoot apex and aim to position the different regulators relative to each other.

NETWORK-BASED INTEGRATIVE GENOMICS REVEALS CONVERGENT TRANSCRIPTIONAL CONNECTIONS BETWEEN DROUGHT AND NITROGEN SIGNALS

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Nitrogen and water are crucial inputs for plant survival. Given their importance, the molecular mechanisms that plants rely on to signal nitrogen or water status changes - i.e. drought - have been under intense scrutiny. However, how plants sense and respond to the combination of nitrogen and drought signals at the molecular level has received scant attention. By utilizing the wealth of publicly available RNA sequencing data, we identify and compare regulatory connections between these signals. Searching online repositories, we found over 500 sequencing libraries relating to nitrogen, drought and abscisic acid (ABA)-hormone treatments. We completed and normalized their metadata profiles, allowing for universal comparisons of treatment details and regimes. Differentially expressed genes (DEGs) were derived for each experiment and further filtered to find consistently regulated genes for a given treatment. We found high and significant overlaps for nitrogen, ABA, and drought treatments genes. More importantly, nitrogen and drought-regulated genes show a strong negative correlation indicating these are opposite signals. We identified convergent transcriptional circuits that control both nitrogen and drought responses through network inference using machine learning tools. We validated NLP7 as a transcription factor that activates the nitrogen signaling and negatively regulates drought responses. This is a powerful approach to interrogating the relationship between signaling pathways and identifying key regulatory components for plant responses to environmental changes.

UNCOVERING NON-CANONICAL FUNCTIONS FOR O₂- AND COLD-REGULATED PROTEINS IN PLANTS

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Changes in O₂ levels function as signals to detect developmental events and environmental stresses such as flooding. Identifying the molecular mechanisms involved in plant O₂-sensing and adaptation to environmental stress is essential to identify agriculturally relevant targets to improve crop development and survival.

The O₂-sensing branch of the PRT6 N-degron pathway is a conserved proteolytic system which targets proteins for degradation based on their Methionine-Cysteine (MC) N-terminal sequence and O₂ availability.

The Group VII of the Ethylene Response Factor Family (ERFVIIIs) were the first proteins identified as substrates of this pathway in plants. In normoxia, these proteins are degraded, whereas in hypoxic conditions, they are not degraded and accumulate in the cell. The ERFVIIIs are transcription factors and some of their identified targets are associated with the response to hypoxia. Interestingly, cold stress produces similar molecular responses as hypoxia.

Despite their known function as transcription factors, a combined ChIP-seq – RNA-seq approach identified novel ERFVII functions. Here we show that ERFVIIIs regulate gene expression also through their involvement in preventing the deposition of the H3K27me₃ mark on certain genes. Remarkably, some of these epigenetically controlled genes are known O₂- and cold-regulated genes, providing a second layer of regulation of gene expression via the ERFVIIIs.

JUMPSTARTING EVOLUTION: THE ROLE OF MOBILE DNA IN THE GENERATION OF ADAPTIVE VARIATION IN RESPONSE TO ENVIRONMENTAL CHALLENGES

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Transposable elements (TEs) are powerful engines of genome evolution, as illustrated by their implication in the rewiring of gene regulatory networks and the creation of new cellular functions. Short-term consequences of TE mobilization can also be particularly dramatic given that TE insertions are a unique source of large effect mutations and that transposition can be exquisitely sensitive to the environment. Despite these considerations, there is a lack of knowledge about the role of ongoing transposition to within-species variation. In this talk I will present our efforts to characterize the impact of TE mobilization to *Arabidopsis thaliana* diversity and how we used this knowledge to forecast the consequences of environmentally-induced transposition in response to ongoing climate change.

OMICS APPROACHES UNDERPIN THE MOLECULAR BASIS OF PEARL MILLET RESPONSES UNDER DROUGHT-PRONE ENVIRONMENTS

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Genetic and phenotypic traits of dryland crops were shaped during their evolution and conquest of harsh environments. Multi-omics approaches can help examine in depth the molecular bases of their adaptive responses. By integrating genomic, transcriptomic and ionomic studies in pearl millet, we studied: i) the allelic variations of flowering time, ii) the differential gene expression network between contrasting drought-tolerant genotypes, and iii) the nutrient acquisition in leaves under low-inputs conditions. Genome-wide association studies identified 18 genes linked to flowering time, plant height, tillering and biomass. A fine-scale genetic difference in *PgPHYC* and *PgMADS11* (SNP and indel, respectively) and a co-variation of their alleles were associated with flowering time. Genotyping-by-sequencing identified two chromosomal regions differentiating two clusters among the early-flowering landraces. Transcriptomic analysis revealed a group of JA-regulated genes that could protect pearl millet at early-stage development and contribute to its production in drought-prone environments. We investigated the relationship between root traits and plant nutrition as the root system is responsible for water and nutrient acquisition. Preliminary results showed a 4-fold variation in Phosphorus concentration within a diversity panel with a broad-sense heritability of 0.72. Our findings give a holistic and a more comprehensive understanding of biological mechanisms taking place in pearl millet evolutive and adaptive responses under drought-prone environments. In the context of climate change and growing population, accelerating the breeding of dryland crops as pearl millet using candidate molecular markers could contribute to food security and resiliency.

MULTI-OMICS NETWORK ANALYSIS IDENTIFIES PUTATIVE REGULATORS OF MOLECULAR RESPONSES TO WATER STRESS AND SULFUR DEFICIENCY IN *PISUM SATIVUM*

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Plants in their physical environment face multiple biotic and abiotic stresses during their life cycle. In nature, environmental stresses often co-occur – such as water deficit and nutrient deficiencies – and can have synergistic, antagonistic or additive effects on the plant molecular responses. In pea (*Pisum sativum*), combination of water stress (WS) and sulfur (S) deficiency showed a mitigation effect on the seed protein composition, as compared to S deficiency occurring alone (Henriët et al., 2019). To better understand how pea responds to WS and/or S deficiency, a multi-omics (transcriptomics, proteomics, metabolomics, ionomics) analysis has been performed from leaf samples collected during a time course, on different lots of plants subjected to either WS, S deficiency, or to the combination of the two stressors. Our analyses revealed that 21% of the stress-responsive mRNAs, proteins, metabolites and ions showed a stronger response when S deficiency occurs with WS, suggesting synergistic or additive effects. Using a weighted-gene co-expression network approach, we evidenced genes strongly regulated by changes in the S concentration in leaves. The comparison between our transcriptomics and proteomics data allows us to formulate hypotheses on the level of regulation (transcriptional or post-transcriptional) of these genes. We also propose candidate genes that might help plants to better tolerate combinatorial stresses.

Reference: Henriët, C., Aimé, D., Térézol, M., Kilandamoko, A., Rossin, N., Combes-Soia, L., Labas, V., Serre, R.-F., Prudent, M., Kreplak, J., Vernoud, V., and Gallardo, K. (2019). Water stress combined with sulfur deficiency in pea affects yield components but mitigates the effect of deficiency on seed globulin composition. J. Exp. Bot. 70: 4287–4304.

TOWARDS A MECHANISTIC MODEL FOR THE CEREAL VERNALISATION PATHWAY

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While we understand much of the molecular regulation of cold induced flowering in *Arabidopsis*, far fewer genetic players are known in temperate cereals. The understanding from *Arabidopsis* also does not translate well because the vernalisation requirement in both groups originated independently. In the model grass *Brachypodium*, which displays strong and varying vernalisation requirements between accessions, we found that loss-of-function of the *VRT2* locus results in delayed flowering regulated by ambient temperature. This is puzzling because *Brachypodium* only shows weak ambient temperature regulated flowering. We learned that the phenotype is in fact devernalisation. So the *VRT2* mutant in fact reverses its vernalized state more easily than the controls, which suggests that *VRT2* regulates the maintained or epigenetic regulation of the vernalization process. Because *VRT2* is involved in (de-)vernalisation, we became interested in understanding the effect of fluctuating temperature on vernalisation in *Brachypodium*. To do this, we grew plants over fall and winter in an unheated greenhouse from different sowing times to flowering and monitored gene expression. We then applied the mathematical modelling framework generated in *Arabidopsis* to the *VRT2*-*VRN1* pair of cold induced flowering regulators in *Brachypodium*. This modelling effort will allow us to predict flowering under current and future climate scenario's. In addition, we plan to translate these findings to wheat.

ANALYSIS OF A LARGE BARLEY PANEL FOR CAPACITY TO GERMINATE AFTER A SHORT SUBMERGENCE STRESS

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Barley is one of the most susceptible crops to submergence and hypoxia stress during germination. However, a large variability can be observed in the germination capacity among different barley varieties after short submergence periods, but the mechanisms behind this response are still unclear. To explore this aspect, we screened a large panel of barley accessions for tolerance to short submergence periods during germination. A Genome Wide Association Studies (GWAS) was performed to identify candidate genes responsible for the trait. Varieties showing opposite phenotypes present different haplotypes for genes that might be involved in tolerance. Some of these genes also show differences in expression under submergence. Additionally, core-hypoxic genes were analysed, suggesting the idea that tolerance to short periods of submergence during germination is a complex trait, which is determined by multiple loci.

Session 7: Molecular mechanisms of adaptation to extreme environments

DESERT PLANT MICROBES FOR ENABLING AGRICULTURE UNDER EXTREME ENVIRONMENTAL CONDITIONS

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Beneficial microbes can enhance plant growth and provide abiotic and biotic stress tolerance to plants in extreme environments, but their underlying mechanisms are poorly understood. *Enterobacter sp.* SA187 is a root endophytic desert bacterium that maintains growth and yield of various monocot and dicot plants under various abiotic stress conditions. We compared the metabolic wirings of *Arabidopsis* and SA187 in the free-living and endophytic state, observing massive changes in sulfur metabolism pathways in both organisms. Plant sulfur metabolism mutants are hypersensitive to salt stress but can be partially rescued by SA187. We show that SA187 provides a small sulfur metabolite to its host plant, which regulates the ethylene signaling pathway and renders plants resistant to salt and heat stress in an ethylene-mediated manner. We further found that thermopriming and SA187 use the same heat stress response pathway regulating the key transcription factor HSFA2 to induce HSPs and anti-oxidative enzymes. SA187 primes plants already in the absence of stress treatment without any growth or yield penalty, showing that a single microbial strain can contribute to secure food production under multiple abiotic stress conditions.

SENSING AND SIGNALLING AT THE EXTREME : ABIOTIC STRESS AND CLIMATE CHANGE SIGNALLING MECHANISMS IN EXTREMOPHILE PLANTS

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Keywords: abscisic acid, calcium, cell homeostasis surveillance, epigenetics, multiple stress, reactive oxygen species, retrograde signalling, stress resilience

Extremophile plants develop and grow in harsh environments with extremes of cold, heat, drought, desiccation, or salinity, which have driven original adaptations. In line with their polyphyletic origins, extremophile plants likely possess core mechanisms of abiotic stress signalling, but novel properties or regulations may also have emerged. Comparative omics of extremophile genetic models such as *Arabidopsis lyrata*, *Craterostigma plantagineum*, *Eutrema salsugineum*, and *Physcomitrium (Physcomitrella) patens*, reveal signalling strategies potentially related to abiotic stress response efficiency, with putative specificities of sensing and with modifications of regulations, at the level of secondary messengers (Ca²⁺, phospholipids, reactive oxygen species), signal transduction (intracellular sensors, protein kinases, transcription factors, ubiquitin-mediated proteolysis) or signalling crosstalk. Involvement of hormone signalling, especially abscisic acid signalling, cell homeostasis surveillance, and epigenetic regulation also shows that large-scale gene regulation, whole-plant integration, and stress memory are important features of adaptation to extreme conditions. It is however difficult to assess whether these specificities may lead to resilience or hypersensitivity under conditions of climate change. Extremophile plants are endowed with stress sensors and with internal signals that may act as molecular integrators of climate change signalling. Stress-related information propagation may lead to stress memory and acquired stress tolerance that could withstand abiotic stress changes. However, functioning of existing sensors, integration of additive constraints and signals, or memory processes can be hampered by conflicting interferences between novel combinations and novel changes of climate change-related stresses. All of this emphasises the need to understand better the diversity and robustness of extremophile signalling in relation to climate change conditions.

AQUATIC PHOTOSYNTHESIS UNDER EXTREME CO₂ CONDITIONS

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CO₂ diffusion is slow in water compared to air. This physical limit has led to a biological adaptation of microalgae and cyanobacteria to low CO₂ levels: CO₂ Concentration Mechanisms (CCM). The industrial bioremediation of CO₂-rich industrial flue gases is desirable and profitable (carbon tax savings), but how adaptable or exploitable are green algae to extreme CO₂ environments? How high can we raise the CO₂ level in the medium and maintain photosynthesizing cells alive? Here we show that *Chlamydomonas* cells can tolerate up to 20% CO₂ (200 000 ppm), and that such extreme conditions induce a complete remodeling of photosynthesis. Cyclic electron flow is boosted to work against the acidification of the medium induced by bubbling high-CO₂. ATP produced by cyclic photophosphorylation likely participates to active proton efflux from the cytosol and maintain the intracellular pH in the physiological range.

PHYLOGENOMICS AND SYSTEMS BIOLOGY APPROACHES REVEAL CONSERVED ADAPTIVE PROCESSES IN ATACAMA DESERT PLANTS

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Throughout evolution, plants adapted to flourish in a variety of ecosystems, including extreme deserts. In the current changing climate scenario, it is essential to identify the underlying molecular mechanisms that enable plant resilience extreme conditions. The Atacama Desert, the driest non-polar desert in the world, offers a unique opportunity to explore plant adaptations to extreme environmental conditions. We characterized the three pristine and

extreme ecosystems along a natural altitudinal gradient of environmental parameters on the western Andes slopes in the Atacama Desert. We recorded low and unpredictable precipitation patterns, large daily temperature oscillations, low humidity, extremely high radiation levels, as well as soils with consistently low nitrogen levels. Despite these harsh conditions, a diversity of plant species coexist. We sequenced the transcriptome of the 32 most important plant species, representing 14 plant families with diverse phylogenetic origins. Using phylogenomics, we compared the protein-coding sequences of these 32 Atacama species to their 32 closest available sequenced species, and found 265 genes under positive selection in Atacama plants versus their non adapted “sister” species. These genes are involved in various developmental, regulatory and metabolic processes associated with environmental adaptation. We chose a set of positive selected genes and based the available functional characterization of their *Arabidopsis* orthologs we exemplify their potential role in the adaptation of plants to the extreme Atacama Desert. Our study provides new insights into plant abiotic stress tolerance, and improves our understanding of the highly unique, undisturbed Atacama Desert ecosystem.

MKK3 MODULE INTEGRATES STRESSES AND NUTRITION SIGNALS IN *ARABIDOPSIS*

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Since its first descriptions in 1995 and its first functional characterization 12 years later, plants MKK3-type MAP2Ks have emerged as an important integrator in plant signaling. If it does not count among the major stress-dependent MAPK actors in plant, recent works shed light on important roles in plant adaptation to environment. Our previous work (Danquah et al 2014, Sozen et al 2020) showed that together with clade-C MAPKs and MAP3K14/17/18, MKK3 constitutes a functional module which is activated by abscisic acid (ABA) and by jasmonic acid (JA) in the drought and wounding responses respectively. This activation requires a strong transcriptional regulation of the upstream *MAP3K* genes, which are virtually not expressed in resting conditions, the protein accumulating upon stimulation. Consequently, C-clade MAPK activation by ABA and JA is rather slow, occurring in hours. We present here a new piece of data suggesting that MKK3 modules are not only activated by stresses but also by nutritional signals such as nitrate (NO₃⁻). Nitrate activated MPK7 in a MKK3 dependent way in plantlets which have been N-starved. This activation kinetic, occurring in 20 minutes, fits the one of NO₃ triggered NLP7-dependent up-regulation of *MAP3K13/14* (Marchive 2013), suggesting that these two MAP3Ks play important roles for module activation. Coherently MPK7 was not activated anymore by nitrate in both *map3k13/14* and *nlp7* mutants. Taken together, this data break the textbook message that MAPK modules define fast responsive signaling pathways and indicate that MKK3 module is a more general responsive signaling mechanism in plants, controlling a second layer of responses, remaining to be identified.

UNCOVERING THE WILD GENETIC DIVERSITY IN *CHENOPODIUM* SPP. FOR IMPROVEMENT OF HEAT STRESS TOLERANCE IN QUINOA.

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Quinoa (*Chenopodium quinoa* Willd.), is a semi-domesticated, pseudo-cereal crop that originated in the Andes. Quinoa has gained increasing interest as a highly nutritious food source that can be sustainably grown on marginal lands due to its ability to be grown in poor soils and tolerance to several abiotic stresses (including drought, salinity and frost). Yet, quinoa performs poorly in environments where temperatures reach above 35°C, which represents a major threat to the production in its native environment, and a major limitation on the expansion of quinoa as a major crop beyond the Andes in the context of climate changes.

We are exploring heat stress tolerance mechanisms in the North- and South-American wild relatives of *C. quinoa*, namely *C. hircinum* and *C. berlandieri* respectively, in order to improve the resilience of quinoa to high temperatures during reproductive developmental stages. **Quinoa wild populations originate from some of the hottest environments in South and North America**, hence we hypothesize that they possess previously **unexplored mechanisms of adaptation to high temperatures**, that are not present, but could be transferred, into cultivated quinoa. **Here we report on the morphological and physiological characterization of the response to heat stress** in a sub-collection of sixteen wild and cultivated quinoa genotypes. Our preliminary results confirm superior heat stress tolerance of the wild relative, and pave the way to further in-depth molecular and genetic analyses to identify the underlying genetic basis for differences in tolerance to high temperatures.

Key words: quinoa, heat stress response, reproductive development, plant physiology.

Posters Presentations

P1

IMPACT OF POST-FLOWERING ENVIRONMENTAL STRESS ON NITROGEN FLUX AND SEED COMPOSITION IN *ARABIDOPSIS*

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Plants are sessile organisms that take nutrients from the soil for growth and development. Direct post-flowering uptake and remobilization are the two nitrogen fluxes set up for seed filling. The proportions of nitrogen coming from these two fluxes constitute a balance that determines the final composition of the seed. These mechanisms are regulated by internal processes (hormones, age etc...) and environmental fluctuations. While the cellular mechanisms of response to the environment are well known, the effects of environmental stresses on the balance between remobilization and uptake remain little studied. In the context of climate change where stress events are predicted to be more frequent and intense, we chose to study the effects of the environment on carbon and nitrogen allocation in *Arabidopsis*. To this end, we conducted a series of cultures of 8 genotypes in various stress environments using ¹⁵N nitrate isotope labelling system. Overall, the results indicate that biotic and abiotic stresses significantly influence both carbon allocation and nitrogen use efficiency (NUE) in plants and generate different bottlenecks in nitrogen fluxes during seed filling.

P2

HYDRAULIC STRESS AT THE MERISTEM-ORGAN BOUNDARIES

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Beyond biochemical factors, plant development is also shaped by physical variables, and notably external and internal mechanical conflicts. Interestingly such conflicts involve hydraulic components, and thus water stress, too. In shoot apical meristems (SAM), the fast growth of new organs creates a local mechanical conflict at the meristem-organ boundaries. We have previously demonstrated that these stresses correlate with nuclear deformation and changes in chromatin physical and chemical properties, as well as gene expression (Fal *et al.*, 2021). In order to understand the morphological deformations in boundary cells, we performed an automated 4D image segmentation analysis over the whole SAM and measure cell volumetric changes. This suggests water flux out of the boundary domain, which correlated with a bias in water-stress gene expression. Through artificially compressing meristems and modifying osmotic conditions, we further show that tissue hydraulics can influence transcriptional activity. While this hydraulic stress occurs under normal growth conditions, we speculate that it is likely dependent on plant water status. Taken together, our integratory analysis suggests that SAM boundaries are defined by mechanical as well as hydraulic stress. Interestingly, boundaries are also largely determining plant architecture, thus potentially relating plant branching to force-derived water-stress.

Fal K, Korsbo N, Alonso-Serra J, *et al.* Tissue folding at the organ-meristem boundary results in nuclear compression and chromatin compaction. PNAS, 2021.

P3

STRESS GRANULES – POTENTIAL FIELD FOR CROP IMPROVEMENT

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Being sessile, plants are challenged with the continuous changes in their environment. These changes are portrayed by different stresses. These stresses have a major impact on agriculture and food production. One of the mechanisms that allow plants to sense stress and adjust resistance to stress are condensate formation. Stress granules are non-membranous organelles formed under stress conditions to sequester mRNA, proteins and metabolites into cytosolic foci, to protect or to regulate their activity under stress. In our group, we are uncovering mechanisms of stress granule formation under high temperature, low oxygen and salt stress. Novel, unpublished data from our lab suggests that SGs have stress-specific composition and stress-specific dynamic. We discovered that mechanism of SGs formation depends from mRNA-protein complexes, protein-protein interactions, prion-like domain composition, depends on posttranslational modifications. We have significant data suggesting that key components involved in SGs formation are required for stress tolerance and might improve stress recovery. This opens a new direction of research where by focusing on those protein candidates we will work on improvement of stress tolerance and recovery in crop plants.

P4

SWEET SLEEP; DORMANCY OF TUBER BUDS IN A CHANGING CLIMATE

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Bud endodormancy (ED) is a crucial stage in the life cycle of many perennial plants and is regulated by genetic and environmental factors. ED release requires the accumulation of a certain amount of cold exposure, measured as chilling units. However, the mechanism governing the effect of cold on ED duration is poorly understood. We used the potato tuber system to investigate the mechanism underlying ED release. Cold-induced sweetening (CIS) is a well-known response of potato tuber to chilling. We developed sugars accumulation measure during CIS and defined it as sugar units. We showed that ED release was better correlated with sugar units' accumulation than chilling units. In turn, CIS was associated with shorter ED. Heat exposure also caused sugar unit accumulation, followed by faster ED release. A logistic function was developed to predict ED duration based on sugar unit measurements. Modifying sugar units level by knockout of the vacuolar invertase gene (*StVInv*) extended ED, while *StVInv* overexpression shortened it. We found that the time gap between sugar accumulation and ED release is associated with abscisic acid (ABA) level that serve as a growth inhibitor. Our results suggest that sugar units are better predictors of ED duration than chilling units. We also found a novel interaction between ABA and sugar metabolism that determine the timing of bud growth. We believe that better understanding of the effect of climate change on bud dormancy will allow the development of cultivars that are more thermotolerant.

P5

TO GROW OR NOT TO GROW: SINGLE CELL SIZE REGULATION IN PLANTS UNDER LOW TEMPERATURE

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How cells regulate their size is one of the most fascinating questions in current biology. Root hairs (RH) are single plant cells that can expand several hundred-fold their original size and they are an excellent model system for learning about cell size regulation. Root hair size determines the surface area/volume ratio of the whole roots exposed to the nutrient and water pools, thereby likely impacting nutrient and water uptake rates. Their growth speed is determined by cell-intrinsic factors like hormones (e.g., auxin and ethylene) and external environmental signals like nutrient availability in the soil (e.g., phosphate and nitrates). Previously, we have determined that the low-temperature treatment at 10°C is able to trigger, unexpectedly, an exacerbated RH growth compared to the room-temperature control condition (22°C). This plasticity in RH growth at low-temperature was linked to a reduced nutrient availability in the media. In this short talk, I will explore the molecular basis of this strong RH growth response. Although nutrient availability in the soil is one of the key factors for a sustained plant growth, the molecular mechanisms behind the perception and the downstream signaling pathway in the roots are still far to be clear.

P6

MOLECULAR ANALYSIS OF BUD DORMANCY REGULATION BY TEMPERATURE IN SWEET CHERRY

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Over the past decades, climate change has already led to a higher frequency of mild winters. In particular, the rising temperatures impact winter dormancy, a resting period which is a key step of the tree life cycle, preventing growth under unfavorable conditions. Indeed, the lack of chilling temperatures during dormancy can cause flowering defaults such as bud necrosis whereas the mild temperatures at the end of winter lead to early flowering, desynchronization with pollinators and a higher risk of frost damages. These effects of mild temperatures will contribute to a decrease in fruit production and could have a major economic impact. Sweet cherry trees, in particular, are very sensitive to temperatures; therefore their production is greatly threatened by climate change. In order to better anticipate the effects of future climatic conditions on sweet cherry cultivation, it is necessary to better understand how dormancy is regulated under constrained temperatures. We investigated the effect of warm temperatures on sweet cherry trees during dormancy onset and maintenance, using both phenological observations and molecular analyses. Potted trees of the sweet cherry cultivar 'Regina' were submitted to cold deprivation from July to March, under long days and short days in order to separate the effect of temperature and photoperiod. We recorded the phenological observations through the entire cycle of seasons. Moreover, we performed global transcriptomic analysis using RNA-Seq on bud tissues sampled throughout the temperature and photoperiod treatments, in order to highlight candidate genes that are regulated by temperatures during dormancy progression. Our results show that cold deprivation induces a marked delay in the flowering date, and strongly affects the expression patterns of dormancy candidate genes. Further analyses will allow the identification of genes and physiology pathways that are involved in the response to temperature.

P7

SPATIOTEMPORAL PERCEPTION OF WATER STRESS IN ARABIDOPSIS

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Water stress, is experienced by plants as soon as the water demand exceeds the water uptake capacity, drop two biophysical parameters of plants—which are extracellular Ψ_{Π} (osmotic potential) and intracellular Ψ_P (turgor potential). Deciphering their transduction mechanism clearly is a huge challenge, and we are committed to understanding their respective effects from a molecular and biophysical perspective.

We have performed preliminary experiments where *Arabidopsis* plants grown in hydroponic cultures were treated with varying concentrations of solutes. With the help of a cell pressure probe, we found the optimal time of transcriptome detection. Then, we found some candidates by analyzing the correlation between gene expression and P and/or Π . Moreover, we have screened some optimal genes by analyzing the temporal variation of their expression and expected model. Next step, we try to develop the biosensor of P and/or Π , identify their roles in regulatory network of water stress and test whether aquaporin participate in this network.

Co-author : Christophe Maurel, Yann Boursiac

P8

THE IMPACT OF MULTIFACTORIAL STRESS COMBINATION ON PLANT GROWTH AND SURVIVAL

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Climate change-driven extreme weather events, combined with increasing temperatures, harsh soil conditions, low water availability and quality, and the introduction of many man-made pollutants, pose a unique challenge to plants. Although our knowledge of the response of plants to each of these individual conditions is vast, we know very little about how a combination of many of these factors, occurring simultaneously, *i.e.*, multifactorial stress combination, impacts plants. In this work, seedlings of wild type and different mutants of *Arabidopsis thaliana* plants were subjected to a multifactorial stress combination of six different stresses, each applied at a low level, and their survival, physiological and molecular responses determined. Our results show that while each of the different stresses, applied individually, had a negligible effect on plant growth and survival, the accumulated impact of multifactorial stress combination on plants was detrimental. We further show that the response of plants to multifactorial stress combination is unique and that specific pathways and processes play a critical role in the acclimation of plants to multifactorial stress combination. Taken together our findings reveal that further polluting our environment could result in higher complexities of multifactorial stress combinations that in turn could drive a critical decline in plant growth and survival.

P9

GRAPEVINE RESPONSE TO HEAT: LINKING TRANSCRIPTION, REDOX STATUS AND K/ACIDS BALANCE

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High temperature (HT) accounts for large yield losses in crops worldwide. In the context of the climate change, understanding how plants cope with HT in their environment while maintaining good crop yield and quality is an important issue. Regarding viticulture, predictive models reported that temperature elevation should cause significant and permanent damage to the vine, affecting its phenology and yield. This results in desynchronizing sugar and organic acid metabolisms and increasing sugars and K⁺ accumulation during ripening. Indeed, HT modifies the fruit content at harvest and the quality of wines resulting in wines with a high alcoholic content, low acidity and ageing potential and poor organoleptic qualities. In this context, there is an urgent need to anticipate the effects of climate change on vineyards, including the consequences of extreme T°. In addition to wine-growing practices, exploiting the genetic diversity of the genus *Vitis* in order to select varieties better adapted to a warmer climate while maintaining the expected quality profiles remains a valid alternative. The ANR PARASOL aims at supporting this action by studying the functioning of relevant heat-modulated transcription factors (TFs) and their interaction with important physiological parameters driving plant response to HT and fruit quality: redox status and K⁺/acid balance. Taking advantage of the biological properties of the microvine, in association with molecular tools valuable for TF studies (CRES-T) or genome editing (CRISPR/Cas), PARASOL should allow to i) assess to what extent an altered genotype in one of the studied parameters (TFs, K⁺/acids, Redox) may be affected in thermotolerance and/or fruit quality, and ii) validate our hypothesis suggesting an interconnection between the identified heat-responsive TFs and key players of both K⁺/Acids and redox homeostasis in the context of HT response in grapevine. Some preliminary results will be presented here.

P10

LSM7 ROLE IN MRNA DEGRADATION AND SPLICING UPON AMBIENT TEMPERATURE CHANGES

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Sm-like (LSm) proteins are highly conserved among eukaryotes with a fundamental role in RNA metabolism. They interact with each other forming a heptameric ring and with other core components of the splicing machinery to ensure RNA splicing integrity in the nucleus and are also involved in the P-body formation and mRNA degradation by promoting decapping in a nonsense-mediated decay (NMD) mechanism in the cytoplasm. By promoting alternative splicing of essential genes and regulating correct RNA levels, LSm proteins are important regulators of development and response to environmental signals. For example, LSm proteins were shown to be important during heat and cold stress.

Here, we report that LSM7, one of the eleven LSm proteins in *A. thaliana*, is essential for embryogenesis and that total loss of *LSM7* expression leads to embryo arrest at the globular stage. In contrast to the *lsm7* null mutant, downregulation of *LSM7* does not affect embryo development, but leads to different developmental defects in a temperature-dependent manner. Performing a comprehensive transcriptome analysis, we observed that downregulation of LSM7 promotes an inaccurate selection of splice sites that leads to a genome-wide increase in alternative splicing. Moreover, we observed that LSM7 modulates the splicing of stress-responsive genes in different ambient temperatures. We propose that LSM7 splicing activity is essential for plant adaptation and survival. This study highlights the essential roles of Sm-like proteins in splicing and RNA degradation during plant development and provides new insights to improve plant adaptation under temperature-associated stress.

P11

HSP90 ALTERS SAM MORPHOLOGY DURING REPRODUCTIVE PHASE TRANSITION IN *ARABIDOPSIS THALIANA*

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The timing of flowering is regulated by environmental and endogenous signals that converge at the Shoot Apical Meristem (SAM). During phase transition SAM takes on an inflorescence meristem (IM) identity that balances two antagonistic processes: to remain undifferentiated and to produce continuously flower primordia. Little is known about the molecular processes controlling meristem cells responses to adverse temperatures- though of critical importance under the projected climate change. Heat Shock Protein 90 (HSP90) is an evolutionary conserved molecular chaperone that modulates many cellular processes under physiological and stress conditions. Previously, we showed that cytoplasmic HSP90s are expressed in the SAM under normal and stress conditions [1] and are essential for the transition from vegetative to reproductive state and for flower meristem formation [2]. To investigate further the involvement of HSP90 in this process, we examine SAM organization and size during vegetative to reproductive phase change under different photoperiodic regimes. Our findings show that HSP90 depletion results in abnormal morphological features and size of the SAM. The transcript levels of key floral integrator genes were analyzed by q-PCR. Additionally, we show that HSP90 modulates the spatio-temporal expression pattern of genes conferring flower primordium identity under normal and heat-stress conditions. Taken together our results suggest that HSP90 integrate extrinsic cues in order to regulate SAM growth and differentiation in a changing environment.

- [1] Prasinos *et al.*, (2005). Tight regulation of expression of two *Arabidopsis* cytosolic Hsp90 genes during embryo development, *Journal of Experimental Botany*, Volume 56(412), 633–644. <https://doi.org/10.1093/jxb/eri035>
- [2] Margaritopoulou *et al.*, (2016). HSP90 canonical content organizes a molecular scaffold mechanism to progress flowering. *Plant J*, 87: 174-187. <https://doi.org/10.1111/tpj.13191>

P12

THERMOPRIMING MITIGATES THE EFFECTS OF HEAT STRESS IN *BRASSICA JUNCEA* (INDIAN MUSTARD) SEEDLINGS

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High temperature is one of the most devastating abiotic stresses, that poses a great threat to the plant growth and productivity. Although plants respond to heat stress by mounting complex, multi-layered mechanisms, it may not often be sufficient to cope with the stress. Interestingly, pre-exposure to a mild stress (referred as priming) has been shown to enable the plants to exhibit remarkable plasticity in adapting to the changing environments. In the present study, we analysed the thermopriming-induced heat stress response in the major vegetable oil seed crop, *Brassica juncea* L. (also known as Indian mustard). Our study demonstrates that thermopriming mitigates the effects of heat stress on the growth and survival of Indian mustard seedlings, which was found to be associated with efficient ROS scavenging mechanism. Gene expression analysis of *BjHSF* family of transcription factors identified *HSFs* that display heat stress- and priming-responsive expression pattern in *B. juncea* v. Pusa Bold. In addition to Pusa Bold, fifteen Indian mustard varieties were screened for their basal and acquired thermotolerance. Based on their survival rate and other growth parameters these varieties were categorized as heat 'sensitive' or 'tolerant'. Notably, almost all the varieties display thermopriming-induced acquired thermotolerance. Taken together, our study shows that thermopriming effectively alleviates the harmful effects of heat stress on *Brassica juncea* seedlings.

P13

REDOX REGULATION OF HISTONE ACETYLATION IN RESPONSE TO ENVIRONMENTAL STRESS IN *A. THALIANA*

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High temperatures impact plant growth and survival. Recent data indicate that chromatin modification is an essential process of gene expression reprogramming during plant response to high growth temperature. Histone deacetylases (HDACs) that regulate histone acetylation levels have been shown to play an important role in plant adaptation to environment. Moreover, HDACs are also involved in the deacetylation of non-histone proteins such as metabolic enzymes to control their activity. In animals, some HDACs are regulated by post-translational oxidation-reduction (redox) modifications involving the oxidation of conserved cysteines. In plants, the redox regulation of HDACs is very little known. A recent study demonstrated that redox modifications affect histone acetylation by inhibiting HDAC activities. This would result in hyperacetylation of genes specifically involved in the response of plants to stress. However, the exact nature of these changes remains unknown. During my thesis, we detected a redox modification of HDA6 under oxidative conditions, affecting the oligomerization state of these proteins. We have also shown that the expression of genes regulated by HDA6 is affected by the redox environment of the cell. Surprisingly, we found that HDA6 localizes to cytoplasmic stress granules under thermal stress of 37°C. To decipher the importance of HDA6 regulation under environmental stress, we investigated the role of HDA6 in thermotolerance and thermomorphogenesis and found that *hda6-6* and *hda6-7* mutants are very sensitive to heat-stress and show thermomorphogenesis defects. Finally, we discussed the emerging link between redox signaling and histone acetylation in response to heat stress.

P14

THE NUCLEOTIDE ALARMONE GUANOSINE TETRAPHOSPHATE (PPGPP)- A CONSERVED REGULATOR OF PHOTOSYNTHESIS DURING PLANT STRESS ACCLIMATION.

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To survive, plants must be able to withstand sudden and sometimes harsh changes in their environment because they cannot move away. An unusual nucleotide called guanosine tetrphosphate (ppGpp) may help plants to do this. ppGpp was discovered more than 50 years ago in bacteria where it acts as an alarmone that mediates acclimation to environmental fluctuations. ppGpp was discovered more recently in the chloroplasts of plants and algae, which are hubs of stress perception and acclimation. Thanks to recent work from several laboratories, ppGpp has now emerged as a major pathway that is capable of regulating chloroplast function. Until recently it was not yet clear to what extent ppGpp was required for stress acclimation. Here, I will present the latest results from our laboratory showing that ppGpp biosynthesis is necessary for acclimation to nitrogen starvation in *Arabidopsis*. To determine the physiological role of ppGpp during nitrogen starvation we grew different mutants lines (deficient for ppGpp accumulation or accumulating ppGpp) on a nitrogen limiting medium, and analysed the effects of this treatment on ROS production, photosynthesis and chloroplast transcription. We found that ppGpp is

required for remodeling the photosynthetic electron transport chain to downregulate photosynthetic activity and for protection against oxidative stress. Global analysis of chloroplast transcript abundance indicated that ppGpp is likely to act by promoting the global downregulation of chloroplast transcription and also that ppGpp is required for coupling chloroplastic and nuclear gene expression during nitrogen starvation. Altogether our work indicates that ppGpp acts as a major regulator of chloroplast activity during nitrogen limitation, and is likely to play a similar role in acclimation to other abiotic stresses. Understanding the role of ppGpp signalling is likely to assist in the development of more effective strategies for making crop plants more tolerant to stress and less reliant on high levels of nutrient provision.

<https://www.cite-des-energies.fr/en/home-biam/search/lgbp/lgbp-research-themes/>

P15

HEAT STRESS TARGETING INDIVIDUAL ORGANS REVEALS THE CENTRAL ROLE OF ROOTS AND CROWNS IN RICE STRESS RESPONSES

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The influence of plant inter-organ communication under heat stress (HS) was evaluated by targeting the stress at individual plant organs (roots / shoots / whole plant) in rice (*Oryza sativa*). Photosynthetic activity, ascorbate peroxidase activity, phytohormones levels, and gene expression revealed different strategies employed by the plant. Whereas HS targeted to leaves elevated levels of JA in roots, cis-zeatin in crowns, and ascorbate peroxidase activity in crowns and roots, HS targeted to roots increased levels of abscisic acid and auxin in leaves and crowns, cis-zeatin in leaves, and JA in crowns, while reducing trans-zeatin levels. Whole-plant HS increased levels of jasmonic acid (JA) and cytokinin cis-zeatin in leaves, while up-regulating auxin indole-3-acetic acid and down-regulating trans-zeatin in leaves and crowns. Ascorbate peroxidase activity and expression of alternative oxidases (AOX) increased in leaves and crowns. The weaker protection of leaves reflects the growth strategy of rice. HS treatment of individual organs induced changes in phytohormone levels and antioxidant enzyme activity in non-exposed organs, in order to enhance plant stress tolerance.

P16

CYTOKININS SYNERGISTICALLY WITH HEAT ACCLIMATION ENHANCE RICE THERMOTOLERANCE AFFECTING VOLATILE EMISSION

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Heat stress is a frequent environmental constraint, which significantly affects plant primary and secondary metabolism. One of the possibilities to increase plant vulnerability against heat stress includes application of phytohormones, the important regulators of growth and stress responses. In this study, the effects of exogenous cytokinin *meta*-topolin-9-(tetrahydropyran-2-yl)purine (mT9THP) on rice (*Oryza sativa*) were compared after acclimation (37°C 2h), heat stress (HS; 45°C 6h) and their combination (AHS). Acclimation had only minor effect on phytohormone levels, gene expression, membrane lipid composition and volatile emission. AHS and HS responses shared some features (increase in levels of abscisic acid and the ethylene precursor aminocyclopropane-1-carboxylic acid in leaves and roots, *cis*-zeatin and cytokinin methylthio-derivatives in whole plants, expression of heat shock proteins, alternative oxidases and superoxide dismutases). However, AHS also induced specific changes, including increase in levels of jasmonic acid and auxin indole-3-acetic acid in leaves, membrane diacylglycerolipids with less double bonds, and volatile emission (acetamide, LOX-derived volatiles). Under control conditions, application of mT9THP negatively affected levels of abscisic and salicylic acids in crowns and jasmonic acid in leaves. In combination with acclimation, mT9THP enhanced its positive impact on plant defence. Together with direct HS, exogenous cytokinin mimicked some beneficial effects of acclimation. Combination of mT9THP and AHS had the strongest thermo-protective effect. The results demonstrated an important role of roots in HS responses, and a substantial contribution of stimulation of volatile production to positive effects of cytokinin and AHS on rice thermotolerance. This work was funded by the Czech Science Foundation (grant no. 20-22875S).

P17

DOES THE MEMORY OF A FIRST WATER DEFICIT ENABLE A MORE EFFICIENT RESPONSE TO A SUBSEQUENT WATER DEFICIT?

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In the current context of climate change, periods of water deficit occur more frequently along the crop cycle, leading to high yield losses. To limit the negative impact of recurrent water deficits, plants can adapt, via the mobilization of “stress memory”, allowing them to respond to a subsequent stress in a faster and/or more intensive manner. After a first stress event, plants can keep an imprint of this stress *via* the induction of epigenetic (e.g. memory gene regulation), physiological (e.g. stomatal closure) and molecular (e.g. compound accumulation) changes. When maintained between two stress periods, these changes may prepare plants for a subsequent water deficit.

This work addresses the potential role of stress memory in plant adaptation to recurrent water deficits with a special focus on plant hydro-mineral uptake by roots. For this purpose, an experiment was conducted on the high throughput phenotyping platform (4PMI, Dijon, France), where several frequencies of water deficits were applied to pea plants. An integrative approach, including a structure-function ecophysiological framework characterizing plant hydromineral nutrition (nutrients and beneficial elements), enriched with root and nodule transcriptomic analyses (RNA-seq), and metabolomic analyses revealed the mechanisms underlying the “memory effect” throughout the plant cycle. We will discuss the role of memory genes during recurrent stresses and plant strategies to acquire water as well as macro- and micro-nutrients more efficiently during recurrent stresses. This work offers the new perspective of considering plant memory in the design of ideotypes better adapted to multiple stress events in a context of climate change.

P18

NATURAL VARIATION OF TEMPERATURE-DEPENDENT METABOLIC CHANGES IN *ARABIDOPSIS THALIANA* IDENTIFIES *TPS1* AS A PUTATIVE NEGATIVE REGULATOR OF THE HEAT SHOCK RESPONSE

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Facing high temperatures, plants respond with a genetically encoded heat shock response (HSR). This HSR results in vast changes of both the transcriptome and metabolome, ultimately leading to the acquisition of a short-term thermotolerance. Transcription factors of the *HEAT SHOCK TRANSCRIPTION FACTOR (HSF) A1* subclass are known to be crucial for a functional HSR. However, the mechanism of initial heat sensing and thus *HSFA1* activation remains to be elucidated as do other, so far unknown, regulators of this response. Here, we utilized the natural variation of temperature-dependent metabolic changes in *A. thaliana* to identify novel regulators of the HSR. Correspondingly, the accumulation of various metabolites in response to moderate heat stress (32°C) was screened across 250 natural accessions. Subsequent genome-wide association studies (GWAS) identified several SNPs in proximity of the *TREHALOSE-6-PHOSPHATE SYNTHASE 1 (TPS1)* locus, a gene crucial for sugar and energy signaling, that were associated with variation in heat-induced accumulation of the trisaccharide raffinose. Seedlings with a weak *tps1* mutant allele accumulated higher raffinose levels and performed superior under long-term heat stress, accompanied by a stronger induction of the two prominent HSR transcription factor genes *HSFA2* and *MULTIPROTEIN BRIDGING FACTOR 1C (MBF1C)* in response to elevated temperature. In conclusion, our data suggest that *TPS1* functions as a negative regulator of the plant HSR.

P19

SM PROTEIN SMD3B IS CRITICAL FOR *ARABIDOPSIS* GROWTH AT LOW TEMPERATURE AND ITS EXPRESSION IS REGULATED BY CIS-INTRONIC DNA.

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Modulation of pre-mRNA splicing has been widely associated with plant acclimation to temperature stress, yet we are just beginning to understand the underlying molecular mechanisms. Sm proteins are core components of the spliceosome small nuclear ribonucleoprotein complex (snRNPs). Sm proteins form a hetero heptameric ring that associates with U-rich snRNAs, around which other factors organise to form the spliceosome. We found that loss of *SmD3B* dramatically impairs growth and development of *Arabidopsis thaliana* at low temperature. This is in agreement with previous reports on the central role of Sm-mediated RNA splicing for survival under that environmental condition. To understand the role of SmD3B in response to low ambient temperature, we studied the *SmD3B* locus. Complementation analyses revealed that introns are required for proper expression of *SmD3B*. Ongoing experiments address the contribution of individual introns to *SmD3B* gene and protein expression, the underlying regulatory mechanism and whether this mode of regulation is specific to low temperature. We are also analysing which pre-mRNA splicing events are mediated by SmD3B at low temperature. Our analysis points towards unexplored mechanisms for plant response to low temperature that regulate core spliceosome components.

P20

ROOTS TRAITS FOR IMPROVED PEARL MILLET NUTRITION AND PERFORMANCE IN LOW INPUT AGROECOSYSTEMS

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Pearl millet is an important crop for food security in arid and semi-arid regions of the world. It is traditionally cultivated in low input agroecosystems where its yield is limited by soil nutrients and water availability. Low yield potential is accentuated by climate change, characterized in these regions by more frequent drought events. Because roots are responsible for water and nutrient capture, they represent good targets to improve pearl millet nutrient acquisition and drought adaptation. To better understand how root traits influence nutrient acquisition and drought adaptation in pearl millet, a panel of 160 newly re-sequenced inbred lines were phenotyped for root architectural and anatomical traits using shovelomics and laser ablation tomography, ion content in leaves using ICP-MS, and yield and yield components traits under irrigated and drought stress treatments in field conditions in Senegal. Mixed linear models were used to correct these data sets for spatial trends and assess heritability, as well as treatment effect. Phenotyping and data analysis pipelines will be presented as well as preliminary results showing high heritability for most traits and interesting correlations between root traits, ion content and agromorphological traits. Further physiological and genetic dissection of these traits, their synergy, and interactions with the environment, will help in identifying markers controlling root traits related to nutrient acquisition and responses to drought, that could be useful for breeding pearl millet plants better adapted to future climates.

P21

UNDERLYING MECHANISMS OF PLANT STRESS GRANULES ASSEMBLY: ROLE OF POSTTRANSLATIONAL MODIFICATIONS

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Stress granules (SG) assembly is a highly conserved mechanism through which eukaryotic organism modulates their transcriptome, proteome, and metabolome as an adaptive stress response against high salt, heat, darkness, hypoxia, and viral infection. Upon stress recovery, the SG disassembly contributes to reprogramming cell signaling and metabolic pathways being essential to reassume cell growth and development. Proteomic studies of SGs isolated from mammals, yeast, and plants have revealed a steady-state interaction network between conserved SGs proteins in unstressed cells, which is suggested to enable fast SGs assembly during the stress response. This finding raises an interesting question of how cells control the behavior of these preexisting complexes. In mammals, the evidence suggests posttranslational modifications (PTMs) of core SGs proteins such as glycosylation, phosphorylation, and ubiquitination act as cellular switches to modulate SG assembly. In plants, there is no direct evidence about the role of PTMs in SGs dynamics. Here, we investigate the role of PTMs on the plant SGs assembly driven by the RNA-binding protein 47b (Rbp47b), an *Arabidopsis* SGs member of the preexisting complex, using a collection of rationally designed Rbp47b mutants, cell imaging, genetics, omics, and high-throughput phenotyping. Our results suggested that point mutation on Rbp47b phosphorylation and acetylation sites unpaired the SGs assembly. These findings give evidence of a potential regulatory mechanism where PTMs act as a molecular switch during SGs assembly.

P22

ACCLIMATION OF PHOTOSYNTHESIS TO THE ENVIRONMENT 1 (APE1) IS A PSII FACTOR THAT MAINTAINS PHOTOSYNTHETIC YIELDS IN HIGH LIGHT IN *CHLAMYDOMONAS REINHARDTII*

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Photosynthetic organisms require high light acclimation mechanisms to regulate light capture for photo-protection and to tune an increased turnover of photosystem II. The protein ACCLIMATION OF PHOTOSYNTHESIS TO THE ENVIRONMENT 1 (APE1) is found in all oxygenic phototrophs and encodes a 25kDa thylakoid protein. The *ape1* mutant was identified in a genetic screen that aimed to isolate regulators of linear electron flow in the model alga, *Chlamydomonas*. We have determined APE1 localisation in thylakoids and co-localisation with PSII using biochemical methods. The *ape1* mutant maintains more PSII supercomplexes in high light and we show that *ape1*'s inability to adapt to high light is due to PSII photoinhibition. Interestingly, the *ape1* mutant sustains CO₂ capture in the photoinhibited state. These phenotypes are accompanied by upregulation of oxidative stress markers and we discuss hypotheses as to APE1's role in ROS detoxification, electron transfer and PSII biogenesis.

P23

THE MOLECULAR MECHANISM OF TEMPERATURE-RESPONSIVE INFLORESCENCE DEVELOPMENT IN BARLEY (*HORDEUM VULGARE*)

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Recent studies related to climate change have shown that temperate crops are increasingly subjected to high ambient temperatures during the growth season. High ambient temperatures can impair inflorescence development and floret fertility, and are thus a major cause of yield loss in temperate cereals, in particular barley and wheat. However, the mechanisms that underpin the inflorescence development under high ambient temperature are not fully understood. Our research has established that high ambient temperature interacts with the major photoperiod responsive gene *Ppd-H1* to control the inflorescence meristem activity, and thus the rate and duration of spikelet and floret primordium initiation. In this study, we aim to unravel the molecular basis of *Ppd-H1* controlling inflorescence development under high ambient temperature. For this purpose, we conducted transcriptome profiling on developing inflorescences and leaves of genotypes differing at *Ppd-H1* at different ambient temperatures. We have identified floral homeotic and hormone responsive genes that were mis-regulated by high temperature in a *Ppd-H1* dependent manner. Further analysis on auxin reporter lines has shown that hormone homeostasis in the floral organs was affected by the interaction of *Ppd-H1* and high ambient temperature. These datasets and findings provide a valuable resource for future investigations into the complex regulation of inflorescence development in barley under high ambient temperatures.

P24

THE DNA GLYCOSYLASE MBD4L CONTRIBUTES TO DNA REPAIR AND PRESERVES SEED VIABILITY AFTER HEAT-INDUCED AGEING

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Germination represents a key developmental transition in plant lifecycle and is critical for seedling establishment. Seed desiccation, storage and imbibition are associated with high levels of reactive oxygen species (ROS) that results in oxidation of DNA bases and strand breaks. Moreover, heat shocks exacerbate ROS-induced genomic damage resulting in aged seeds with decreased vigor and viability. Consequently, DNA must be repaired prior germination to maintain genomic integrity and ensure cell survival and accurate transmission of genetic information. The base excision repair (BER) contributes to this end by using DNA glycosylases to excise damaged bases from the genome. The analysis of the expression pattern of *Arabidopsis* DNA glycosylases showed that MBD4L (*methyl-binding domain protein 4 like*) mRNAs strongly accumulate during seed desiccation, keeping a high abundance during first hours of imbibition. Moreover, MBD4L transcripts were strongly up-regulated during imbibition of seeds pre-incubated at 37°C or 45°C. We further analyzed germination phenotypes associated with the deficiency/ overexpression of MBD4L under control and heat stress conditions. Interestingly, *mbd4l* mutants showed late germination under basal conditions, and lost viability after heat treatments. These phenotypes were rescued or improved by overexpressing MBD4L in the *mbd4l-1* mutant background. Further expression analysis and comet assays showed that both BER system and DNA repair were significantly compromised in the *mbd4l-1* mutant. Our results suggest that MBD4L contributes to DNA repair required for proper seed germination, playing a major role in preserving seed viability after heat shock episodes.

P25

IS RIP₁, AN RSH₁ INTERACTING PROTEIN, INVOLVED IN PPGPP SIGNALING AND STRESS ACCLIMATION IN PLANTS?

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The unusual hyperphosphorylated nucleotide guanosine tetraphosphate (ppGpp) is a secondary messenger that plays a major role in growth rate regulation and stress acclimation in bacteria. ppGpp along with the bacterial RelA/SpoT Homolog (RSH) enzymes, that synthesize/hydrolyze ppGpp, are also present in the chloroplasts of plants. An equilibrium between the ppGpp synthesis and hydrolysis is necessary to regulate chloroplast gene expression, photosynthesis, plant growth and development under standard conditions. Studies have proposed that abiotic stresses and hormones induce ppGpp accumulation in the chloroplast. Recent findings from our group demonstrated that ppGpp is necessary for acclimation to nitrogen limitation in *Arabidopsis*. However, it is not known how ppGpp biosynthesis is activated in the chloroplast biosynthesis in response to nitrogen limitation or other stresses. In order to understand the mechanism behind the activation of ppGpp biosynthesis, we searched for target proteins that interact with the RSHs and thus may be involved in the regulation of ppGpp signaling. Using a Yeast Two-Hybrid screen we identified RSH₁ Interacting Protein 1 (RIP₁) that specifically interacts with the ppGpp hydrolase RSH₁ and not with the other RSH enzymes present in *Arabidopsis*. We confirmed the interaction by co-immunoprecipitation and BiFC (Bimolecular Fluorescence Complementation). Then we focused on investigating the physiological function of plant RIP₁ and the physiological relevance of the RIP₁/RSH₁ interaction. Understanding how ppGpp biosynthesis is regulated will allow us to better understand how plants are able to acclimate to environmental stress. This in turn will help in the development effective strategies to help crops become resilient to stress and less dependent on high nutrient inputs.

P26

THE THYLAKOID-MEMBRANE PROTEIN FIP IS INVOLVED IN THE ACCLIMATIVE RESPONSE OF PLANTS TO CHANGING LIGHT CONDITIONS.

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Plants are constantly exposed to environmental stress conditions, such as water and nutrients availability, temperature and light variance, etc. Consequently, plants need to adjust their photosynthetic processes to avoid photoinhibition and maximize yield. Photosynthetic acclimation involves the dynamic regulation of the biogenesis, folding, trafficking, and degradation of proteins. FIP belongs to DNAJ-related proteins, and was first described as a FtsH5 protease Interacting Protein, involved in abiotic stress response (Lopes et al., 2018). The absence of a conserved J domain in FIP, indicates the chaperone activity non-dependent of Hsp70, by substrate interaction and stabilization. Our results suggest that under low light conditions, FIP could act as a chaperone stabilizing the structure of PSII and could be related to the formation and organization of photosystems. Under conditions of high light intensity, FIP is down-regulated, allowing the repair system (e.g. FtsH protease) to function efficiently. Moreover, our results indicate also that FIP is crucial for efficient electron transport and excitation energy distribution between PSII and PSI. Thus, FIP can act as an important element of proteostasis network in thylakoids, optimizing photosynthesis under changing conditions.

Lopes KL. et al., (2018). The Zinc-Finger Thylakoid-Membrane Protein FIP Is Involved With Abiotic Stress Response in *Arabidopsis thaliana*. *Front Plant Sci.* 2018; 9: 504.

P27

INVOLVEMENT OF PECTIN MODIFICATIONS AND OLIGOGALACTURONIDES IN PLANT DEFENSE AGAINST APHIDS

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Aphids are phloem-feeding insects that transmit viral diseases in commercial crops, a major threat to world agriculture. The feeding strategy of aphids consists of the use of a slender stylet that probes the host tissues using intercellular spaces as the main pathways to reach the phloem. It is thought that pectin degradation facilitates stylet penetration through the cell walls. However, host plants may take advantage of this feeding mechanism since they have evolved to sense the byproducts of pectin degradation called oligogalacturonides (OGs) and trigger a defense response. OGs are well-known damage-associated molecular patterns (DAMPs) that enhance plant resistance against pathogens. However, whether plants use this defense mechanism against aphids is unknown yet. Here we show that pectin and pectin-modifying enzymes are significantly altered during the plant-aphid interaction. As a result, *M. persicae* infestation induced a significant increase in total PME and PL activities, concomitant with a decrease in the methyl-esterification degree of pectin. In addition, OGs treatments in *Arabidopsis* increase the resistance to *M. persicae* infestation by reducing their offspring number, settling preference, and feeding performance. This enhanced resistance was related to a high accumulation of ROS and callose deposits and an increase in the transcript level of genes related to the salicylic acid pathway, such as SARD1 and PR1.

This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico [ANID-FONDECYT regular 1210320] - Programa Iniciativa Científica Milenio - ICN17_022, NCN2021_010, and ANID PIA/BASAL FBO02 to F.B-H. [ANID-FONDECYT Postdoctorado 3200902] to C.S-S.

P28

ABA AS A VACCINE FOR BETTER DROUGHT TOLERANCE? - BARLEY PHYSIOLOGICAL AND TRANSCRIPTOME RESPONSE TO DROUGHT AT THE PRE-FLOWERING STAGE

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Considering the predicted increase in drought stress occurrence and its global impact, research about drought tolerance mechanisms in cereal plants is of utmost importance. Barley (*Hordeum vulgare*) is one of the leading and most widely adopted cereal crops worldwide. It is ranked fourth in terms of harvested acreage and production. Pivotal regulator of plant drought response and adaptation is abscisic acid (ABA) which is extensively synthesized in response to drought and its signalling leads to stress response. Here we asked a question whether the pre-treatment with ABA followed by drought stress in pre-flowering stage of development will act as a vaccine in terms of better drought adaptation in barley plants. The experimental set-up included 4 sets of plants batches growing under different conditions including: optimal water conditions, pre-treated with ABA and followed by drought and recovery, treated only with ABA, treated only with drought followed by recovery. Combining physiological and transcriptomic data we were able to discern molecular pathways underlying alterations that were observed during plant growth in different conditions. Clearly, we were able to demonstrate better photosynthetic protection under drought stress when plants experienced ABA treatment before drought exposure at both physiological and molecular level. Currently these data are analysed in terms of alternative splicing events that might be crucial for observed changes. The overall findings expand our knowledge related to ABA role under drought stress and will likely serve as a basis for deeper investigations.

This work was supported by the National Science Centre, Poland project SONATA BIS10 '(QUEST) Quest for climate-smart barley - the multilayered genomic study of CBC function in ABA signaling' (2020/38/E/NZ9/00346).

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LASER MICROIRRADIATION AS A TOOL TO STUDY DNA DAMAGE RESPONSE IN MODEL AND CROP PLANTS

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Genomes of plants are constantly subject to the effects of endogenous and exogenous genotoxic agents, which create DNA lesions that have to be resolved by the cells. DNA repair is a fundamental cellular physiological process that is influenced by environmental conditions. We have recently adapted a flexible experimental system, with which we can induce targeted DNA lesions in plant protoplasts, which can be extended to crop plants (Franek et al., 2022). This technique is based on transient transformation of plant protoplast with putative DNA repair proteins and laser irradiation of regions of interest in the nucleus of plant protoplasts with a 405 nm UV-laser (based on previous research in mammalian models; Dinant et al., 2007; Lan et al., 2005). With this technique, we can monitor the dynamics of DNA repair in plants and probe the effects of higher temperature or changes in ionic strength on the recruitment of DNA repair proteins to sites of DNA damage. While the implementation of this technique was tested in *Arabidopsis thaliana*, we are extending its functionality to crop plants, including *Medicago sativa*, *Hordeum vulgare*, *Triticum aestivum* or *Solanum Lycopersicon*. In a two-step process of the optimization of protoplast isolation and DNA damage induction, we aim to observe the dynamics and turnover of proteins facing adverse effects of extreme temperature, toxic compounds in the medium or transient UV-exposure.

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P30

A VACUOLAR ION CHANNEL IN *ARABIDOPSIS* GUARD CELLS INVOLVED IN THE CONTROL OF LEAF TRANSPIRATION

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Stomata are pores localised at leaf epidermis. They consist of a pore surrounded by a pair of specialised cells – the guard cells. Guard cells modify their volume and turgor pressure to tune the aperture of the stomata pore, allowing plants to control gas exchanges and water transpiration. This process, regulated by the perception of the environmental conditions, plays a significant role in plant adaptation to climate modifications. Indeed, it impacts on plant biomass production and tolerance to water stress. The regulation of the pore aperture is under the control of ions fluxes through the plasma and the vacuolar membrane. Our recent data showed that *AtALMT5*, a member of the aluminium activated malate transporter family, is a vacuolar anion channel mediating chloride and malate influx into the vacuole during stomatal opening. *Almt5* loss-of-function plants present reduced stomatal aperture in response to light. Our data introduce *AtALMT5* as a new player in the regulation of stomatal aperture and thus of leaf transpiration. Understanding its function in plant cells will allow to understand the importance of *ALMT5* for the adaptation to climate changes.

Keywords: Stomata, malate, *AtALMT5*, tonoplast, electrophysiology

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ACTIVATION OF AN ALTERNATIVE PATHWAY TO SUCROSE METABOLISM INCREASES PLANT TOLERANCE TO ABIOTIC STRESS

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Plants deal with abiotic stress by combining antioxidants with osmotic protection. Exposing storage organs such as potato tuber to cold stress causes a rise in the concentration of soluble sugars due to starch breakdown. In this sweetening process, sucrose is cleaved to hexose, mainly by *vacuolar acid invertase (StVInv)*, raising the osmotic concentration of the tuber parenchyma. The potato plant is tetraploid. CRISPR knock out of *StVInv* in potato protoplasts allows us to produce non-transgenic mutants with a various number of mutated alleles. Sugar analysis showed that the mutant tubers produce a much lower hexose concentration in response to cold stress and maintain low invertase activity. A dramatic phenotype occurred even if part of the alleles were mutated. To our surprise, the mutant tubers showed much lower lipid oxidation and lowered H₂O₂ levels in response to cold stress. Moreover, the mutant plants also displayed higher drought tolerance. Transcriptomic and biochemical analysis of the mutant tuber parenchyma during cold stress showed an increase in antioxidants and galactinol pathway. These findings show the relevance of the galactinol pathway as an alternative metabolic pathway leading to ROS detoxification, cold and drought tolerance. Mechanisms that can be used to improve plant osmoprotection during abiotic stress.

P32

**THE CRK₅ RECEPTOR-LIKE KINASE AFFECTS
TRANSPIRATION AND SALT TOLERANCE IN
ARABIDOPSIS**

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Receptor-like kinases (RLKs) constitute a large family of signaling molecules in plants, with more than 600 members in *Arabidopsis*. These membrane-localized proteins possess both external receptor domain and internal kinase domain, thus they are able to perceive various stimuli and proceed signaling pathways controlling growth and development. One of the largest subgroups of RLKs is formed by cysteine-rich receptor like kinases (CRKs). Reverse genetic screen of recessive mutants for the whole CRKs family revealed that especially one member, *crk5*, showed a striking phenotype manifested by retarded growth and accelerated leaf senescence. Interestingly, gas exchange analysis revealed that stomatal conductance was significantly higher in *crk5* plants compared to the wild type, whereas there were no differences in the assimilation rate, suggesting less efficient water management, indicated by lower water use efficiency in the mutant plants. In accordance with that the *crk5* mutant plants displayed greater susceptibility to osmotic stress, which was reverted in complementation lines. Our molecular analysis, based on yeast two hybrid library screening, identified 32 proteins interacting with CRK₅. Intriguingly, as many as six of these interactors were previously described in terms of their role in response to water deprivation or calcium signalling. Among the other identified CRK₅ interactors we found three proteins associated with cell wall modification, which might affect the composition of guard cell walls and regulate stomatal movements in response to abiotic stress. Taken together, the CRK₅ kinase arises as an important regulator of transpiration and salt tolerance in *Arabidopsis*.

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A VACUOLAR ION CHANNEL IN *ARABIDOPSIS* GUARD CELLS INVOLVED IN THE CONTROL OF LEAF TRANSPIRATION

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Stomata are pores localised at leaf epidermis. They consist of a pore surrounded by a pair of specialised cells – the guard cells. Guard cells modify their volume and turgor pressure to tune the aperture of the stomata pore, allowing plants to control gas exchanges and water transpiration. This process, regulated by the perception of the environmental conditions, plays a significant role in plant adaptation to climate modifications. Indeed, it impacts on plant biomass production and tolerance to water stress. The regulation of the pore aperture is under the control of ions fluxes through the plasma and the vacuolar membrane. Our recent data showed that *AtALMT5*, a member of the aluminium activated malate transporter family, is a vacuolar anion channel mediating chloride and malate influx into the vacuole during stomatal opening. *Almt5* loss-of-function plants present reduced stomatal aperture in response to light. Our data introduce *AtALMT5* as a new player in the regulation of stomatal aperture and thus of leaf transpiration. Understanding its function in plant cells will allow to understand the importance of *ALMT5* for the adaptation to climate changes.

Keywords: Stomata, malate, *AtALMT5*, tonoplast, electrophysiology

P34

THE GUANDINE EXCHANGE FACTOR 14 (GEF14) IS A SPECIFIC ACTIVATOR OF ROP6/RBOHS COMPLEX FOR THE OSMOTIC SIGNALING

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Plant responses to water stress, particularly to osmotic signals, have been largely described. This is including long term physiological and developmental acclimation; but also fast and reversible changes like modification of stomatal conductance or the regulation of root water permeability (Maurel et al., 2015; Afzal et al., 2016). Surprisingly, molecular actors that control plant osmotic perception and early signaling are still poorly understood (Nongpiur et al., 2020). Reactive oxygen species (ROS) are accumulated in cells minutes after osmotic stress perception. It acts as a secondary messenger to activate plant responses (Boursiac et al., 2008; Martinière et al., 2019; Smokvarska et al., 2020). In root, we found that a single isoforme of Rho Of Plants (ROPs), ROP6 is necessary and sufficient to trigger osmotically-induced ROS. Upon an osmotic stimulation, ROP6 is activated and accumulates in plasma membrane nanodomains together with RBOHD and F, that are two key enzymes for ROS production in plant cells. Whereas ROP6/RBOHs nanodomains formation trigger osmotic signaling, ROP6 is also required for others processes like auxin or pathogen signaling (Platre et al., 2019). Here, we search for ROP6 activators that can convey specifically osmotic signal. By a reverse genetic screen, we found that the Guanine exchange factor 14 (GEF14) is needed for ROS accumulation after osmotic stimulus but not for other signals. Based on genetic evidences and FRET sensors experiments, we show that GEF14 is an activator of ROP6 in planta. We further describe the effect of GEF14 on ROP6 and RBOH nanodomains organization. Our results suggest that the activation of a specific GEF protein can determine early steps of osmotic signaling in plant. This concept will be extended to other ROP6 dependent signaling pathway to understand how protein membrane organization can determine signaling processes in plant.

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P35

A GENETIC AND MOLECULAR APPROACH TO IDENTIFY TRANSCRIPTION FACTORS CONTROLLING MAIZE ROOT ADAPTIVE RESPONSE TO WATER DEFICIT

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Water stress is recognized as the most severe abiotic stress for agricultural productivity. Root traits play a key role in tolerance to water stress but have largely been neglected in selection schemes. In order to identify the maize genetic bases of the root adaptive responses to water deficit (WD), we used a MAGIC mapping population of 400 lines based on the intercrossing of 16 genotypes. The fine phenotyping of the different genotypes was performed under contrasting water supply on the French root phenotyping platform (4PMI). On the 16 founder genotypes, in addition of phenotyping, we sampled different root tissues daily over 7 days after irrigation arrest and performed RNAseq. On the basis of these 448 transcriptomes, we identified 6945 differentially expressed genes between axial and lateral roots and in response to WD and inferred a regulatory gene network to identify transcription factors (TF). Using a hierarchical clustering, we split the network in 35 clusters homogeneous in their expression pattern. Fine analysis of individual cluster pointed out, without prior knowledge, already known FTs responding to WD and identified new candidates. Functional validation of Arabidopsis orthologues has been initiated and many genotypes have an altered root developmental response to *in vitro* osmotic stress. In parallel, the phenotyping and a transcriptomic analysis by RNAseq of the genotypes of the mapping population under optimal conditions and water deficit enabled a GWAS and an eQTL analysis. Both approaches identified polymorphisms in genes of interest and identified SNPs colocalizing near transcription factors also identified by the gene network approach. Taken together all the data identified candidate genes and alleles potentially controlling adaptive root development that can be interesting target for breeding.

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DIFFERENTIAL PHYSIOLOGICAL, TRANSCRIPT AND METABOLIC RESPONSES OF WINTER WHEAT GENOTYPES TO DROUGHT STRESS

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Globally, wheat yield is threatened by adverse climate conditions, mainly drought and heat. This study investigated physiological, transcriptional and metabolic changes in three winter wheat genotypes (Turandot, Midas and Fakir) to drought with emphasis on the differential response affected by treatment, genotypes or both. A continuous drought was applied by withholding water to gradually dry until wilting point, where the soil moisture reduced to <30%. Dynamic quantification of side projected leaf area and chlorophyll fluorescence kinetics showed gradual growth reduction and declined photosynthetic performance, respectively, along with decreased CO₂ assimilation, transpiration rate, stomatal conductance and yield due to drought. The negative effects of drought were most pronounced in genotype Turandot, while genotypes Midas and Fakir were able to retain better physiology and yield as compared to their respective controls. RNA sequencing revealed changes in the transcriptional response of drought stressed leaves in all the genotypes. In total, 5493 drought-responsive differentially expressed genes (DEGs) were identified, belonging to critical biological functions. The categories like carbohydrate metabolism and protein phosphorylation process were enriched across genotypes. The genotype-specific DEGs represented by the ketone, urate and fatty acid metabolism, oxidation-reduction process and transmembrane transport processes. Further, metabolomic analysis reflected on major differences in metabolite composition between the treatments. Drought impacted the metabolites of the citrate cycle, amino and nucleotides while the phenolics were more affected by genotype. Overall, we have identified genes and metabolites that function in a common response mechanism against drought stress while others as a part of genotype-specific response responsible for variable drought tolerance characteristics.

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IMPACT OF HIGH CO₂ LEVELS AND HEAT WAVES ON VIRAL INFECTION/RESISTANCE: *PHASEOLUS VULGARIS* AS A MODEL PLANT

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One of the major issue of this century is the impact of climate change, notably on crop cultures. Experts of climate change have forecast an increase in atmospheric CO₂ level from 400 $\mu\text{L.L}^{-1}$ in 2014 to above 1000 $\mu\text{L.L}^{-1}$ in 2100 in the worst case scenario, as well as a raise of 3.3-5.7°C in temperature (IPCC, 2021). Accordingly, heat waves will increase in frequency and intensity (IPCC, 2021). Plants are directly impacted by these changes as well as pathogen populations including viruses (Jones, 2009). In that context, an important question is to what extent the increase in CO₂/temperature will affect plant-virus interactions, whether susceptibility or resistance? The objective of our work is to study the impact of high CO₂ level and heat waves on viral susceptibility/resistance using common bean (*Phaseolus vulgaris* L.) as a model plant. Indeed, many viruses infect common bean including Bean common mosaic virus (BCMV, *Potyvirus*) and Bean pod mottle virus (BPMV, *Comovirus*) (Meziadi *et al.*, 2017 for review). Here, we use the *P. vulgaris*/BPMV pathosystem to make preliminary experiments on the impact of high CO₂/heat waves on the level of infection/resistance to BPMV.

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CHLOROPLAST PROTEIN IMPORT DETERMINES PLANT PROTEOSTASIS AND RETROGRADE SIGNALING

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Only a few prion-like proteins with polyglutamine regions (polyQ) are characterized as molecular sensors that integrate internal and external cues contributing to plant striking ability to adapt to ever-changing environments. In humans, mutations in genes that translate into an abnormal expansion of the polyQ tract of a protein cause distinct pathologies. For instance, Huntington's disease is caused by an abnormal expansion of the polyQ tract of Huntingtin (HTT) protein. PolyQ-expanded HTT (> Q35) is prone to aggregation, leading to proteotoxicity and subsequent neurodegeneration. Here, we ask how plants maintain the proteostasis of prion-like proteins containing polyQ regions, which are intrinsically enriched in the plant proteomes. To this end, we overexpressed the polyQ aggregation-prone fragment of human HTT (Q69) in plant cells. In contrast to other invertebrate and mammalian transgenic models, we find that *Arabidopsis thaliana* plants suppress Q69 aggregation. This elevated proteostasis ability is mediated through the import and degradation of Q69 in chloroplasts. Conversely, inhibition of chloroplast protein import either genetically or pharmacologically reduces the capacity of plant cells to prevent Q69

aggregation. We find that Q69 interacts with the stromal processing peptidase (SPP) in chloroplasts. Notably, ectopic expression of synthetic *Arabidopsis* SPP is sufficient to suppress Q74 aggregation in human cells, supporting a role of chloroplast proteases in proteostasis of polyQ-expanded proteins. Similar to ectopically expressed Q69, endogenous polyQ-containing proteins aggregate in *Arabidopsis* upon inhibition of chloroplast import. Among them, the plastid casein kinase 2 (pCK2), a protein involved in chloroplast-to-nucleus (i.e. retrograde) signaling that localizes both in plastids and nuclei, contains a polyQ-prion-like domain next to the chloroplast targeting peptide. Upon inhibition of chloroplast import, pCK2 forms diamond-shaped amyloid-like fibrils surrounding the chloroplasts and accumulates at higher levels in the nucleus. Thus, our results indicate that the differential conformation and redistribution of pCK2 to the nucleus depends on chloroplast import efficiency. Our findings on plant proteostasis mechanisms can open new avenues towards the use of plant proteins to generate stress-resistant plants and to target Huntington's disease and other incurable diseases that involve protein aggregation.

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GENOME WIDE ASSOCIATION STUDY IDENTIFIES CANDIDATE GENES INVOLVED IN THE MINERAL DEPLETION OF *ARABIDOPSIS THALIANA* UNDER ELEVATED CO₂

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In the decades to come, atmospheric CO₂ concentration is expected to steadily increase. This comes as a serious threat to food security, as the ionome of C₃ plants declines when exposed to high CO₂ conditions [2]. Even though several physiological hypotheses have been put forward, the reasons behind this mineral depletion are still largely unclear. Recent studies examined several genetically diverse lines of cultivated rice and concluded that genetic diversity could be a source of variability in iron, zinc, and protein changes under elevated CO₂[3]. To identify polymorphisms underlying such intra-specific diversity could not only be a way to understand this response, but also to breed more resilient crops. In this work, we screened three populations of *Arabidopsis thaliana* ecotypes, originating from local, regional and world-wide geographic scales, and characterized the phenotypic variability observed in their ionome response to high CO₂. We could confirm a global decline of mineral status happening jointly with an increase in carbon content, and identify a subset of more tolerant lines with a preserved nutrient content. We further performed GWAs on mineral elements under elevated CO₂ for the REGMAP accessions [1], based on linear mixed models. Our analysis brought out haplotypes of interest, and candidate genes for the control of iron and nitrogen accumulation in *Arabidopsis* leaves under elevated CO₂.

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ROOT TRANSCRIPTOME AND CO-EXPRESSION NETWORKS FOR THE IDENTIFICATION OF CANDIDATE GENES AT MAJOR QTLs FOR ROOT GROWTH ANGLE IN DURUM WHEAT

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Root systems architecture (RSA) is crucial for crop performance, particularly under non-optimal water and nutrients supply conditions. Root ideotypes that facilitate water or nutrient absorption and their use efficiency have been proposed to enhance crop stress resilience. However, knowledge of genes, developmental programs and regulatory circuits governing these ideotypes in crops is largely missing or not ready to be exploited in breeding. Seven QTLs controlling root growth angle (RGA) were identified in durum wheat (*Triticum turgidum* ssp. *durum*) combining linkage mapping and association mapping (Maccaferri et al., 2016). RGA determines the direction of root growth and narrow RGA generally results in deeper root systems that could better capture water and nitrogen in deep soil layers. This study reports new results on the genetic control of RGA based on a wider genome wide association study and integrated with RNA-Seq and Gene Coexpression Network (GCN) analyses. Transcriptome analysis was performed on root tips (including root cap and meristematic region), elongation and transition/maturation zones. Roots were collected from four shallow RGA and four narrow RGA genotypes, carrying contrasting haplotypes at the three major QTLs confirmed by an improved GWAS pipeline applied to a collection of 755 accessions of tetraploid wheat. Differentially expressed genes (DEGs) showing RGA-related expression levels and mapping in the RGA-QTL intervals resulted involved in auxin signalling, melatonin biosynthesis, hormone signalling and cross-talk and cell wall biosynthesis. These candidate genes were predicted as hub genes in modules associated to auxin-mediated root development. Complete analysis of co-expressed genes and regulatory networks associated to wide or narrow RGA in durum wheat are underway, with preliminary results pointing to a main role for abscisic acid signalling components.

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GENETIC AND EPIGENETIC CONTROL OF HEAT STRESS MEMORY IN *CYANIDIOSCHYZON MEROLAE*

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Plants can memorize non-lethal doses of stress and are thereby primed to tolerate stronger stress doses in the future. This applies to various types of stressors including heat. The small genome and short reproduction time of the thermophilic unicellular red alga *Cyanidioschyzon merolae* makes it an excellent model organism to study heat stress (HS) memory at a genetic and epigenetic level. *C. merolae* grows optimally at 42 °C, but can survive temperatures below 60 °C. We revealed that *C. merolae* cells can be primed at a sub-lethal temperature to survive a triggering HS at 60 °C. It thus acquires a HS memory, which is mitotically stable to a certain extent. To discover the mechanisms that underlie HS memory, we performed a comparative transcriptome analysis of algae that experienced either the priming or the triggering stimulus, or a combination of both. We showed that the establishment of HS memory is orchestrated by the differential expression of several nuclear and plastidic HS trainable genes. In addition, we performed a genetic screening revealing that both epigenetic and RNA processing mechanisms, particularly splicing, are required for HS memory. In the future, we will make use of forward and reverse genetic techniques to identify further regulators of stress memory. Overall, our results provide a view how phototrophic organisms adapt to rising temperatures, a topic that gains interest in times of changing climate conditions.

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FUNCTIONAL CHARACTERIZATION OF GENES INVOLVED IN IODINE CONTENT REGULATION IN PLANTS

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Iodine is a scarce yet essential element in human diet, acting as a key component of the thyroid hormones. Insufficient iodine intake results in various Iodine Deficiency Disorders (IDDs). The prophylactic consumption of iodized salt has contributed, but not enough, to mitigate IDDs. Alternatively, the development of iodine biofortified crops has become a promising strategy to solve iodine deficiency. In plants, iodine is not considered a nutrient, but it is beneficial for plant growth and the response to environmental stress. Surprisingly, the molecular mechanisms of iodine regulation in plants are not understood. Based on a comparative genomics strategy, we have established several parallelisms for iodine regulation between plants and non-plant organisms. Here, we will explore a gene family involved in the homeostatic control of iodine in plants. First, a phylogenetic overview of this gene family will highlight the degree of conservation in plants. For a functional genomics analysis, *Arabidopsis* T-DNA insertion mutants and overexpression lines will be established. A developmental map of these lines will be performed and iodine will be quantified. Moreover, *Arabidopsis* seedlings will be subjected to iodine excess and depletion. The impact of these treatments will be studied by analyzing morphology/physiology parameters and expression of stress-related marker genes. Using promoter::GUS report assays, we will establish the spatial expression patterns and response to iodine treatments.

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INTEGRATIVE OMIC APPROACHES FOR FUNCTIONAL ANALYSES OF ABIOTIC STRESS RESPONSIVE PATHWAYS IN PLANTS

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The impacts of global climate changes have been spreading all over the world for several decades, leading to environmental changes such as global warming, high salinity, drought, and floods to name a few. These drastic changes greatly affect the developmental processes of all living organisms, especially sessile organisms like plants, forcing them to quickly adapt to the stresses, or otherwise face a great risk of extinction. Soil salinity is considered as one of the most concerning problems for worldwide agriculture, as approximately 1,125 million hectares of arable land is affected by high salinity, making it unsuitable for agriculture. In this study, we explored new candidate genes involving in salt-responsive pathways in rice cultivars, by investigating multiple quantitative trait loci mapping datasets of the Chromosome Segment Substitution Line (CSSLs) populations, to find the common and unique salt-responsive QTLs related to different physiological traits involving salt stresses in rice. We also gathered and combined publicly available transcriptomic datasets, and asked whether the salt-related candidate genes identified by the CSSL studies tend to show differential expression characteristics in the salinity conditions, as compared to the controls. New transcriptomic data of CSSL parents after being treated with salt stress have been obtained. The candidate genes that are linked to salinity were validated using the mutants of their orthologous genes in *Arabidopsis* in order to investigate the functions in other plant species. We envisage that this work will contribute new insights and salinity-related candidate genes and their potential functions in mitigating the stresses.

Keywords: Quantitative Trait Loci (QTL) mapping, Chromosome Segment Substitution Line (CSSL), multi-omics, salinity stress

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A SYSTEM BIOLOGY APPROACH TO IDENTIFY GENES INVOLVED IN CROWN ROOT DEVELOPMENT IN RICE.

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The root system of cereals is mainly composed of shoot borne crown roots. To identify new master regulatory genes involved in crown root development in rice, we developed a systems biology approach to determine the gene regulatory network involved in crown root formation in rice. An original crown root inducible system based on the inducible expression of *CROWN ROOT LESS 1 (CRL1)* gene in the *crl1* mutant background was engineered in rice (1) and used to acquire transcriptomic kinetic data during the early steps of crown root formation (2). A gene regulatory network was inferred from these data using the TDCor algorithm (3) and topological and dynamical analysis (4) of the gene regulatory network were conducted to identify key genes corresponding to the major hubs of this network. We developed CRISPR-cas9 mediated knock-out mutation in the identified genes. The plants mutated in eight of the nine selected genes showed a modification in crown root number. In addition KO or Over-expression plants for some of these genes presented a modification of their root architecture. The impact of these root architecture modifications on the capacity of rice mutant plant to better tolerate water deficit is under investigation.

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P45

MOLECULAR AND GENOMIC CHARACTERIZATION OF THE *PSEUDOMONAS SYRINGAE* PHYLOGROUP 4: AN EMERGING PATHOGEN OF *ARABIDOPSIS THALIANA* AND *NICOTIANA BENTHAMIANA*

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Environmental fluctuations such as increased temperature, water availability, and air CO₂ concentration triggered by climate change influence plant disease dynamics by affecting hosts, pathogens, and their interactions. Here, we describe a newly discovered *Pseudomonas syringae* strain found in a natural population of *Arabidopsis thaliana* collected from the southwest of France. This strain, called *Psy* RAYR-BL, is highly virulent on natural *Arabidopsis* accessions, *Arabidopsis* model accession Columbia 0, and tobacco plants. Despite the severe disease phenotype caused by the *Psy* RAYR-BL strain, we identified a reduced repertoire of putative Type III virulence effectors by genomic sequencing compared to *P. syringae* pv *tomato* (*Pst*) DC3000. Furthermore, *hopBJ1_{Psy}* is found exclusively on the *Psy* RAYR-BL genome but not in the *Pst* DC3000 genome. The plant expression of HopBJ1_{Psy} induces ROS accumulation and cell death. In addition, HopBJ1_{Psy} participates as a virulence factor in this plant-pathogen interaction, likely explaining the severity of the disease symptoms. This research describes the characterization of a newly discovered plant pathogen strain and possible virulence mechanisms underlying the infection process shaped by natural and changing environmental conditions.

This work was supported by the Fondo Nacional de Desarrollo Científico y Tecnológico [ANID-FONDECYT regular 1210320] - Programa Iniciativa Científica Milenio - ICN17_022, NCN2021_010, and ANID PIA/BASAL FB0002 to F.B-H. [ANID-FONDECYT Iniciación 11200944] to A.H-V

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BRANCHING OUT IN NEW DIRECTIONS: INTEGRATION OF ENVIRONMENTAL AND DEVELOPMENTAL SIGNALS TO MODIFY ROOT ARCHITECTURE

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Root plasticity helps the plants to change root system architecture according to the intrinsic and extrinsic cues and maximize the absorption of water and mineral nutrients. Lateral roots (LRs) are often maintained at specific angles with respect to the gravity vector, a quantity called gravitropic set-point angle (GSA) that serves as a crucial adaptation. Abiotic stresses affect root branching wherein plant roots direct their growth away from stressful conditions by affecting local auxin levels and towards more optimal soil micro conditions. Recent studies have identified GSA to be highly auxin dependent. However, currently, not much is known about the role of other phytohormones and their interaction with auxin in controlling GSA. Our study has identified the role of methyl jasmonate (MeJA) in altering the branching angle of *Arabidopsis* LRs. MeJA inclines LRs to a more vertical orientation and this response is found to be dependent on JAR1-SCF^{COL}-MYC2, 3 and 4 signalling. We also demonstrate that light perception through phytochrome A and B (PHYA and PHYB) and the downstream transcription factor LONG HYPOCOTYL5 (HY5) are necessary for inducing vertical angle by MeJA. Light indirectly via photosynthesis may also lead to the production of sugars. In this study, we have found that Glucose (Glc) induces wider branching angles and antagonizes the MeJA response via TARGET OF RAPAMYCIN (TOR) signalling pathway and by stabilizing JAZ9 protein. Moreover, physiological and cellular assays using auxin mutants and *DR5::GFP* and *PIN2::GFP* pinpointed the role of an intact auxin machinery required by MeJA for vertical growth of LRs. Thus, this study highlights the intimate connections between environmental and phytohormone signals and dual role of light to optimize branching angle which is a key determinant of foraging capacity of plants. The present study in *Arabidopsis* can be used for crop improvement strategies via the manipulation of LR growth angle.

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MORPHOLOGICAL AND MOLECULAR PHENOTYPES OF TOMATO CULTIVARS IN RESPONSE TO A HEAT WAVE

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Demographic growth and global warming are two of the major issues that humanity will have to face in the next decades. In the last years, there was an exceptional number of extreme heat waves around the world, which caused severe damages to ecosystems, human society and crop production. There is thus a strong need to design new strategies to maintain high yields of fruits and vegetables produced in harsh temperature conditions. Tomato is one of the most produced and consumed fruit in the world. In years with a very warm summer, remarkable losses occur in tomato yield, due to a decreased fruit set, leading to small fruits of low quality. Pollen development seems to be among the most temperature-sensitive process within the life cycle of plants, and its inhibition results in poor fertilization and reduced fruit and seed yield. By using Tomato as a model for fleshy fruit crop, we studied the effect of elevated ambient temperatures on flower development in laboratory and commercial tomato cultivars. We compared their response at morphological, cytological and molecular level. This analysis highlighted different strategies that tomato cultivars set-up for facing heat waves.

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AMBIENT TEMPERATURE AND PLANT DEVELOPMENT: A VIEW FROM THE SM CORE ASSEMBLY

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Splicing is a co-transcriptional process thanks to which plants react quickly to external changes by modulating the identity of the mature RNAs available in the cell. Thus, it is not surprising that many splicing related genes have been linked to the response to abiotic and biotic stresses, with mutants showing an unpaired ability to properly respond to stimuli compared to the wild type (Dikaya *et al.*, 2021). This is the case of SmEa, also named PORCUPINE and orthologue of human SNRPE and yeast SME, whose mutants show a wildtype phenotype when grown at 23°C but important developmental defects at 16°C (Capovilla *et al.*, 2018; Huertas *et al.*, 2019). SmEa is part of the heptameric Sm-ring, the core of the SnRNP spliceosome structure. In metazoan, the assembly of the Sm-ring is not spontaneous, but it proceeds in steps and it is assisted by multiple factors, members of the methylosome and the SMN-complex (Matera and Wang, 2014). Trying to shed light on the link between splicing and abiotic stress, we decided to specifically focus on the Sm-ring biogenesis to understand whether this process is involved in the response to temperature. So far, only two components of the methylosome and the SMN-complex have been described in plants, PRMT5 and GEMIN2, respectively. Interestingly, GEMIN2 mutants have been already reported to show a temperature-related phenotype (Schlaen *et al.*, 2015). We aim at understanding if other proteins exist in *Arabidopsis* that could be involved in the Sm-ring assembly and if this stepwise process is overall modulated by the temperature. Preliminary results from genetics, molecular and biochemical approaches suggest that high ambient temperatures help the plant to overcome defects in the Sm core assembly.

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STRUCTURE IN THE DISORDERED - THE PLANT THERMOSENSORY PROTEIN EARLY FLOWERING 3 FORMS ORDERED BIOMOLECULAR CONDENSATES

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The increased average temperatures and prolonged periods of extreme heat due to global warming have already altered plant phenology for both wild and domesticated species, presenting a critical challenge for food security in the coming decades. How plants are able to perceive and respond to temperature is a fundamental question that is still not fully answered. One important mechanism that allows a fast response to temperature stimuli is the formation of dynamic liquid-liquid phase separated (LLPS) states. LLPS is often guided by proteins with intrinsically disordered regions and low complexity prion-like domains (PrD). Recently we demonstrated that EARLY FLOWERING 3 (ELF3), a PrD containing protein, acts as a direct temperature sensor via LLPS. We use a combination of *in vitro* biophysical and structural techniques to investigate the dilute and condensed phases of ELF3 PrD in order to investigate the biophysical characteristics of the condensates.

We demonstrate that the length of the polyQ repeat in the PrD in ELF3 alters the properties of the condensate as shown by scattering techniques and fluorescence and atomic force microscopies. Furthermore, we demonstrate that the condensed phase assumes a semi-ordered structure as determined by small angle scattering, electron microscopy and X-ray diffraction. The ability of ELF3 to toggle between active and inactive states through phase transition represents a novel thermosensory mechanism and here we describe the underlying biochemistry and biophysics of this process.

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AMELIORATION OF DROUGHT STRESS BY APPLICATION OF BETAINE ENCAPSULATED CHITOSAN NANOPARTICLES IN MAIZE (ZEA MAYS L.)

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Water deficiency results in drought stress that severely affects crops productivity, thus considered as one of the major contributors to food insecurity worldwide. Maize is one of the major cereal crops, but its growth and production are also negatively affected due to drought stress in most parts of the world. The characterization of biodegradable betaine encapsulated chitosan nanoparticles (GB-CNPs) and chitosan nanoparticles alone (CNPs) was performed through UV–vis spectrophotometry, scanning electron microscopy, dynamic light scattering (DLS) and Fourier-transform infrared spectrometry. We analyzed the role of foliar application of glycine betaine encapsulated chitosan nanoparticles (GB-CNPs) and chitosan nanoparticles (CNPs) alone in the amelioration of drought stress in maize. To meet the objectives, we studied the physiological, biochemical, and genetic responses of nanoparticles in maize varieties HNG and CZP132001 grown under drought stress. Initially, a screening experiment was performed in petri plates to select the meaningful treatment levels of drought (5%,10%, and 20%) and nanoparticles (10ug NPs,20ug NPs, and 40ug NPs). For further experiment 20% drought stress, 20 ug NPs glycine betaine encapsulated chitosan nanoparticles (GB-CNPs) and 20 ug NPs chitosan nanoparticles (CNPs) alone were selected for pot experiment to study the physiological, biochemical, and molecular parameters under drought stress and nanoparticles treatments. Drought stress significantly reduced fresh and dry weight, relative water content, root and shoot lengths, photosynthetic pigments, antioxidant enzymes activity such as catalase, superoxide dismutase, ascorbate peroxidase, and proline content as compared to control plants. Moreover, foliar application of GB-CNPs increased photosynthetic pigments, antioxidant enzymes activity, and proline content, under both well-watered and drought-stressed conditions. However, glutathione and MDA contents were significantly

decreased by the foliar application of GB-CNPs and chitosan nanoparticles alone under stress conditions. Gene expression analysis revealed that foliar application of GB-CNPs upregulated *ZmAPX*, *ZmCAT*, and *ZmSOD* genes under drought stress. Our results showed that GB-CNPs have shown to be more effective than CNPs alone. Moreover, the positive and effective response of Maize to GB-CNPs under drought stress conditions causes to consider the GB-CNPs as a potential protective agent to enhance plant growth and mitigate drought stress which will be an innovative prospect for its use in agriculture.

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MECANO-ECO-SENSING: SENSING WATER AVAILABILITY THROUGH MECHANICS

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While the responses of plants to drought are well documented, the way they assess the amount of water in their environment is poorly understood. Mechanically speaking, water availability is strongly linked with the osmotic pressure and thus cell wall tension. Plant cells must perceive this tension to reinforce their cell wall accordingly, and maintain their mechanical integrity. Here, we find that the receptor-like kinase FERONIA (FER) is required for the mechanical integrity of the cell (1). The *feronia* mutant presents burst cells and small cotyledons, two phenotypes that can be partially rescued by reducing water potential and tensile stress levels. To investigate the contribution of FER in mechanical integrity, we propose new tests integrating cell wall properties and the regulation of osmotic pressure. The pleiotropic role of FER, both in *Arabidopsis* and in crop species, could be explained by the role of FER in the regulation of water management. If FER perceives cell wall tension, and consequently water availability, then it could integrate multiple signals in a mechanotransduction pathway and play a broader role in the perception of the environment (mechano-eco-sensing).

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INVESTIGATING THE eCO_2 IMPACT ON ROOT SYSTEM ARCHITECTURE AND NO_3^- ASSIMILATION IN *ARABIDOPSIS THALIANA*

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Since the Industrial Revolution, the CO_2 concentration in the atmosphere has increased from 280 ppm to more than 400 ppm today. Elevated atmospheric CO_2 (eCO_2) can lead to increased biomass for many C_3 species (the so-called 'CO₂ fertilization' effect). However, an unexpected outcome is that increases in biomass under eCO_2 is associated with a lower plant nutritional quality. The reasons and mechanisms underlying the negative impact of eCO_2 on the mineral nutrition of C_3 plants are still unclear. One hypothesis is that eCO_2 could lead to a decrease in the efficiency of mineral acquisition by plant roots. Compared to the huge amount of data on the responses of the shoots to eCO_2 , only a fragmentary knowledge is available about how eCO_2 affects root physiological and developmental processes, and even less under nitrate (NO_3^-) starvation. Our project aims to address two hypothesis to explain the plant mineral status (especially nitrogen N) decline under eCO_2 . Our main results showed that: i) Increase in shoot biomass in response to eCO_2 is independent of NO_3^- condition but leads to a huge decrease in N levels in plants grown on 0,5 mM NO_3^- ii) Gene of NO_3^- absorption and assimilation enzymes are affected by eCO_2 especially under very low NO_3^- condition suggesting an important role of eCO_2 on the high affinity transport system iii) The root system architecture is stimulated in response to eCO_2 with an increase in primary root growth and a lateral root density in low NO_3^- condition. These results suggest that eCO_2 promoted root morphological development and expansion, implying that the sensitive responses of root morphology and function to eCO_2 would increase long-term belowground C sequestration but cannot be an explanation for the reduced efficiency of root nutrient uptake.

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REDOX CONTROL OF ARABIDOPSIS ROOT DEVELOPMENT ADAPTATION TO ENVIRONMENTAL CONSTRAINTS VIA THE GLUTAREDOXIN ROXY19

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The plant root system plays pivotal functions in soil foraging and water and nutrients supply to the whole organism. Plants survival and fitness under unfavorable growth conditions therefore highly depends on the root system adaptability that relies on its developmental plasticity via the combinatorial modulation of undetermined growth and branching. Glutaredoxins (GRXs) are generally known as thiol-disulfide proteins involved in the control of reactive oxygen species homeostasis, which is critical for plant survival and adaptation in response to changing environmental conditions. They also modulate the activity of several enzymes implicated in plant development, although quite little is known about their functions in root development. The ROXY family is a land plant-specific family of GRX (classIII GRX), composed of 21 members in *Arabidopsis*. All members harbor a Cys-Cys (CC) putative active site, and a L**LL motif important for binding to TGA transcription factors. Some of the ROXY also share a terminal ALWL motif important for binding to the TOPLESS (TPL) and TOPLESS RELATED (TPR) transcriptional co-repressors, which leads to ternary ROXY/TGA/TPL complexes. This suggests an important role of this family in transcriptional regulation of downstream pathways. Although mainly expressed in roots, ROXY19 has never been implicated in root development so far. In this work, we show that ROXY19 controls in opposite ways primary and lateral root growth in *Arabidopsis thaliana*, by modulating cell elongation. We also report that ROXY19 is required for proper root development responses to Abscisic Acid (ABA) treatments, thus suggesting a key role for this GRX in root system adaptation to water availability. A detailed transcriptomic analysis has been conducted to better understand the molecular mechanisms enabling ROXY19 to fine tune root system architecture under optimal conditions and in response to ABA.

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EXPLORING THE IMPACT OF DROUGHT STRESS ON BARLEY *HVERA1* MUTANTS PHENOTYPE

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Drought stress adversely affects plant growth and productivity and severely hinders agricultural production worldwide. An important focus of plant breeding is to engineer more water use-efficient plants to improve crop performance under water-limiting conditions. ERA1 (Enhanced response to ABA1) mediates protein farnesylation to promote the interaction between the modified signaling protein and membrane lipids and/or other proteins, and acts as a negative regulator of ABA signaling. It has been shown in several species that plants lacking functional ERA1 show increased tolerance to drought stress. In this study, we subjected to drought stress two barley TILLING *HvERA1* mutants, *hver1.b* and *hver1.d*, and measured stomatal conductance and photosynthetic parameters in control and drought treated plants. In addition, samples for transcriptome analysis by RNA sequencing were harvested. Our results showed that both studied mutants were more drought resistant and had more efficient photosynthesis performance compared to the WT. Our transcriptome analysis suggests that ERA1 is involved in the regulation of photosynthesis pathway as we previously published. Analysis of chloroplast ultrastructure revealed that the *hver1* mutants subjected to drought stress had smaller plastoglobuli compared to WT. Furthermore, to confirm the drought resistant phenotype of ERA1, we have developed introgression lines for our TILLING mutants in different high yielding barley varieties (e.g. Barke, Golden promise, RGT Planet) and subjected them to drought stress. We have also confirmed ERA1 role in very early response to rapid dehydration stress. Together, these results indicate the possible mechanism of the primary cause of the observed alterations in the *hver1* mutant.

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PLASTICITY OF ROOT AND ROOT HAIR DEVELOPMENT IN RESPONSE TO NUTRIENT DEFICIENCY AMONG MODERN AND ANCESTRAL WHEAT GENOTYPES

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Reducing the ecological costs of conventional agriculture, including fertilization and irrigation, is a major objective of research in plant science.

High-throughput phenotyping of root traits as predictors of crop performance under limited water and nutrient availability are now available but these still often ignored root hairs (RHs), although these cells provide a major contribution to plant nutrient, water uptake and interactions with soil microbes.

We have developed a non-destructive root procedure to phenotype wheat RHs development on whole root systems. A modern wheat variety *Triticum turgidum* ssp. *durum* cv. Oued Zenati and an ancestral wheat variety *T. t. L. spp. dicoccum* cv. Escandia. Current experiments aim at investigating the root system and root hair development and their responses to nutrient availability. The developed methodology allows for instance to analyze on a single scan root system and RH developmental responses to low P or low K conditions. Analysis of root images revealed that nutrient shortage resulted in an increase of root area that was mainly due to a large increase of root hair area. The root hair contribution to the increase of total root area upon Pi deficiency was especially significant in *dicoccum* seedlings

Such analyses pave the way to comparison of wheat genotypes that recapitulate wheat domestication in different abiotic conditions (nutrient availability) and in presence or absence of PGPR bacteria

P56

RNA STRUCTURE DISCOVERY AND FUNCTION IN PLANT ADAPTATION TO ELEVATED TEMPERATURES

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Rises in global average temperature is a major consequence of climate change, with significant impact on plant development, growth, and defence. However, our understanding of how plants perceive and adapt to elevated temperatures is still recent and far from complete. Although temperature is a major parameter for RNA structure, the biological role of temperature modulation of RNA structure has not been thoroughly explored in biology yet.

Plants are not homeotherms and their cell's environment, with constant shifts in temperature, likely have created ideal conditions for RNA structures to be selected and genetically transmitted as an adaptive mechanism to elevated temperatures. The main objective of my upcoming research group is to understand how the plant model *Arabidopsis thaliana* adapts to elevated temperatures, focused on the conformational changes of secondary structures in the transcriptome. For this, we will identify and study the conserved RNA structures that have evolved to perceive elevated temperatures with critical role in plant adaptation. We will apply cutting-edge high-throughput approaches to study RNA structural dynamics *in vivo*, and a suite of approaches to dissect conserved structures that are critical for plant adaptation.

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CITRUS TOLERANCE TO SALT STRESS IS AFFECTED BY SCION STOMATAL DENSITY AND TONOPLAST CHLORIDE TRANSPORTERS

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Citrus is one of the major fruit crops affected by climate change that, among other effects, increases salinity in the irrigation water. Despite the clear role that rootstocks have on the citrus tolerance to adverse soil conditions, scions can be also determinant for the plant performance under salt stress as different citrus varieties have contrasted capability to regulate transpiration, accumulate and compartmentalize toxic ions in the vacuole. In this work, 4 rootstock/scion combinations (using Carrizo citrange (CC) or *Citrus macrophylla* (CM) as rootstocks, and Navelina (NA) or Oronules (OR) as scions), were watered with a 90 mM NaCl solution for 30 days. CC-NA plants accumulated higher Cl⁻ quantities in leaves, while no differences were observed in CM-OR plants, being this accumulation highly related to the higher stomatal density of NA scions compared to OR ones. Expression of genes encoding for proteins involved in the vacuole uptake of toxic ions indicated that the Cl⁻ transporters, *CsDTX35.1* and *CsDTX35.2*, were overexpressed only in leaves from CM-OR plants, whereas no differences were observed in Na⁺ transporters *CsHKT1.2* or *CsNHX1*. This reveals the importance of scion selection according to the environmental conditions, concluding that CC-NA combination is the most sensitive to salinity, whereas CM-OR is the most tolerant. Additionally, this work suggests that genes *CsDTX35.1* and *CsDTX35.2* can be good candidates for breeding programs.

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REGULATION AND CONSEQUENCES OF GENE EXPRESSION VARIABILITY BETWEEN PLANTS FOR NITRATE NUTRITION IN *ARABIDOPSIS*

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Proper gene expression is needed for development and response to the environment. This is

particularly true for plants that cannot move to avoid environmental changes. However, there is an important observation that is still not understood: **multiple phenotypes can derive from one genome in one environment**. This unexpected phenotypic variability between genetically identical individuals in the same environment has been overlooked for a long time. It is important to keep in mind that phenotypic and gene expression variability are not the same as plasticity (i.e. the response to environment) or genetic variation (e.g. different genotypes) as it is observed in absence of environmental and genetic differences. There are now some evidence that inter-individual phenotypic and gene expression variability could be at least partly genetically controlled and be biologically relevant.

It has been proposed that phenotypic and transcriptional variability could be beneficial in the context of unexpected and sudden environmental stress. In unicellular organisms, it has been shown that transcriptional variability allows a single genotype to generate multiple phenotypes where one of these phenotypes can survive a stress. In desert plants, a delayed or absence of germination for a proportion of the seeds after rain is a strategy to respond to the risk of a potential extensive drought right after the rain. These strategies using inter-individual variability could be particularly beneficial in the context of climate change, which will increase the frequency of extreme and unexpected environmental variations. **However we still lack information about the mechanisms involved in strategies using inter-individual variability in plants.**

Using nitrate nutrition in plants as a model, we will show **our results of the analysis of inter-individual gene expression variability, its phenotypic consequences and the role of chromatin in its regulation.**

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**EXPLORING CHROMATIN-BASED GENE
REGULATORY MECHANISMS CONTROLLING PLANT
ARCHITECTURE**

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Plants' exceptional phenotypic plasticity is nicely shown by the unique form and shape each plant takes; its potential is genetic, but its ultimate phenotype results from interactions with the environment. In order to understand the gene regulatory networks that underly plant architecture, the molecular signals provided by the ontogenic and environmental inputs, and the impact climate change will ultimately have on plant shoot architecture regulation, we have been studying the process of axillary bud dormancy initiation by the plant hormone strigolactone. Using functional genomics and systems biology tools in the model plant *Arabidopsis*, we have identified early transcriptomic changes in response to dormancy-inducing strigolactone signal and identified some of the key components of the gene regulatory network controlled by strigolactone, including some of the key hormone signaling integrators, as well as heat shock response factors. We discovered chromatin remodellers and epigenetic modifiers as some of the early targets of strigolactone signaling, which prompted us to look at changes to chromatin accessibility and modifications in response to strigolactone. We identified hundreds of putative cis-regulatory components and several novel trans-acting transcription factors of the strigolactone gene regulatory network, including heat shock factors and heat shock proteins. By deciphering the strigolactone gene regulatory network and its genetic and epigenetic components, we have also discovered potential vulnerability in bud dormancy, and by extension, plant architecture to climate change.

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IDENTIFICATION AND CHARACTERIZATION OF MUTATIONS CONFERRING RESISTANCE TO HEAT STRESS IN *ARABIDOPSIS*, TOWARD APPLICATION TO CROP SPECIES

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In agriculture, abiotic stresses lead to severe yield losses. One of the urgent challenges of agriculture is to improve crop resilience to heat stress to guarantee yield stability. Heat Shock Proteins (HSPs) represent a widely conserved class of proteins involved in plant stress response and development. Elucidation of the molecular mechanisms responsible for the regulation of HSPs is essential to improve the tolerance of crops to abiotic and biotic stresses. HSFA2 (Heat Shock Transcription Factor A2) is involved in mediating multiple abiotic stress resistance in *Arabidopsis*. To identify novel HSFA2 regulators, we performed a forward genetic screen using an EMS mutagenized *pHSFA2::GFP Arabidopsis* line. This screen allowed us to isolate a loss of function mutant of ACCH4 (ACC-oxidase Homolog 4), a putative 2-oxoglutarate dioxygenase enzyme of the AtDOXC31 clade. The *acch4* loss of function mutants exhibited an enhanced response to ACC treatment and overproduced ethylene, indicating that ACCH4 exerts a negative feedback on ethylene production. The amplified ethylene response in *acch4* mutants improved tolerance to heat but also to cumulated heat and high light stress and increased resistance against the necrotrophic pathogen *Botrytis cinerea*, without negatively affecting biomass. *acch4*-induced thermotolerance and resistance to combined heat and light stress required the ethylene receptor EIN2 (ETHYLENE INSENSITIVE2) and HSFA2. Moreover, we found that other members of the AtDOXC31 clade were also involved in thermotolerance. In tomato, introduction of a loss of function mutation in the close orthologous gene *SLALP* (*ACCH4-LIKE PROTEIN*) improved thermotolerance and resistance to combined heat and high light stress. Overall, we conclude that targeting *AtDOXC31* genes in crops is a valuable strategy to enhance stress tolerance by modulating ethylene signalling in *planta*.

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UNDERSTANDING THE MODE OF ACTION OF THE SYMBIOSIS-SPECIFIC NF-YA1 TRANSCRIPTION FACTOR *MEDICAGO TRUNCATULA*

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Plants belonging to the legume family are able to interact symbiotically with nitrogen fixing bacteria named rhizobia, allowing these plants to grow in agricultural systems with low nitrogen inputs and thus to be important actors of sustainable agriculture. This interaction leads to the formation of a new organ on the roots host plants, called nodule, inside which N_2 is fixed for the benefit of the plant. Nodule development is specifically controlled by the nuclear factor Y (NF-Y) transcription factor (TF) NF-YA1. The heterotrimeric NF-Y complex is composed of the DNA-binding subunits NF-YB and NF-YC. Interestingly NF-Y is both a sequence specific TF binding CCAAT boxes, but also shows nucleosome-like properties, promoting chromatin accessibility for other master regulators. Indeed, NF-YA1 in *M. truncatula* is rapidly, strongly and specifically expressed during nodule development and knock-out mutant lines only form few small nodules that lack a functional meristem. In order to better understand the model of action of this TF, and to identify and characterize potential MtNF-YA1 targets, we performed a comparative RNAseq analysis between the *nf-ya1* KO mutant and wild type plants. This analysis identifies a total of 956 differentially expressed genes whose expression significantly changed between the samples analysed. Among these potential targets of MtNF-YA1, we focused on the TF of the ethylene response factor (ERF) family called LEP (Leafy Petiol), proposed to play a role in the regulation of cell division in the leaf petioles in *A. thaliana* (van der Graaff et al., 2000, 2002). Functional and expression analysis strongly suggest that this novel TF, is a positive regulator of nodule initiation. We thus propose to name it NODIN (nodule initiation), as a new component of the network of TFs regulating nodulation and acting downstream of NF-YA1. We characterized the expression pattern of NODIN in different organs of *Medicago* and during nodule development using RT-qPCR and promoter reporter constructs. In order to gain insight into the role of NODIN during root infection and nodule organogenesis, we performed a functional analysis of NODIN using RNAi, CRISPR/CAS9 and ectopic expression approach. Data of this project could help defining new strategies to promote better nodule development in legume crops adverse conditions and to transfer nodule development into non-nitrogen-fixing crops thereby contributing to the ongoing worldwide effort to increase biological nitrogen fixation in agriculture

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THE SELECTIVE KNOCKOUT OF *OSDIS1* (RING E3 LIGASE) CONFERS DROUGHT TOLERANCE BY REDUCING STOMATAL DENSITY IN RICE

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Global climate irregularities and freshwater shortage have become major environmental problems that pose serious threats to global food security. Among stresses, drought negatively influences every stage of plant development, altering water and nutrient relations thereby causing huge crop loss. Rice is the major staple food crop, mainly cultivated in the lowland irrigated ecosystems, and is highly susceptible to drought. Drought resistance is governed by complex mechanisms involving post-translational protein modifications. E3 ubiquitin ligases play a very critical role in stress response and adaptation through protein ubiquitination and subsequent degradation. Here, we manipulate a negative regulator of drought response gene *OsDIS1* (E3 ubiquitin ligase) through CRISPR/Cas9 genome editing in indica rice. The loss of function of *OsDIS1* showed a high degree of survival rate under severe drought treatment. Further, the *dis1* mutants exhibited improved drought stress tolerance by decreased stomatal conductance and transpiration rate. Due to reduced stomatal density in the *dis1*, the leaf water retention was relatively high under dehydration stress. Additionally, infrared thermal imaging of *dis1* rice lines displayed a relatively cooler canopy under drought stress. The biomarkers including relative water loss, electrolyte leakage, and lipid peroxidation were found significantly reduced in the *dis1* mutant under drought conditions. The *dis1* plants exhibited a slight semi-dwarf phenotype without affecting its yield through modulation of the auxin-cytokinin ratio. Thus, the *dis1* mutant generated in this study showed improved water use efficiency and will be used as a resource in crop improvement programs for the development of climate resilient varieties.

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IDENTIFYING THE SODIUM-RESPONSIVE MIRNAS FROM RICE

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Recent years have witnessed severe implications of climate changes and environmental stresses on global crop production, and these impacts are projected to be worsened further. Hyper soil-salinity (NaCl) is an important environmental stress which severely hampers the crop yield and quality of produce. Amongst the constituent ions, sodium is known to be more toxic. However, the number of attempts to investigate impacts of sodium alone are very less. Current investigation was proposed to understand the rice responses and adaptive strategies to the sodium toxicity, particularly microRNA mediated post-transcriptional ones. One salt tolerant (Pancel-3) and one sensitive (Sahyadri-3) Indica rice cultivar were treated with the sodium-specific treatments (mixture of sodium dominant salts or sodium gluconate) and NaCl at the reproductive phase. Physiological, biochemical and agronomic investigation confirmed immature grains at the milk stage most prone to the Na-induced toxicity. Post-transcriptional regulation during the sodium toxicity was evaluated in terms of the differential behavior of miRNAs in the immature grains. Total 140 different miRNAs were identified via small-RNA sequencing followed by downstream processing of the data, amongst which the miR1861e was proved to be top-responsive to the sodium toxicity. Target prediction and validation was conducted using RLM RACE, which revealed the probable involvement of identified miRNA in transport, phytohormonal signalling and stress responses. Target confirmation and validation was carried using overexpression based transgenic approach. Expression levels of *Osa-miR1861e* were increased whereas expression levels of target genes were decreased in the transgenic plants in response to the Na⁺-stress.

Keywords: Sodium toxicity; miRNA; Genetic engineering; Post-transcriptional regulation; Stress-responses

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IDENTIFICATION AND CHARACTERIZATION OF CYTOSINE-5 DNA METHYLTRANSFERASE GENES IN PSEUDOCEREAL *AMARANTHUS CRUENTUS* L. UNDER HEAVY METAL STRESS

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The plant genome is known to be highly methylated in both, symmetrical and asymmetrical, contexts. Cytosine-5 DNA methyltransferases (*C5-MTases*) are involved either in *de novo* methylation of unmethylated cytosines or in maintenance of pre-existing DNA methylation.

Amaranth (*Amaranthus cruentus* L.) is a pseudocereal native to Central America, that has been known for its nutritional value since ancient times. In addition, amaranth is able to cope with rapidly changing environmental conditions and pollution. Amaranth also thrives in less fertile and nutrient-poor soils, which makes this pseudocereal attractive for marginal lands.

Identification and characterization of *AcC5-MTases* will contribute to a better understanding of epigenetic regulation in amaranth plants under heavy metal (HM) stress. Structural characterization of *AcC5-MTase* genes was performed together with phylogenetic analyses. In addition, the expression of *AcC5-MTase* genes was investigated in root and leaf tissues at the different developmental stages and under the HM-induced stress. Toxicity was induced by the administration of cadmium/lead/zinc/manganese in hydroponic solution. The majority of *Acc5-MTases* were down-regulated in response to the HM stress. Thus, the results suggest a decrease in DNA methylation, which may contribute to the induction of gene transcription as part of the defence mechanisms.

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RESPONSE OF NITROGEN NUTRITION TO CHALLENGING ENVIRONMENT IN *ARABIDOPSIS*

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Because of the dramatic effects of nitrogen on growth and development, plants have evolved to adapt to a challenging environment. At the level of root nitrate uptake it results in a highly integrated process, which is determined by nitrate availability, the nitrogen demand of the whole plant and the carbon produced by photosynthesis. This last regulation enables plants to adjust nitrogen acquisition by roots and carbon acquisition by shoots, which is necessary for the incorporation of inorganic nitrogen into amino acids and proteins. The results we obtained revealed that the control of root nitrate uptake by carbon signalling depends on a signal coming from the Oxidative Pentose Phosphate Pathway (OPPP). Interestingly, OPPP is a major source of NADPH and is critical to maintain redox balance under stress situation. This led to the hypothesis of an OPPP-related redox regulation of root nitrate uptake. This hypothesis is supported by recent data showing that root nitrate transporters in *Arabidopsis* are also regulated by the redox status of the plant. Furthermore, preliminary data indicate that the signalling mechanism is linked to the first step of the OPPP, coded by *G6PDH* and involved in the production of NADPH. Finally, it seems that oxidative stress could be a more general hidden player in the regulation of nitrogen nutrition. Indeed, recent results suggest that at least part of the regulation of root nitrate transporters by N signaling is also link to the redox status of the plant. In this context, further developments, in collaboration with the group of Yves Gibon in Bordeaux and Rodrigo Gutierrez in Chile, will concern the implication of such signalling pathways for plants adaptation to extreme environment in the Atacama desert.

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EMERGING ROLE OF PLASTIDIAL STRESS GRANULES IN PLANT TOLERANCE

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Climate change and weather variability have a significant impact on agricultural production. One mechanism that plants use to respond to external stimuli is by forming biomolecular condensates. Several studies have found that various environmental stresses such as heat, salt or hypoxia can trigger formation of plant cytoplasmic stress granules (cSG).

SGs are membrane-less organelles composed of proteins, translationally repressed mRNAs and small molecules. They consist of a stable core, which is fundamental for SG assembly, and an outer dynamic fluid shell that can differ between species, cell type, and developmental stage. Recent discovery shows that in addition to cSGs, heat can induce formation of stress granule in the chloroplasts (cpSG) suggesting their involvement in stress-induced regulation of plastidial machineries.

The aim of this work is to further broaden our current knowledge on cpSGs and to understand the dynamics, composition and cpSGs formation in the context of stress response. We show that cpSGs can be formed in response not only to heat but also to different stresses. Understanding molecular mechanisms underlying plants' response to such variability will help us to strengthen plants' resilience.

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TRH1/KUP4 PROTON COUPLED HAK/KUP/KT POTASSIUM TRANSPORTER CONFERS ROOT RESISTANCE TO ACIDIC CONDITIONS

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Root evolution allowed plants to move from aquatic habitats and conquest the land. In higher plants, roots are firmly anchored in the soil, showing positive gravitropism to acquire water and nutrients. Auxin coordinates root plasticity, providing developmental adaptation and allowing plants to cope with adverse soil conditions. The loss-of-function mutant of *TRH1*, a potassium transporter of the HAK/KUP/KT family, results in impaired auxin homeostasis at the root tip causing defective root hair elongation and root agravitropism. Here, we show that in acidic conditions (pH 4.5), the developmental response of the *Arabidopsis* primary root was reminiscent of the *trh1* mutant grown at normal pH 5.7. Low pH disturbed further the auxin distribution within *trh1* roots and worsened agravitropism. Exogenous application of membrane-permeable synthetic lipophilic auxin, 1-naphthaleneacetic-acid (NAA), restored the root hair phenotype of wild-type plants under acidic conditions. Wild-type roots moderately acidified the rhizosphere to establish a proton motive force for nutrient acquisition, while *trh1* seedlings exhibited a significantly increased media acidification when grew on pH 5.7 media without a buffering agent or in acidic conditions (pH 4.5). The elevated accumulation of protons in the apoplast leads to rhizotoxicity, causing inhibition of primary root elongation in *trh1* young seedlings, while the size and weight of the rosettes of mature plants were also diminished. These findings support that rhizosphere acidification under low-pH conditions interferes with TRH1-mediated cell-to-cell polar auxin transport. Hence, they highlight a novel developmental role of HAK/KUP/KT transporters towards an increase of root fitness on overly acidic highly-toxic soil layers.

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ECTOPIC EXPRESSION EITHER CHLORIDE CHANNEL (VvCLC₄) OR BOTH SODIUM ANTIporter AND CHLORIDE CHANNEL (VvNHX₁/VvCLC₄) INFLUENCE POTATO DEVELOPMENT AND NITRATE LEVELS IN TUBERS.

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Potato, *Solanum tuberosum* L., is one of the foremost staple and world's fourth largest food crop. In advance, its need is increasing because of its high-industrial value and population blast. However, potato culture is sensitive to multiple abiotic stresses, particularly salt stress the most limiting factor for plant development and yield. In order to improve both potato growth and behavior under harsh environmental conditions, we produced transgenic potato plants overexpressing either *VvNHX* (a sodium proton antiporter from *Vitis vinifera*), *VvCLC* (a chloride channel from *Vitis vinifera*), or both. Control and transgenic plants were grown in greenhouse and field in order to characterize their phenotype and evaluate their agronomic performance. The results showed that transgenic plants display an improved growth with better tubers quality (nitrate, protein and starch sugar contents) and composition.

Keywords: Potato, CLC, NHX, overexpression, nitrate, transgenic plant.

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**MANGANESE HOMEOSTASIS IN *ARABIDOPSIS* :
POST-TRANSLATIONAL REGULATION OF THE
NRAMP1 TRANSPORTER**

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Although plants only require small amounts of manganese (Mn), its availability in soils is often a limiting factor for their proper development. Mn is essential for important metabolic pathways such as photosynthesis but can be toxic when accumulated in excess. The maintenance of cellular Mn homeostasis is therefore crucial. In *Arabidopsis* root cells, Mn is taken up by the high-affinity transporter NRAMP1 (Cailliatte *et al.*, 2010). Our recent studies characterized the regulation of the subcellular dynamics of this transporter in response to Mn availability. NRAMP1 is stabilized at the plasma membrane under Mn deficiency and internalized in response to Mn excess in a phosphorylation-dependent manner (Castaings *et al.*, 2021). The actors of this signaling are however still unknown. This thesis work aims (i) to identify the kinase(s) responsible for the phosphorylation of NRAMP1 in response to Mn and (ii) to characterize their role in Mn homeostasis. Exposure to excessive Mn amounts triggers calcium signaling by kinases from the CIPKs (CBL-Interacting Protein Kinase) and CPKs (Calcium-dependent Protein Kinase) families to promote Mn detoxification in the vacuole (Zhang *et al.*, 2021; Ju *et al.*, 2022). Given that these kinases are also involved in the regulation of numerous transporters at the plasma membrane (Tang *et al.*, 2020), they are prime candidates to be responsible for NRAMP1 phosphorylation under Mn stress. We developed several approaches to test this hypothesis and our latest results will be presented in this poster.

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DECIPHERING THE MOLECULAR MECHANISMS THAT CONTROL IRON-MOBILIZING COUMARIN BIOSYNTHESIS, TRAFFICKING AND STORAGE

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Iron (Fe) is essential for most living organisms and is the most commonly deficient micronutrient in the human diet, with an estimated 1 billion people worldwide suffering from Fe deficiency. Increased atmospheric carbon dioxide concentration due to global climate change is predicted to reduce the amount of Fe present in several crops and thus may reinforce Fe nutritional issues. Although Fe is one of the most abundant elements found in soil, it is generally poorly available to plants since it is mainly present in the form of insoluble Fe (hydr)oxides. This is for instance the case in calcareous soils that represent one-third of the world's cultivated lands. Bio-fortification by, for instance, improving the capacity of plants to absorb Fe is one way to provide adequate dietary Fe to the growing global population. Recently, it has emerged that non-grass species have evolved a reduction-based mechanism to mine Fe from the soil and cope with its poor bioavailability. This mechanism relies on the secretion of Fe-mobilizing coumarins by the plant root system via the PDR9 transporter. Within the frame of this project, we aim at identifying and characterizing novel molecular actors involved in catechol coumarin biosynthesis, trafficking and storage by using untargeted approaches (GWAS and expression studies).

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A LOW COMPLEXITY DOMAIN CONTRIBUTES TO THE UBP1C PROTEIN ASSEMBLY INTO STRESS GRANULES IN *ARABIDOPSIS*

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Stress granules (SGs) are ribonucleoprotein condensates transiently assembled in all eukaryotic cells during the stress response. SG formation is mediated mainly by the liquid-liquid phase separation (LLPS) of protein-containing low-complexity or prion-like domains. In *Arabidopsis*, the OLIGOURIDYLATE BINDING PROTEIN 1C (UBP1C) is responsible for the selective sequestration of mRNAs into SGs during hypoxia. Here we demonstrate that UBP1C moves into SGs also under heat and salt stress. One of the intriguing questions is what is the cause of UBP1C aggregation into SG? The UBP1C protein contains an N-terminus low complexity polyglutamine (polyQ) repeat, which might serve as a coiled-coil region involved in protein-protein oligomerization. Mutation of this UBP1C polyQ repeat interferes with the seedling establishment and reduces survival during stress. To uncover the molecular basis of the UBP1C aggregation, we analyzed the SGs behavior of wild-type and generated mutants with extended polyQ length of UBP1C protein during stress using cell biology and genetic approaches. Our analyses revealed that the shape and number of UBP1C condensates depend on polyQ length. Moreover, glycine substitution in the polyQ repeat revealed that the amino acid composition of the UBP1C low complexity domain is determinant for the properties of SGs. Our research work discloses a mechanistic basis that underlies the protein LLPS in plant SG and suggests the employment of protein LLPS as an approach for increasing plant stress tolerance.

Key words: Plant stress granules, low-complexity domain, polyQ repeat.

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ROLE OF *ARABIDOPSIS* ECT FAMILY IN STRESS TOLERANCE AND STRESS GRANULE ASSEMBLY

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Stress granules are evolutionarily conserved condensates mainly composed of proteins and untranslated mRNA transiently assembled in response to stress. Although their composition in *Arabidopsis* under certain stress conditions has been reported, the physiological consequences of their assembly remain poorly understood.

On the other hand, posttranscriptional N6-adenosine (m6A) methylation of mRNA has emerged as an important gene regulatory mechanism in diverse physiological processes. The m6A mark is recognized for RNA binding proteins, acting as anchors. Among them, “m6A readers” containing the YTH (YT₅₁₂-B homology) domain are the most well characterized, but their role in plant stress tolerance is not well-known.

Independent pull-down assays reported the presence of YTH domain proteins of the ECT (EVOLUTIONARILY CONSERVED C-TERMINAL) family in *Arabidopsis* stress granules among the group of prion-like domain (PrLD)-containing proteins. PrLD is an essential characteristic for SG formation, since it provides structural flexibility and polymer-like behavior to the proteins. Nevertheless, the identification of ECTs is limited to the election of an appropriated “bait”, and also biased for the particular stress condition assessed.

Here, we show the localization and behavior of *Arabidopsis* ECT family members under stress conditions and their co-localization with AtRbp47b (a *bona fide* stress granule marker) of complete and modified versions of ECT proteins. Finally, we report initial assessments of their relevance for plant stress tolerance employing single and multiple ECT knockout lines. Our findings provide new insights in stress granule biology and also in the relevance of methylation readers for plant stress tolerance.

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THE ROLE OF RNA METHYLATION IN COLD STRESS RESPONSES IN *ARABIDOPSIS*

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At the posttranscriptional level, the stress-responsive transcripts are regulated by the alternative splicing, miRNA-mediated regulation, alternative polyadenylation etc. The epitranscriptome or mRNA modifications is yet another novel layer of post-transcriptional regulation that affects the fate of mRNA life cycle in multiple pathways. The importance of m⁶A in plant growth and development have been appreciated but its significance under stress conditions is still underexplored. To assess the role of m⁶A modifications on transcriptome during cold stress responses, we analyzed Me-RIP-seq profiles. The results revealed large scale shifts in this modification under cold stress. The m⁶A is known to affect transcript stability, degradation as well as translation. Therefore, we examined these possibilities which revealed that the cold-enriched m⁶A-containing transcripts are more stable and positively correlated with the translation. These recent findings as well as the phenotypic and physiological analyses of the *mta* mutant will be presented.

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IDENTIFICATION OF COUMARIN IMPORT TRANSPORTERS REGULATING COUMARINS TRAFFICKING WITHIN *ARABIDOPSIS* ROOTS

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Iron (Fe) is an essential micronutrient for a wide range of physiological functions in plants. Nevertheless, plants are frequently threatened by Fe deficiency because the majority of Fe in natural soil is in the form of insoluble ferric (hydr)oxides. To overcome this limitation, plants secrete specialized metabolites capable of solubilizing ferric Fe. In non-grass species, Fe-mobilizing coumarins (FMC) play a key role in this process. Recently, multiphoton microscopy imaging revealed that FMC are highly accumulated in root cortex and epidermal cells, and that the ABC transporter PDR9 (ABCG37) mediates their export into the rhizosphere. However, it remains unknown how FMC are delivered into these cells, thereby forming their cell-type-specific distribution in roots. Here, we attempted to identify Coumarin Import Transporters (CIT) involved in FMC trafficking within *Arabidopsis thaliana* roots. From transcriptome analyses, we isolated transporter genes whose expression was enhanced in Fe deficient roots as CIT candidates. Among them, *cit1* mutations were found to inhibit coumarin secretion. Heterologous expression of *CIT1* in yeast cells demonstrated an uptake activity of CIT1 for a major FMC, fraxetin. Promoter analyses showed that *CIT1* is expressed in root tip epidermal cells. Taken together, these results suggest that CIT1 contributes to Fe acquisition in *Arabidopsis* by enhancing fraxetin flow across the epidermal cell layer in coordination with PDR9.

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CAN HIGH TEMPERATURE EFFECTS ON GRAIN YIELD AND BIOCHEMICAL COMPOSITION IN SORGHUM BE ALLEVIATED BY A 'PRIMING' HIGH TEMPERATURE STRESS?

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One observable effect of climate changes is higher temperatures and in particular, recurrent and more intense periods with high temperatures (HT). These events affect plant physiology and development and, therefore, final performances in terms of yield and production quality. Sorghum, the 5th grain cereal, is known for its tolerance to drought and low nutriment requirements. In the climate change context, it may present advantages compared to other crops. However, as for other cereals, HT occurring at anthesis affect grain number whereas HT occurring during grain filling affect grain size and/or biochemical composition.

We hypothesized that modifications in grain size and/or composition due to a single HT stress imposed during grain filling could be partly alleviated if plants experienced an earlier HT stress during the cycle. Such recurrent HT stress scenario occur in natural conditions and their effects on plant performance are not well-understood mainly because of the difficulty to anticipate how plants can recover between two periods of stress and how far plants subjected to a first stress can be acclimated for a second one. The objectives of this study were to quantify the effects of HT stresses occurring during anthesis and grain filling periods on plant production-associated traits. Two genotypes were grown in controlled conditions with four thermal scenario: optimal, single HT stress at grain filling, and two recurrent HT stresses: one at heading and grain filling; the other one at flowering and grain filling. At plant maturity, morphological and biochemical analyses were performed on seeds. For SC748, both grain weight and biochemical composition were affected by HT stresses. For Btx623, only grain biochemical composition was affected by HT stresses. This preliminary study paves the way for future experiments with an original approach which combines multiscale phenotyping and molecular analysis to understand plant heat stress acclimation.

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DIVERSITY OF C₂ PHOTOSYNTHESIS PHENOTYPES ACROSS WILD ROCKET (*DIPLLOTAXIS TENUIFOLIA*) CULTIVARS

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Diplotaxis tenuifolia (wild rocket) is the only crop in commercial production currently known to exhibit the C₂ photosynthetic pathway. This interesting salad green improves well-being by offering a wide range of important human health benefits owing to its particularly high nutrient content. The C₂ photosynthetic pathway recycles carbon waste within the leaf, to improve the plant's overall photosynthetic efficiency. They do this by optimising the oxygen fixation pathway across two leaf cell types to retrieve lost carbon under environmental conditions that promote high rates of photorespiration, where many C₃ plants would show signs of stress. This unique physiological capability enhances the resilience of C₂ plants across a broad range of challenging environments. However, identifying this rare C₂ physiology is challenging, requiring multiple lines of evidence. Furthermore, intraspecific variation for the anatomical and biochemical characteristics of this photosynthetic type further complicates this characterisation. This study investigates the intraspecific photosynthetic diversity present in *D. tenuifolia* by characterising the C₂ phenotype across 15 cultivars via examinations of leaf anatomy, stable isotopes, and gas exchange evidence. We found uncharacteristically low CO₂ compensation point values across all the cultivars, similar to those measured in C₄ plants. However, all *D. tenuifolia* cultivars also showed very negative $\delta^{13}\text{C}$ values, which indicate C₂ physiology and firmly reject any C₄ pathway engagement. Despite evidence of some intraspecific variability of C₂ cycle engagement, our results suggest that *D. tenuifolia* cultivars universally possess particularly efficient C₂ photosynthetic mechanisms. How this species achieves such high C₂ pathway efficiency and whether this could be engineered into other crops should be investigated.

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INTRACELLULAR SIGNALLING UNDER OSMOTIC STRESS: FROM THE ENDOPLASMIC RETICULUM TO THE NUCLEUS, EXPLORING THE ROLE OF RHOMBOID PROTEASES.

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Questions about a role of the unprocessed form of AtbZIP60 have been raised, as a key component of the endoplasmic reticulum to nucleus signaling in plants, but until now no evidence for such a role has been provided. In the present study, we show that mannitol induces the expression of AtbZIP60, but in contrast to treatment with chemical agents that trigger the canonical unfolded protein response, no processing of the mRNA of this gene is observed. Furthermore, treatment with mannitol leads the AtbZIP60 protein to a nuclear localization. Transgenic plants harboring a silent mutation that inhibit the processing of this transcription factor mRNA by IRE1, show an increase in the abundance of the protein in the nucleus in a manner that seems to be dependent on a rhomboid protease. Moreover, ChIP-seq analyses of nuclear fractions from transgenic plants expressing a GFP-tagged version of AtbZIP60u show that this protein binds to the promoter of genes responding to abiotic stress, and qPCR data indicates that also it regulates their expression. Since we observed that the localization of AtbZIP60u was dependent on a rhomboid protease activity, the role of three rhomboid proteases predicted to be localized in the endoplasmic reticulum where analyzed. Among them, one of these rhomboid proteases exhibited a compromise regulation of AtbZIP60u targets genes and preliminary results suggest that mutants on this gene are more tolerant to osmotic stress than wild type plants. Current research is focus on the characterization of this rhomboid protease and it is apparent tolerance to osmotic stress.

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CELLULAR RESOLUTION OF REGULATORY NETWORKS UNDERPINNING ARBUSCULAR MYCORRHIZAL SYMBIOSIS UNDER WATER DEFICIT IN RICE.

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A large proportion of rice cultivation relies entirely on rainfall for water. Consequently, drought stress is a serious limiting factor to rice production, resulting in significant yield losses which are expected to increase under current climate change models. However, the intimate association of plant root systems with beneficial fungi, such as arbuscular mycorrhizal (AM) fungi, can improve resilience to water deficit. The relationship between the symbiont and the host root is mutually beneficial with the fungus delivering minerals and water to the plant, and the plant providing the fungus with organic carbon. These reciprocal exchanges happen within specific cells and result in the overall promotion of plant growth. In this project, sponsored by the US National Science Foundation, Plant Genome Research Program, we aim to identify regulatory networks that promote plant growth at cellular resolution in rice. By combining Isolation of Nuclei Targeted in Specific Cell Types (INTACT) and Translating Ribosome Affinity Purification (TRAP) techniques with RNAseq and spatial single cell transcriptome analyses, we aim to identify transcription factors underlying improved drought tolerance of mycorrhizal rice at single cell resolution.

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NEW STRATEGY TO STUDY PLANTS' EPIGENETIC PLASTICITY AGAINST COLD STRESS: A CHEMICAL-BASED APPROACH

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Plants, as sessile organisms, have to face changing environments with recurrent adverse conditions, such as drought, salinity, and extreme temperatures, which severely affect growth and development. Cold stress is a priority, as it limits the geographical distribution of plants as well as crop productivity, and persistent cold extremes have been observed in agricultural regions worldwide. Epigenetic machinery allows plastic responses to environmental conditions, providing tolerance and adaptation within the on-going climate changes. Different strategies are used to study epigenetics in plants. Chemical inhibition of epigenetic regulators provides many advantages: it avoids the problem of paralog proteins functionally redundant and mutants' lethality, and it can be applied to species in which genetic information is limited. So far, few compounds have been applied in plant epigenetic studies, mostly belonging at just two categories: DNA methyltransferases and histone deacetylase inhibitors. Taking advantage of high conservation of the epigenetic regulators between animals and plants, we proved the efficacy of a human Polycomb Repressive Complex 2 (PRC2) inhibitor in *Arabidopsis thaliana*.

We have also assessed the efficacy of a set of inhibitors, derived from a lead compound against human P300/CBP acetyltransferases, against the *Arabidopsis* homologs, the HAC proteins. We selected the most active compounds by a phenotypic screening. Efficacy of the compounds has been proved by analysing the expression level of target genes.

Currently, we are using this pharmacological approach to investigate the role of PRC2 in cold response, setting up a new experimental design. First, we assessed the inhibitor effect in inducing cold response, at molecular level. Expression analysis of cold-induced genes in samples induced at 4 ° for 12h, following inhibitor treatment, revealed that inhibitor-treated samples show an increased cold response compared to the untreated.

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Lettuce (*Lactuca sativa*) is a popular leafy vegetable grown globally under the field, greenhouse, and vertical farming conditions. The public-private partnership LettuceKnow aims to obtain basic knowledge about the regulation of development, architecture, and stress resilience in this crop. This consortium includes three Dutch Universities, University Medical Centre Utrecht, Centre for Genetic Resources in the Netherlands (CGN), and seven breeding companies. Research in our teams involves extensive characterization of 500 lines from four *Lactuca* species at the level of phenotypic and gene expression responses related to abiotic and biotic stress tolerance. Together with the Big Data and genetic transformation teams, we develop descriptive and predictive models of lettuce development and resilience. At the poster, we could talk about the overall organization of the LettuceKnow consortium and its connection to the Netherlands Plant Eco-phenotyping Centre (NPEC). Also, I am glad to tell you about work to develop sustainable resistance against downy mildew and to mitigate the costs of activated immunity on physiology in lettuce. Finally, we could chat about my experience of teaming up with the industry to address fundamental questions in plant biology.

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BRASSINOXIDE: DECIPHERING THE MECHANISMS OF BRASSINOSTEROIDS AND NITRIC OXIDE CROSSTALK DURING HEAT STRESS RESPONSES IN PLANTS

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Plant growth and development are controlled by environmental changes dominated by climate change. Sudden and prolonged heat waves limit the life cycle of plants and compromise their survival. Plants have evolved molecular mechanisms to cope with high temperatures. The plant hormone Brassinosteroids (BRs) (1) and the gasotransmitter Nitric Oxide (NO) (2) show an important role in the success of plants in coping with extreme temperatures. We have shown that the BR-regulated transcription factor BES1 can contribute to heat stress signaling (3). BES1 is activated in response to heat even in the absence of BRs and binds directly to heat shock elements (HSEs). BES1 does this through interaction with HSFA1a (HEAT SHOCK FACTOR A1a), a member of the HSF family and the main regulator of the heat signaling pathway. HSFA1a facilitates the activity of BES1 in binding to HSE. Another signaling component involved in heat stress responses is NO (4). Following sudden increases in temperature, NO activates the oxidative response and promotes the accumulation of heat shock proteins (HSPs), which are crucial for the acquisition of heat stress tolerance. A model study that integrates BRs and NO in the regulation of the HSFs is being investigated to activate the response of the plants under extreme temperatures.

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MOLECULAR CHARACTERIZATION OF A PHYTOSTEROL-BASED BIOSTIMULANT IMPROVING PLANT RESISTANCE TO WATER DEFICIT CONDITIONS

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Elicit Plant is a French agrobiotech company whose objective is to provide farmers with alternative solutions to phytosanitary products. To secure crop production in the context of global warming, the company has developed BEST-a, an innovative plant-derived product composed of phytosterols. Foliar application of BEST-a stimulates the plant's defence mechanisms to help it cope with drought stress.

Phytosterols represent a large class of molecules derived from the primary metabolism. Given their chemical diversity, phytosterols fulfil a wide range of functions. They play an important role in membrane fluidity and permeability, being an integral part of the lipid bilayer. Sterols also act as bioactive signals modulating plant growth and development in response to environmental cues and stresses.

Field and laboratory experiments performed by Elicit Plant on soybean and other agricultural plants have shown that spraying BEST-a substantially improves the physiological resilience of crops facing water deficit. Plant water management is optimized, and as a result, seed yield increases. My thesis project aims to study the molecular mechanism by which the sterols derived from BEST-a increases plant resistance to drought stress. To achieve this goal, a multi-OMIC analysis has been performed in soybean and the plant model *Arabidopsis* to identify the gene-metabolite network activated by BEST-a in response to drought.

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