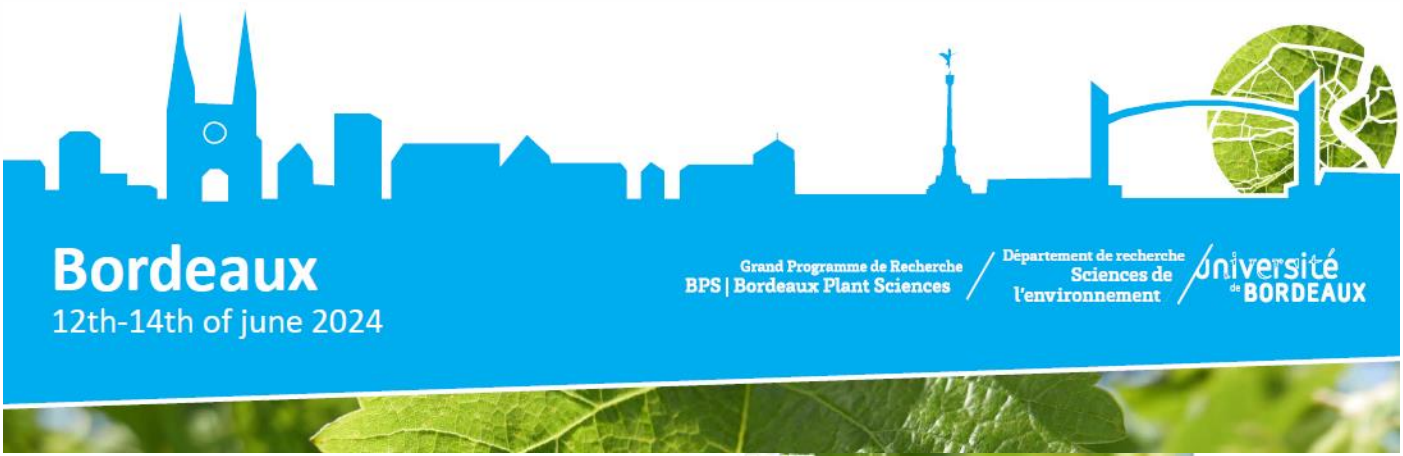
14th International Congres of the French Society of Plant Biology

ABSTRACT BOOK



14th International Conference of the French Society of Plant Biology SFBV

June 12-14, 2024

Amphi Duguit, place Pey Berland, BORDEAUX

Introduction

The 1972 Meadows report: A wake-up call for plant science

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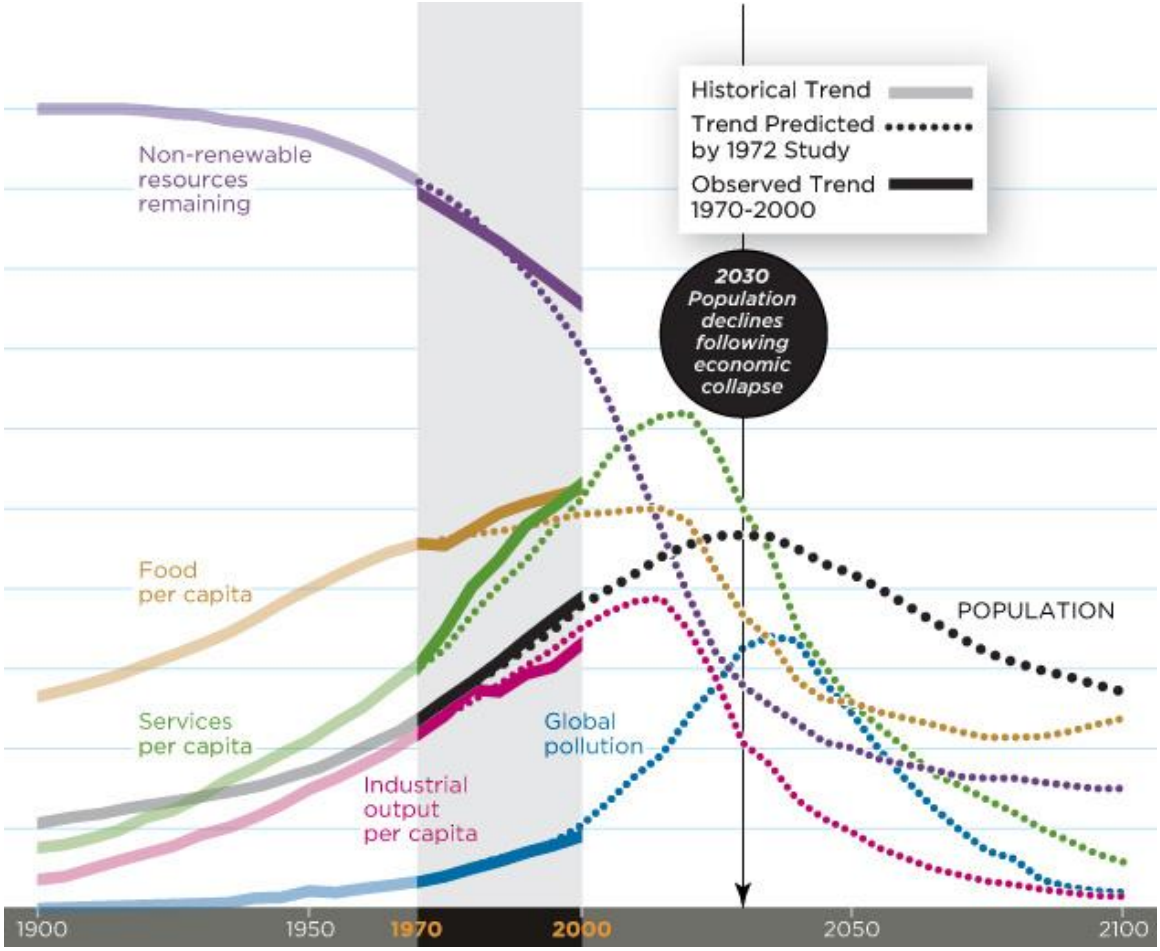
The 1972 Meadows report, "the limits to growth", predicted a global socio-economic tipping point during the 21st century. Now supported by 50 years of empirical evidence, this work is a tribute to systems thinking and an invitation to take the current environmental crisis for what it is: neither a transition nor a bifurcation, but an inversion. For instance, we used matter (e.g. fossil fuel) to save time; we will use time to preserve matter (e.g. bioeconomy). We were exploiting ecosystems to fuel production; production will feed ecosystems. We centralized to optimize; we will decentralize to support adaptability. In plant science, this new context calls for new research on plant complexity (e.g. multiscale robustness, benefits of variability), also extending to new scientific approaches (e.g. participatory research, art & science). Taking this turn reverses many paradigms and becomes a new responsibility for plant scientists as the world becomes increasingly turbulent.



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Agricultural systems in the agroecological transition

Botanicals: A Path to Greener Pest Control?

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Plant extracts or purified plant components, known as botanicals, are increasingly employed as biopesticides in agronomy to address a wide spectrum of applications, including eliciting plant responses and serving herbicidal, fungicidal, insecticidal, bactericidal, virucidal, and nematicidal purposes. These botanicals offer several advantages, such as generally low toxicity to users and consumers, minimal ecotoxicity, and good biodegradability. Although these botanicals have great potential and could, in some cases, prove to be more effective than synthetically produced pesticides, certain disadvantages may arise. Many questions could indeed be raised about their use, particularly regarding their sustainability. In this presentation, we will endeavor to address questions such as:

- Should agricultural land be utilized to grow plants for protecting other plants?
- Is it necessary to excessively harvest naturally growing plants to produce botanicals?
- Should wood or other fossil energy sources be used in tropical regions for distilling plants?
- Are biopesticides, whose co-formulants are mostly derived from chemical synthesis, truly sustainable?

In exploring these considerations, various approaches can be examined:

- The use of by-products or invasive tropical plants as starting plant material.
- The direct utilization of whole plant parts rather than essential oils.
- The co-cultivation of essential oil plants in horticultural plots, protecting consumable plants from insect pests, and subsequently generating essential oil for additional income.
- The study of induced allelopathic traits where crops produce their own metabolites, reducing weed germination and growth.

The utilization of plants to protect other plants through the valorization of botanicals is a compelling subject that draws upon various disciplines, with potential applications in both Northern and Southern countries. However, a substantial journey lies ahead to gain a comprehensive understanding of the mechanisms at play and to develop sustainable valorization strategies to replace conventional pesticides with bio-based solutions.

O1

Does rhizospheric microbiome contribute to common bean tolerance to drought and tropospheric ozone ?

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Drought and elevated tropospheric ozone episodes are expected to be more frequent and severe in the near future. Both are threats to crop yield through their negative impacts on plant carbon and nitrogen metabolisms. In addition, they affect the composition of the soil microbial communities, both directly and via changes in plant root exudation.

Common bean (*Phaseolus vulgaris*), is one of the most widely cultivated crop in the world. Its nutritional composition makes this crop an interesting source of protein for human consumption. Its ability to fix atmospheric nitrogen through bacterial symbiosis contributes to sustainable cropping systems. However, common bean is sensitive to abiotic stresses.

In experiments conducted in 2023, two common bean genotypes differing in their sensitivity to ozone, were grown on a natural soil and subjected to controlled drought, elevated ozone or the combination of both stresses, during the seed filling stage. We wondered if the physiological and yield discrepancies between the genotypes were related to their N use efficiency and/or to the structures of their respective rhizospheric microbial communities.

Physiological parameters, such as gas exchange, chlorophyll content, stomatal conductance, chlorophyll fluorescence, were measured. N remobilisation process was studied using the stable isotope ^{15}N labelling method and by studying foliar proteolysis. Moreover, rhizospheric and non rhizospheric soil samples were collected separately and metabarcoding was carried out on the 16S rRNA gene of the respective DNA extracts.

The two genotypes developed contrasting strategies in response to the treatments, which led to various impacts on yield parameters. However, the N remobilisation efficiencies remained unaffected. Furthermore, rhizospheric microbial communities of the two genotypes were very similar and the stresses induced equivalent modifications. Further analysis will be carried out on the N cycle genes to figure out whether the structural modifications of microbial communities correspond to functional changes in soil N cycle.

O2

Unlocking the potential of biostimulants for greenhouse horticultural crops: insights from NIR spectroscopy fingerprinting and Machine Learning analysis.

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Biostimulants are a very diverse family of products with a lot of different formula. Its current definition by the EU regulation (2019) is "a product that stimulates plant nutrition processes independently of the product's nutrient content, with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere : (a) nutrient use efficiency; (b) tolerance to abiotic stress; (c) quality traits; or (d) availability of nutrients confined in the soil or rhizosphere". Although climatic conditions can be controlled in some greenhouses, this is not the case in all companies, and there are some growing incidents. Biostimulants could be tools used to enhance crop performance or to reduce climatic stresses.

Facing a huge number of commercial products, farmers are claiming for independent effectiveness tests and guidelines to use biostimulants. ASTREDHOR Sud-Ouest is working on this purpose for a long time in its greenhouses, testing different products on cyclamen, poinsettia or tomato jointly with UMR 1332 BFP. The aim of the trials led since 2016 were to assess the effectiveness of the products according to company claims in comfortable crop conditions at first. This work enables to highlight good results certain years, but it is difficult to generalize or to observe stable effects. To address this issue, we carried out multi-year factor analysis to identify a recurring effect of certain biostimulants or certain families of products. Secondly, we complemented 2022 phenotypic data with Near Infrared Spectroscopy (NIRS) measurements. Then, Crop performance was modelled using Machine Learning tools (GLMNET).

Finally, the multi-year statistical analyses showed a major effect of the climatic conditions on the Poinsettia development and did not highlight the effectiveness of a particular biostimulant category. However, machine learning tools made it possible to predict the biostimulant effectiveness using plant canopy surface as proxy, thanks to NIRS data. These results should be confirmed with an independent dataset.

This approach will be developed on cyclamen and tomato trials to exploit the wealth of data collected. It would permit to confirm Poinsettia results and the interest of NIRS fingerprinting to assess the effectiveness of biostimulants.

O3

Impact of the inoculation with marine plant growth-promoting rhizobacteria on the native grapevine rhizosphere microbiome

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Climate change poses new challenges to viticulture. Regenerative viticulture practices are at the forefront of the new, more sustainable approaches to counteract biodiversity loss and both abiotic and biotic stresses. And so, there is an effort to identify new plant growth-promoting rhizobacteria (PGPR) that improve grapevine resilience. Salt marshes are characterised by their harsh environment from high salinity to temperature fluctuation, though the rhizosphere of plants inhabiting it are potential PGPR reservoirs.

Grapevine roots were inoculated with a marine synthetic PGPR consortium (SynCom) and subjected to heatwave stress to evaluate the potential ameliorative effect of these SynCom. Inoculated plants significantly exhibited improvements in plant fitness when exposed to heatwave stress, demonstrating SynCom bioaugmentation capabilities. Nevertheless, it is of utmost importance to evaluate the effects of SynCom addition in the native grapevine microbiome within a regenerative approach. SynCom impact in the native grapevine rhizosphere microbiome was evaluated 45 days after inoculation (maintaining the plants in pots with the native soil), through 16S and 28S regions long-read sequencing in two vineyards soils with contrasting characteristics (loamy sand and clay soil). When comparing sandy and clay native rhizosphere significant differences were observed in microbiome composition, particularly considering Bacilli bacteria. When comparing native and SynCom inoculated rhizosphere, for both the clay and sandy soil, significant differences were found in the abundance of bacteria capable of fumarate respiration, with no impact to other putative functions. Within the fungi community, functions associated with endophytic interactions and different fruit body characteristics were also significantly altered by the inoculation. Demonstrating that the overall functionality of the rhizosphere is maintained despite the addition of exogenous marine bacteria. Our work shows that not only are these microorganisms capable of improving plant resilience against abiotic stress, but also that their inoculation in vineyard soil doesn't impact the native microbiome overall functions.

O4

Unraveling A. thaliana Variation efficiency in Biostimulation: Insights into Metabolic Signatures of Biostimulant Success

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Modern agriculture must balance crop yield maintenance or improvement with climate change and sustainable practices. Plant biostimulants offer a promising solution to these challenges but suffer from mistrust, probably due to a lack of knowledge about their compositions and mechanisms of action. Among others, the company-specific compositions of some biostimulants together with Inter and Intra-specific plant diversity remain as prevalent sources of variability, and can influence the biostimulant efficiency. In this study, we address the effect of intra- specific variations of A.thaliana response on the efficiency of two Ascophyllum nodosum extracts (ANEs), a prominent class of biostimulant known to promote plant growth and improve their tolerance to abiotic stresses. The phenotypic variation of 54 A.thaliana natural accessions under heat stress (HS) and treated with both ANEs, was firstly examined through the evaluation of plant growth and 11 primary metabolic traits. The two ANEs tested exhibited similar efficiencies in term of number of accessions positively affected under stress, with a higher efficiency in HS-sensitive accessions. However, both ANEs showed different metabolic pattern responses, possibly in link with their differing extraction processes. For in-depth metabolic profiling analyses, we kept the plants treated with the ANE generating clear variations of the primary metabolism across the different accessions. Initial multivariate analysis revealed a distinct separation between biostimulated plants and control plants under stress conditions, indicating an intermediary metabolic state between non-stressed and stressed controls. To refine our focus on specific metabolic targets, and taking advantage of the intra-specific diversity of the response, a predictive metabolomic approach is being used to simulate the biostimulation efficiency. The identification of a core-set of metabolites through this approach will serve as valuable parameters for conducting genome-wide association studies (GWAS) across a broader spectrum of accessions, a project in progress.

Genome structure & expression

Endings in the middle: uncovering the structure, evolution, and epigenetic status of interstitial telomeric repeats in *Arabidopsis thaliana*

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Regulation of chromatin compaction and genome topology are both influenced by linker histone (H1) and Polycomb repressive Complex 2 (PRC2). We recently established that, in the Arabidopsis Col-*0* model organism, H1 favors PRC2 activity at genes while having the opposite effect at telomeres and at large interstitial telomeric repeats (ITRs). Repression of PRC2 activity at ITRs relies on H1's capacity to hinder DNA association of Telomeric Repeat Binding proteins (TRBs), a family of proteins that combine an H1-like DNA binding domain and a coiled-coil domain mediating PRC2 *cis*-recruitment. Thus, H1 acts as a safeguarding mechanism allowing plants to cope with the strong attractiveness of telomeric motifs for TRB-PRC2 activity, possibly preventing deleterious consequences of ITRs on the epigenome homeostasis. ITRs contain thousands of telomeric motifs but, contrary to terminal telomeres that end the chromosomes, ITRs are located near the centromeres. Their origin and function in plants have long been ignored. Using newly assembled telomere-to-telomere genome sequences, we identified a great variability in ITR content, structure, and distribution among *A. thaliana* accessions - with total ITR size ranging from 0.2 to over 2 Mb. Yet, despite this variability, ITRs tend to share similar higher-order structures composed of regularly interspersed telomeric and centromeric repeats. Based on these findings, I will present hypotheses on how ITRs could seed at centromeres and how ITR neo-formation or expansion may impact the plant 3D chromatin landscape.

O5

Does genetic load impact adaptive and productive traits in grapevine?

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Theoretical predictions and empirical studies have demonstrated the prevalence of purifying selection as an evolutionary force to remove new deleterious mutations that arise in a population. However, the efficacy of purifying selection may be compromised under certain circumstances resulting in the accumulation of harmful mutations (i.e. genetic load). Demographic bottlenecks, such as those experienced during domestication, hitchhiking of mildly deleterious mutations or artificial positive selection of deleterious mutations, can favour genetic load in domesticated genomes. This phenomenon is known as the evolutionary concept of the "cost of domestication", which has been recently studied in several crops, leading to contrasting results. Nevertheless, little is currently known about the impact of genetic load on adaptive and productive traits for crop species. In this study, we aimed at i) testing for the cost of domestication hypothesis in grapevine, and ii) estimating the impact of genetic load on adaptive and productive phenotypes for cultivated varieties. For this purpose, we estimated genetic load based on whole-genome sequencing of 571 grapevine accessions, including 25 wild *Vitis* species originating from three geographical regions (North America, Asia and Europe), and 296 commercial varieties of *V. vinifera ssp. Sativa*. Genetic load estimates were correlated with phenotypes for adaptive and productive traits of 36 of the sequenced grapevine cultivars obtained in an experimental common garden in Bordeaux (VitAdapt). We observed more deleterious mutations in wild accessions than in domesticated ones. Genetic load was positively correlated with the flowering date of cultivated varieties. The results of this study will contribute to the forethought on how we can best account for deleterious alleles and genetic load in new-generation grapevine breeding.

This study received financial support from the French government in the framework of the IdEX Bordeaux University "Investments for the Future" program / GPR Bordeaux Plant Sciences, and from the PurVitis project.

O6

Genetic architecture of *Tobacco mosaic virus* tolerance in *Arabidopsis thaliana*

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Resistance to viruses is often effective only in the short term as the high rate of evolution of viruses allow them to bypass resistance mechanisms. Conversely, tolerance (i.e., the ability of plants to accumulate pathogens in their tissues without causing disease symptoms or loss of fitness) could be more durable as the viruses are subjected to less selective pressure. *Tobacco mosaic virus* (TMV), an RNA virus from the genus *Tobamovirus,* is able to infect a wide range of hosts, including *Arabidopsis thaliana*. Various Arabidopsis ecotypes exhibit diverse responses to TMV; for instance, Shahdara was described as susceptible with a distinct leaf curling symptom (Figure 1), Tsu-0 was resistant, and Col-0 had a substantial viral accumulation without exhibiting the curly symptom (1). In the GreenTolerance project we aim to investigate the genetic architecture of tolerance to TMV in Arabidopsis. First, the tolerance phenotype was characterized by measuring symptoms, viral load and fitness traits (seed set, biomass, size), alongside an untargeted metabolomics approach. Then, two strategies have been chosen. The first is to use F2 and RIL populations derived from crosses between Shahdara, Col-0 and Tsu-0. Out of 40 F2 plants derived from a Tsu-0 × Shahdara cross, 11 plants showed levels of virus similar to that of Shahdara without visible symptoms, indicating that those plants are tolerant to the virus. Linkage mapping will be performed to find genetic loci associated with this tolerance. The second strategy is to perform genome-wide association mapping (GWAS) using 135 diverse ecotypes. Among all ecotypes, only Shahdara had visible symptoms and had the highest viral accumulation. TMV inoculation disrupted the metabolism of Shahdara to a greater extent in comparison to Col-0 and Tsu-0. Finally, the role of putative genes identified by GWAS and linkage mapping will be analyzed by phenotyping CRISPR/Cas9-engineered mutants.

1. Dardick, C.D., Golem, S., Culver, J.N., 2000. Susceptibility and Symptom Development in Arabidopsis thaliana to Tobacco mosaic virus Is Influenced by Virus Cell-to-Cell Movement. MPMI 13, 1139–1144. https://doi.org/10.1094/MPMI.2000.13.10.1139

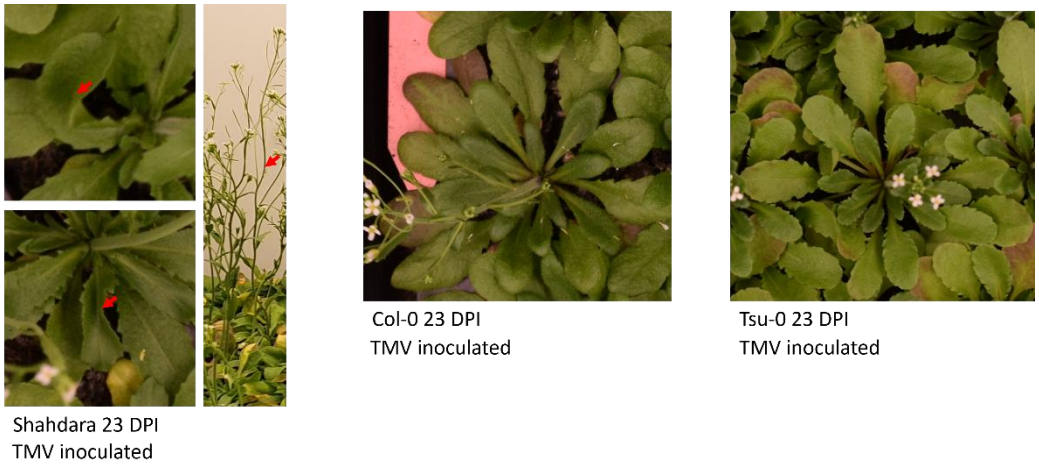

Figure 1 : Shahdara, Col-0 and Tsu-0 plants at 23 days post TMV inoculation.

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O7

Conserved Roles of GDSL-Domain Proteins in Suberin Dynamics During Root Organogenesis

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Improving crop resilience through enhanced root system architecture (RSA) is crucial in adapting to changing environmental conditions. Lateral root (LR) formation, predominantly regulated by auxin signaling pathways, is a complex process. We recently presented the first developmental atlas of LR development in the model grass species *Brachypodium distachyon* (*Bd*), revealing the reactivation of the endodermis cell cycle, contributing to the formation of root cap and columella cells in emerged LRs. Interestingly, our findings suggest that auxin signaling, indicated by DR5 promoter activity, is not evident in the phloem pole pericycle or during early LR development, but correlates with cell wall modifications during LR primordium (LRP) emergence.

Conducting a comparative analysis on Bd21 and Bd21-3 accessions, we observed similar LR primordia synchronization post-root tip removal, but distinct of the LR architecture thereafter. RNAseq analysis unveiled distinct early and late responses in both accessions, with significant overlap of differentially expressed genes at later time points. Enrichment of cell-wall-related processes was observed, and patterns of cell wall remodeling gene families varied during early LR formation.
Additionally, we identified several GDSL/GELP genes showing similar expression patterns during LRP formation in both Brachypodium and Arabidopsis, along with conserved GDSL protein structures.
Notably, the orthologous copy of *At*GELP38, identified as a suberin synthase in Arabidopsis, *Bd*GELP38, was expressed in the endodermis overlaying LRPs and functionally rescued the absence of suberin phenotype in the *gelp*^{quint} mutants. These findings shed light on conserved and divergent mechanisms in LRP development, thereby advancing our understanding of root branching regulation in plant biology research.

O8

Functional Characterisation of *VvAP2-4*, a Transcriptional Factor Involved in Response to Heat Stress in Grapevine

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The effects of climate change include an increase in the average annual temperature and the accumulation of severe heat waves (IPCC 2023). Elevated temperatures affect almost all aspects of plant growth and development, particularly in grapevine (Vitis vinifera L.), one of the most economically important fruit crops worldwide. Heat stress alters fruit composition at harvest, resulting in wines with a high alcoholic content, low acidity, and decreased aging potential.

The selection or development of cultivars that are better suited to withstand heat stress and maintain desirable metabolic traits is essential to address heat stress challenges. Understanding the specific effects of heat stress on berry physiology and development requires the identification of key heat stress responsive genes that can also serve as molecular markers for heat stress tolerance.

Transcription factors play pivotal roles in regulating plant responses to HS. However, functional characterisation of specific regulators remains incomplete in grapevines, similar to other woody perennial plants. In this context, we focused on grapevine transcription factor *VvAP2_4*, a member of the APETALA2/ETHYLENE RESPONSIVE FACTOR (AP2/ERF) family, which was selected for further functional characterisation. A metabolic engineering approach was employed by overexpressing either the dominant version of *VvAP2_4* (HA version) or its dominant-negative version (SRDX) in the microvine model system. In the present study, its involvement in the heat stress response and grapevine thermotolerance was assessed by a non-targeted metabolomic study. Our first results obtained on the leaves of transgenic or wild-type microvines subjected or not to heat stress, highlighted distinct metabolites and biochemical pathways impacted on the one hand by the overexpression of *VvAP2_4* and by the effect of heat stress. These results, supported by previous complementation experiments in *A.Thaliana* and RNA-seq analysis, show that *VvAP2_4* is an interesting candidate for thermotolerance in grapevine.

Plant and pathogen interactions

Cellular lockdowns: plasmodesmal regulation of trade-offs between growth and defence

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In plants, a variety of stimuli trigger short-range and long-range signals that travel to distal tissues. Plasmodesmata are intercellular cytoplasmic connections and are one pathway by which molecules can carry information and resources between cells. As plasmodesmata open and close, their dynamics are a critical component of a range of plant developmental and environmental response processes. In the context of immune responses, we identified that specialized signaling machinery controls plasmodesmal aperture, allowing independent control of plasmodesmata and posing the question of why plasmodesmal dynamics benefit immune execution. We have observed that whether plasmodesmata close in response to immune-associated signals is dependent on the age of the tissue, with young leaves keeping their plasmodesmata open under the same conditions when mature leaves close them. This led us to hypothesize that young leaves might benefit from keeping their plasmodesmata open to allow the maximum flow of soluble sugars from the phloem, provided by source tissues, into growing tissues to sustain growth. Supporting this possibility, forcing these younger leaves to transition between from a sink to a source tissue activated their capacity to close their plasmodesmata. Further, forcing plasmodesmata closed in young leaves induces signatures of a starvation response. Our data suggests that young leaves prioritise growth over defence, and we aim to uncover what processes are executed in source tissues that enable a stronger immune response when plasmodesmata are closed.

O9

Deciphering the responses of *Solanum pimpinellifolium* to *Cucumber mosaic virus* and heat stress

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In the context of global climate change, plants are facing occurrences of heat waves together with various pathogenic threats. Heat directly impacts plant physiological behavior, while pathogens can benefit or not from the consecutive effect of heat. Pathogens like *Cucumber mosaic virus* (CMV) contribute to yield losses in many crops of agronomic interest, including tomato. Tomato is also sensitive to temperature, and heat stress (HS) is known to reduce crop productivity. HS also shows potential for enhancing or inactivating viruses' multiplication. To explore the genetic basis of trade-offs between growth and tolerance, we set up an experiment in a greenhouse with a wild tomato population (*Solanum pimpinellifolium*) displaying a large genotypic diversity. We phenotyped plants responses to CMV inoculation under optimal or HS conditions. Data collected englobed differences in growth through dry weight and gas exchanges, symptom appearance, viral accumulation, and metabolite profiling. The observed differences in plant development offer insights into genotype-specific responses to both stresses and their combination. The metabolite profiling will allow us to identify the signals implicated in defense pathways and their link with physiological development. Moreover, it will enable us to use them as biomarkers or predictors of tolerance. Genome wide association studies will be performed to link the phenotypic and genetic variability and provide insights into the genetic architecture of tolerance to multiple stresses.

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O10

Arabidopsis CPK3 function in anti-viral response: importance of plasma membrane nanoscale dynamics

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Viruses propagate in plant cells through the plasma membrane channels plasmodesmata. Using transient expression in tobacco leaves, we previously showed that the calcium-dependent protein kinase 3 (AtCPK3) from Arabidopsis was able to restrict viral cell-to-cell movement of the potato virus X. Importantly, AtCPK3 phosphorylated the potato Remorin StREM1.3, which was required for its anti-viral function. Using the plantago asiatica mosaic virus able to infect Arabidopsis, we now showed that this mechanism was conserved in Arabidopsis. We tested several higher order *cpk* knockout mutants and discovered that AtCPK3 displayed a specific ability to hamper viral propagation over 5 other CPK isoforms (AtCPK1/2/5/6/11) involved in immune response to bacteria and fungi. We confirmed that Arabidopsis AtREM1.2 was also an in vitro substrate of AtCPK3. Both AtREM1.2 and AtCPK3 are membrane-associated, which is required for their ability to restrict viral propagation. Using single particle tracking photo-activated localization microscopy, we showed that CPK3 diffusion in the plasma membrane was reduced upon activation as well as upon viral infection and that such immobilization depended on AtREM1.2. By contrast, AtREM1.2 diffusion was increased by viral infection in an AtCPK3-dependent manner. This study unveils a complex membrane protein nanoscale dynamics as part of plant defence against viruses.

O11

Vectorized salicylic acid bioprecursors to stimulate plant natural defenses against disease

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Agriculture faces increasing pressure to innovate in response to the challenges presented by global change. The primary challenge implies reconciling increased agricultural production to meet growing nutritional demands of an expanding world population while concurrently reducing environmental impact. This requires a shift in the use of plant protection products and, consequently, the development of new systems for managing plant diseases. One such strategy involves the indirect manipulation of plant disease resistance by stimulating or priming their innate defense mechanisms. Numerous studies have demonstrated the key role played by salicylic acid (SA), an endogenous phytohormone, in orchestrating these defense mechanisms in plants[1]. However, the exogenous application of SA is constrained by its phytotoxicity and inactivation by compartmentation, thus restricting its practical efficacy in agricultural contexts[2,3]. Addressing this limitation, we propose the vectorized bioprecursor strategy. This approach involves conjugating SA with nutrients such as amino acids or glucose, facilitating its systemic distribution *via* the phloem sap and gradual release at target sites to activate defense mechanisms. Promising results have already been documented in a previous study utilizing a conjugate of SA with L-glutamic acid and a triazole spacer on maize. Pre-treatment of maize plants with this conjugate prior to inoculation with the phytopathogenic fungus *Fusarium graminearum* resulted in a significant reduction in disease symptoms[4]. Building on this basis, a set of conjugates between SA and amino acids has been developed by multi-step synthesis. Their biological efficacy is currently being investigated, and initial assessments of their impact on stimulating plant defense mechanisms suggest a clear relationship between their chemical structure and the modulation of SA-responsive genes.

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O12

The quest for grapevine metabolite localization during interaction with *Botrytis cinerea* through Mass Spectrometry Imaging

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Every year, viticulture is facing several outbreaks caused by various pathogens with different life cycles and modes of infection. To cope with these different aggressors, grapevine must recognize them and arm itself with an arsenal of defense strategies.

The regulation of secondary metabolites is one of the first reactions of plants upon pathogen challenge. Their rapid biosynthesis can highly contribute to strengthen the defense mechanisms allowing the plant to adapt, defend and survive. Most of the works published so far have focused on the accumulation of untargeted and/or targeted metabolites in a sample pool of infected tissue. However, with these approaches it is not possible to obtain knowledge about the actual localization of the accumulated metabolites or their specific sites of action.

Mass spectrometry imaging (MSI) analytical techniques enable to visualize and map the spatial distribution of metabolites within plant tissues allowing to a better understanding of metabolite biosynthesis, localization and functions.

We have studied the spatial distribution of different metabolites in grapevine leaves infected with *Botrytis cinerea*, using Matrix Assisted Laser Desorption Ionization-MSI. Our results demonstrated that, depending on the metabolic class, there is a specific pattern of accumulation in infected samples. Results showed that lipids and carbohydrates mainly accumulated at the pathogen infection site, while flavonoids and stilbenes accumulated around the infection site, with a high molecular diversity being observed for all classes.

Our work opens new doors for the scientific community to gain a comprehensive understanding of the dynamics and variations of metabolite profiles in grapevine organs, at different developmental stages and under various stress conditions. This knowledge is crucial for elucidating the role of specific metabolites in grapevine defense mechanisms, identifying specific regions of high or low metabolite production, which can contribute to targeted breeding to improve disease resistance traits and impact grapevine productivity and quality.

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Intra- and inter-cellular communications in plants

Decision making processes in the Arabidopsis seedling: a story of good governance.

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Plants often adapt to adverse or stress conditions via differential growth. We are studying growth tradeoffs – or differential growth decisions - in Arabidopsis in response to future climate scenarios consisting of multiple stress conditions. We have deployed two tools that have been used in decision theory: a well-defined yet limited budget, as well as conflict-of-interest scenarios.

The trans-Golgi network (TGN) has been implicated in stress responses, but it is not clear in what capacity it mediates adaptive growth decisions. We assess the role of the TGN in stress responses by exploring the previously identified interactome of the Transport Protein Particle II (TRAPPII) complex required for TGN structure and function. We identified physical and genetic interactions between AtTRAPPII and shaggy-like kinases (GSK3/AtSKs) and provided in vitro and in vivo evidence that the TRAPPII phosphostatus mediates adaptive responses to abiotic cues. AtSKs are multifunctional kinases that integrate a broad range of signals. Similarly, the AtTRAPPII interactome is vast and considerably enriched in signaling components. An AtSK–TRAPPII interaction would integrate all levels of cellular organization and instruct the TGN, a central and highly discriminate cellular hub, as to how to mobilize and allocate resources to optimize growth and survival under limiting or adverse conditions.

We are currently (1) elucidating the impact of post-translational modifications on the assembly and interactome of TGN-associated TRAPP tethering complexes, (2) characterizing functional interactions between TRAPP complexes and their possible substrates, and (3) assessing how these instruct sorting and trafficking decisions at the TGN. Mechanistic insights gained here are laying down a foundation for understanding plant cell division, adaptive growth, allocation decisions, and resilience to future climate conditions that include erratic or contradictory stimuli.

O13

Organization in nanodomains and function in the regulation of water transport of *Arabidopsis* HIR2 protein

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HIR are plant-specific proteins belonging to the superfamily of SPFH domain-containing proteins that are proposed to play scaffolding functions in membranes. HIR2 isoform organizes into plasma membrane (PM) nanodomains that correspond to nanoscale structures enriched in specific lipids and proteins, acting as signaling/regulation platforms. Using state-of-the-art microscopy techniques, we investigated the mechanisms governing the trafficking and the organization into nanodomains of Arabidopsis HIR2 protein. Our findings demonstrated that the S-acylations of two N-terminally located cysteines act redundantly to target HIR2 to the PM, although they are not essential for HIR2 nanoclustering. Moreover, we provided evidence that the lipid composition of the PM in sterols and very long-chain fatty acids influences HIR2 nanodomain organization. Importantly, we showed that HIR2 oligomerization via its C-terminal region was essential for its organization into nanodomains and for maintaining HIR2 lateral stability in the PM.

So far, the molecular functions of HIR proteins remain largely unknown. Interestingly, HIR proteins were identified in the interactome of Arabidopsis aquaporins from the PIP subfamily that facilitate the transport of water and H_2O_2 across the PM. We investigated a putative role of HIR2 in the regulation of PIP proteins and showed using co-immunopurifications and the split-ubiquitin method that HIR2 forms a complex with PIP proteins. Additionally, HIR influence the radial transport of water in the root, which is largely insured by PIP proteins, as attested by a strong reduction of the root hydraulic conductivity (Lp_r) in Arabidopsis *hir* mutants. Interestingly, co-expression of HIR2 with PIP2;1 protein in yeast enhances the PIP2;1-mediated transport of H_2O_2, suggesting a positive regulatory role of HIR2 on PIP. Ongoing research aims to decipher the role of HIR2 in PIP regulation by exploring a putative impact of HIR2 on PIP dynamics in PM nanodomains and/or on the modulation of PIP activity by phosphorylation.

O14

ROP6-mediated auxin signaling relies on plasma membrane lipid interleaflet coupling

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In cell signaling, extracellular signals are transmitted across the plasma membrane to activate downstream elements inside the cell. These signaling processes are based on protein complexes e.g. activation of receptor-like kinases (RLKs), transmembrane kinases and/or membrane associated kinase regulators. However, it now appears that lipids play a major role for signal transduction across membranes. Indeed, they could act on protein activity or activation, mobility, clustering or interaction with other proteins. More particularly, at the inner leaflet of the PM, phosphatidylserine (PS) is a crucial lipid that acts in the auxin-induced nano-clustering of the Rho-GTPase ROP6, thereby triggering auxin signaling[1]. However, PS nanodomains exist prior to any cell stimulation and a mechanism is lacking to explain how PS nanodomains are primarily formed within the lipid matrix of the PM. In this work, we used a combination of Fluorescence Recovery After Photobleaching (FRAP), Total Internal Reflection Fluorescence (TIRF) microscopy, super-resolution PhotoActivated Localization Microscopy (PALM) and molecular dynamics modeling to address the role of lipids in the lateral mobility and nano-clustering of PS and ROP6 at the PM. Our work reveals that the very long chain fatty acids (VLCFAs) of lipids are crucial in this process. In plants, VLCFAs are found in two main pools of membrane lipids, i.e the sphingolipids and PS. We show that VLCFAs of both sphingolipids and PS are involved in the lateral mobility and nano-clustering of PS and ROP6. Molecular dynamics simulations confirmed these experimental observations and further revealed that sphingolipids, that are located in the outer leaflet of the PM, interact with PS that are located in the inner leaflet of the PM. Computational simulations show that sphingolipid-PS interactions are dependent on their acyl-chain length and create a coupling between the two leaflets of the PM through lipid-lipid interdigitation. Altogether, our results identified an interleaflet lipid coupling mechanism that creates an orthogonal membrane organization that stabilizes or anchors PS and ROP6 signaling nanodomains.

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O15

Identification of a cytochrome P450 involved in apocarotenoid-mediated signaling of oxidative stress in plants

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β-Cyclocitral (β-CC) is a volatile apocarotenoid compound derived from the oxidation of β-carotene in the chloroplasts under oxidative stress conditions. β-CC converts *in vivo* to its oxidized form, β-cyclocitric acid (β-CCA). Both β-CC and β-CCA induce a retrograde signaling pathway that confers stress tolerance to plants, but the molecular mechanisms underlying this signaling are still elusive. A comparative transcriptomic analysis of leaves from β-CC- and β-CCA-treated Arabidopsis plants revealed a small group of 10 genes whose expression was significantly modified by short (4 h) and long (9 h) exposure to each compound. Interestingly, all those genes were related to cellular detoxification and were controlled by TGAII transcription factors. None of the selected genes were inducible by β-CC in a *tga256* triple mutant. Among the 10 genes, a gene coding for a cytochrome P450 was the most induced by β-CC and by high light (about x 100). Overexpressing this cytochrome in Arabidopsis led to a marked increase in photooxidative stress tolerance as shown by the suppression of lipid peroxidation after high light stress compared to the wild type (figure). These effects are specific to this cytochrome since they were not observed with other genes identified as responsive to both β-CC and β-CCA. Our results show that a cytochrome P450 plays a key role in photoprotection and its modulation by apocarotenoids. In this presentation, we will examine different aspects of the regulation, localization and function of this photoprotective cytochrome P450.

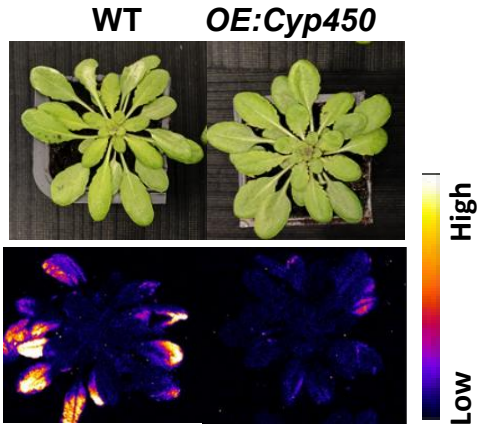

Figure: Lipid Peroxidation measured by autoluminescence of WT and *OE:Cyp450* after photooxidative stress treatment.

O16

Unlocking bud dormancy: exploring cell communication in aspen's adaptive response to temperature

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Plants are unable to escape unfavourable conditions and must instead adapt their growth and development to survive in the face of variable climate. To accomplish this, they must detect and integrate fluctuations in temperature and translate this information into robust decision making, at the multicellular level. Here we describe the mechanisms that facilitate accurate temporal control of bud dormancy release by temperature signal that is crucial for survival of trees in temperate regions. We show that accurate control of bud dormancy release is mediated by regulation of plasmodesmata (PD) in respons to low temperatures and identify a crucial role for FT1, a tree ortholog of Arabidopsis flowering time regulator *FLOWERING LOCUS T*. We identify FT1 as a temperature responsive regulator of PD-mediated cell communication that facilitates PD opening by downregulating callose levels to link temperature input with PD dynamics and bud dormancy release. These results thus identify a novel function of FT1 in symplastic trafficking and reveal how the accurate temporal control of bud dormancy is achieved to ensure tree adaptation to variable temperatures.

Plant Imaging

Spatiotemporal control of cell division in plants

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Spatial control of cell division and elongation is particularly important in plant tissues where cells are glued together by the pectocellulosic cell wall and are not capable of migration. Their shape and position within the organ (and thus the shape and architecture of the plant) largely depend on these two processes and their spatiotemporal coordination, orchestrated by a complex interplay of molecular mechanisms. Among these, cortical microtubules emerge as key regulators, playing pivotal roles during cell elongation and division. For example, in elongating interphase cells, cortical microtubules are responsible for the orderly deposition of the cell wall. As mitosis approaches, this cortical network is completely reorganized to form the preprophase band (PPB), whose position precisely predicts the position of the future division plane that will be formed at the end of mitosis during cytokinesis.

Our team aims at shedding light on the molecular and cellular processes involved in the spatial organization of cortical microtubules in plant cells, and on the connections of these processes with the cell cycle. In the course of our studies, we have identified a large protein network, the TTP complex, controlling the spatial arrangement of cortical microtubules both during interphase and at the PPB stage. This protein complex not only provides an excellent and unique entry point to better understand transitions of the cortical microtubule cytoskeleton, but it also allows us to explore regulatory circuits connecting these transitions to the cell cycle. I will present our latest findings on the function of the PPB and the pre-mitotic definition of the division plane using a variety of imaging approaches and quantitative image analysis.

O17

Deciphering the roles of reactive oxygen species and calcium during viral infection in Arabidopsis

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Abstract

As sessile organisms, plants always have to deal with different environmental stimuli, the generation of reactive oxygen species (ROS) and calcium (Ca^{2+}) flux as second messengers are one of the common strategies to response against such stimuli. Upon perception of pathogen, molecular warning signals propagate inside the cell and throughout the plant tissue to trigger defense mechanisms. However, the role of ROS and Ca^{2+} during viral infection process and associated signaling mechanism remains largely unknown. Viruses are obligate intracellular pathogens that hijack host machineries to facilitate their replication and propagation across the plant through plasmodesmata, communication channels bridging the plant cells. Here, we explored the mutual role of generated ROS and Ca^{2+} signals using biosensors and study the crosstalk between the two signals during viral infection. The results suggest that virus induces the ROS and Ca^{2+} elevation in virus infected and distal area on plant leaves and later virus hijacks the host ROS/Ca^{2+} machineries to invade the host plant. Using genetics, genome editing technology and advanced microscopy techniques including ROS biosensors, we showed that virus perturbs ROS production and the plasma membrane (PM) organization by interrupting PM localized protein nanodomains that contribute to the ROS and Ca^{2+} signaling.

O18

Do cell division patterns constrain monocot leaf patterning?

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Organization and patterning of the leaf venation network in flowering plants varies greatly between species. In particular, unlike the diverse reticulated vein networks that develop in the variably shaped leaves of eudicotyledonous plant species, monocotyledonous grass leaves are unified by a linear shape and a pattern of striated longitudinal veins that extend from base to tip. However, differences exist between monocot species in the number and type of veins that develop across the leaf. Notably, some features of the venation pattern are closely related to the type of photosynthesis performed by the plant.

The process that patterns veins relies on molecular and cellular events being co-ordinated in both space and time. Because of the shape (curled around the stem) and position (wrapped inside the older leaves) of monocot leaf primordia, the underlying mechanisms are difficult to dissect and poorly understood. We developed a 3D imaging method which allowed us to study the spatiotemporal complexity of venation patterning at the organ scale in monocots. Using this method, we conducted a kinetic analysis of vein formation during leaf growth in C_3 and C_4 photosynthesising model grass species - *Oryza sativa* (rice) and *Zea mays* (maize).

Development of the leaf venation network requires the specification of procambial cells within the ground meristem of the primordium and subsequent proliferation and differentiation of the procambial lineage to form vascular strands. By following cell divisions during early leaf growth, we investigated how growth in the leaf influences the parallel venation patterns observed in both species. Together our approaches enabled revealed distinct relationships between cell divisions and venation patterning in C_3 vs C_4 grasses.

This project is funded by the C4 Rice Project Grant (INV-002970) from the Bill & Melinda Gates Foundation to the University of Oxford.

O19

LCAT3 and LCAT4: dual key players in the final stages of autophagy in Arabidopsis

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Autophagy is an intracellular degradation process conserved across eukaryotes and critical for plant development and physiology. Autophagy relies on the formation of specialized membrane vesicles which traffic cargo to the lytic vacuole. Upon fusion with the tonoplast, autophagic bodies are released inside the vacuolar lumen and rapidly hydrolyzed to guarantee cargo degradation. How plant vacuoles deal with the large influx of autophagic bodies upon autophagy induction and how the membrane of the autophagic body is specifically hydrolyzed while maintaining the integrity of the tonoplast remain completely unknown in plants so far. Upstream of this project, immuno-isolation of autophagy compartments identified an atypical phospholipase, LCAT4, as a putative component of the autophagy machinery. Studying the subcellular localization of LCAT4, we mainly found this enzyme in the cytosol in addition to its association with early and late autophagy compartments, including the autophagic bodies. Further, our results show that upon starvation, LCAT4 massively relocate from the cytosol to the vacuole lumen using autophagy as a trafficking pathway. Given the enzymatic activity of LCAT4 and its optimal working environment in acidic pH, these results suggest that LCAT4 could be involved in the disruption of autophagic membrane and/or cargo in the vacuole. Seedlings knocked-out for *LCAT4* do not show defects in physiology or autophagic flux suggesting that the activity of LCAT4 could be compensated by additional phospholipases. In fact, we found that LCAT3, the closest homolog of LCAT4, also co-localizes with autophagic bodies upon starvation; lines knocked down for *LCAT3*, show a slight decreased in autophagic flux and LCAT3 is able to hydrolyze autophagic bodies when expressed in a heterologous system. Analyses of the double *lcat4 lcat3* mutant by electron microscopy suggest an accumulation of autophagic bodies inside the vacuole under starvation and this is correlated with a significant slowdown in the autophagic flux, demonstrating the importance of these enzymes in the recycling and degradation of components of autophagic bodies. Together, this work characterizes novel actors of the autophagy machinery thus shading light on the penultimate step of this critical process for plant tolerance to environmental stresses.

O20

Developmental controls of ER/PM tethering by phosphatydilinositol-4-phosphate in plants

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Eukaryotic cells are composed of different organelles that can communicate with each other by forming contacts in order to respond to different developmental and environmental changes. Plasma membrane (PM) forms extensive contacts with the endoplasmic reticulum (ER) at the ER-PM contact sites. These contacts play crucial functions in lipid homeostasis, Ca^{2+} regulation and signaling in all eukaryotes. In plants, one of the best-known tethering proteins at ER-PM contact sites are synaptotagmins (SYTs) that are orthologs of mammalian extended synaptotagmins (E-Syts) and the yeast tricalbins (Tcbs). Synaptotagmin1 (SYT1) is important for the maintenance of plasma membrane integrity upon various abiotic stresses. However, the mechanisms of SYT1 tethering to the plasma membrane and the dynamics of these contact sites remain elusive. Here, we investigate the importance of anionic lipids in the establishment and dynamics of SYT1 contact sites in plants. We found that phosphatydilinositol-4-phosphate (PI4P), rather than $PI(4,5)P_2$, is required for SYT1 association with the plasma membrane. Furthermore, we uncovered a PI4P-phosphatase called SUPPRESSOR-OF-ACTIN7 (SAC7) that associates with ER/PM contacts and controls both the quantity of PI4P at the cell surface as well as ER/PM tethering dynamics in a cell-type specific manner. Hence, we propose that PI4P regulation at the PM is a developmentally-controlled process that is critical for the establishment and dynamics of ER/PM contact sites and their related functions.

Plant development and nutrition

Checkpoints in cellular programming during root regeneration

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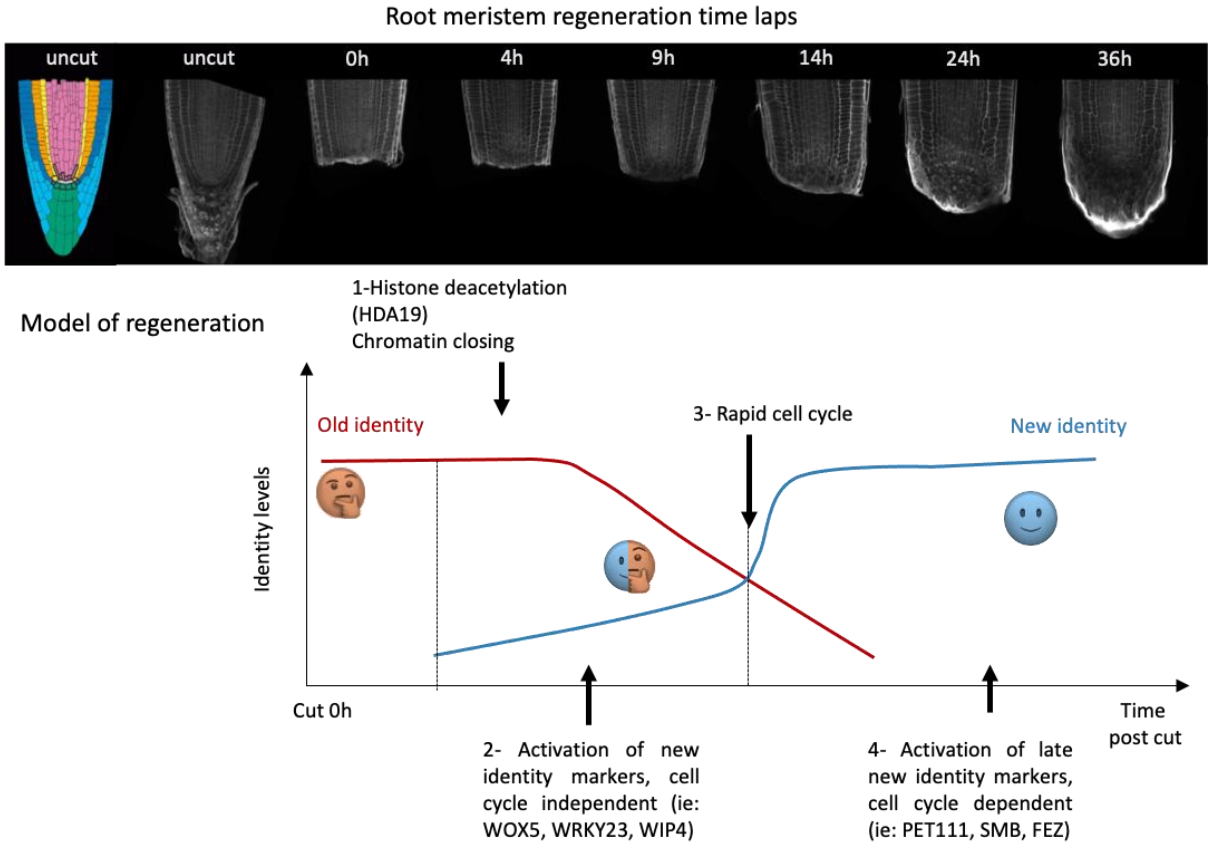
Plants have a remarkable capacity for regeneration. In *Arabidopsis*, the entire root tip—housing stem cells and specialized cells like the gravity-sensing columella—can be cut off and the remnant tissue will rapidly divide and differentiate to replace these missing identities. However, the mechanisms underlying this capacity of plant to fully regenerate are still poorly understood. In this project, using single cell RNAseq, microscopy and pharmacological approaches, we define a timeline of the major steps of regeneration and investigate the role of division and chromatin remodeling in this process. We show that while cell cycle inhibition blocks regeneration, some partial reprogramming can still occur. We outline three broad processes during regeneration—ectopic stem cell niche gene expression, loss of remnant identities, and gain of new identities—and show that some reprogramming events like ectopic stem cell niche gene expression are division-independent. Moreover, we show that histone deacetylase (HDAC) activity is critical at the very early stages of regeneration, potentially preceding the role of cell division. We propose that Class I HDACs, and specifically HDA19, are the prime mediators of early reprogramming in root tip regeneration. Altogether, this work provides new clues on how each cell identity is changing during regeneration.



O21

PUCHI regulates very long chain fatty acid metabolism and tissue functional patterning in developing lateral roots

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The architecture of the root system is an important trait for the plants to explore the soil, interact with its abiotic and biotic components and uptake of water and nutrients. Root branching, which consists in the formation of a new root organ, the lateral root primordium (LRP), from selected cells within existing roots, is a key process influencing this architecture.

The AP2/ERF transcription factor PUCHI was shown to participate in this organogenesis process. Its loss of function alters LRP initiation density, LRP morphogenesis and the regulation of the LRP developmental program and functional patterning. Inference of PUCHI-dependent regulatory networks suggested that PUCHI induces the very long chain fatty acid (VLCFA) biosynthetic pathway in branching roots, which was later confirmed by experimental data. By using a combination of gene network inference and functional genetics experiments, we seek to dissect the role of this PUCHI-dependent regulation of the VLCFA biosynthesis pathway in LRP development.

Our recent results show that PUCHI also regulates the incorporation of VLCFA into lipids, specifically in the cutin biosynthesis pathway. Because the LRP was recently shown to be covered by an atypical VLCFA-rich cuticle layer that is relevant for its development, the influence of PUCHI-controlled VLCFA and cutin biosynthesis on LRP development and morphogenesis is currently under investigation.

In addition, data mining allowed us to identify several candidate genes potentially involved in mediating PUCHI action on these metabolic pathways. Their role in LRP development is explored.

O22

Unravelling physiological and molecular mechanisms of leaf nitrogen uptake in *Arabidopsis thaliana*

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Foliar fertilization, a key agronomic practice, enhances crop growth and yield by facilitating nutrient uptake through leaf surfaces. This practice involves that plants absorb fertilizers from the leaf surfaces before assimilation. Nitrogen (N) is an important nutrient supplied to crops via the foliar route, mainly as nitrate (NO_3^-, mineral) and urea ($CO(NH_2)_2$, organic). Despite the well-established understanding of root nitrate perception and absorption, the molecular mechanisms underlying nitrate uptake by plant leaves remain elusive. Adressing this gap, our study focuses on characterizing nitrate and urea absorption by *Arabidopsis thaliana* leaves, aiming to identify the molecular actors involved in transport, sensing and signalling of these small N-based molecules in leaves. Our research objectives encompass critical aspects: (i) the dynamics of foliar nitrate absorption over short periods (0-240 min); (ii) the genome-wide transcriptional response to shoot nitrate supply; (iii) the role of foliar structures - cuticles, stomata and trichomes - in mediating absorption. Through meticulous experimentations using wild genotypes and mutants with altered leaf structures, we aim to untangle the intricate molecular pathways governing nitrate uptake through leaves.

Our findings confirmed nitrate is uptaken in leaves, in consistence with the rapid activation of known nitrate-responsive genes. Employing RNA-sequencing techniques, we identified novel molecular candidates potentially involved in both leaf nitrate uptake and the leaf-specific nitrate response pathway. Furthermore, when using a combination of the two N forms, this revealed a synergistic effect on leaf N accumulation. However, looking at the physiological effect of these distinct forms when sprayed on leaves, plants demonstrated different responses.

Our investigation extended beyond absorption kinetics to investigate whether plants responded differently to N from foliar versus root sources, addressing long-standing uncertainties surrounding nutrient acquisition mechanisms. By elucidating the molecular underpinnings of foliar nitrate absorption, our study contributes to the broader understanding of plant physiology and agriculture.

O23

How SlCCS52s, activators of the APC/C complex, influence tomato fruit growth.

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Plant growth and development rely on fundamental cellular processes such as cell division, cell expansion and cell differentiation which impact on plant yield and consequently on the quality of plant products. Endoreduplication, the process by which a cell replicates its genome but does not subsequently divide, leads to an increase in cell DNA content and is also essential for growth. However, still very little information is available on the gene networks controlling these processes contributing to productivity in crop species such as tomato. The Anaphase-Promoting Complex/Cyclosome (APC/C), a major E3 ubiquitin ligase complex, is essential for proper cell division, through the specific targeting to degradation of proteins with a direct role in cell cycle progression. Regulation of APC/C activity is facilitated by association with different proteins including CELL CYCLE SWITCH 52 (CCS52). In plants, CCS52 proteins were subdivided into three sub-groups based on studies in Arabidopsis: CCS52A1, CCS52A2 and CCS52B. By mutating, with CRISPR-CAS9 gene editing, the three SlCCS52s, we showed that these proteins have important roles in tomato fruit growth through the regulation of cell division and/or endoreduplication, with very distinct phenotypical outputs. Our findings indicate that translatability to crops is not straightforward.

O24

Cellular coumarin uptake mediated by CIT1 plays a key role in root iron acquisition of Arabidopsis

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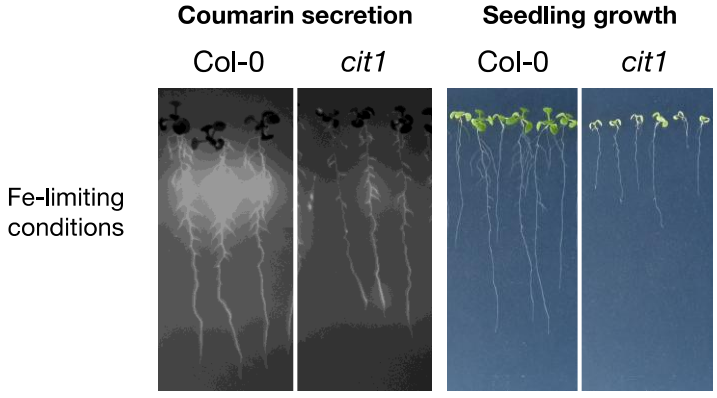
ABSTRACT

Iron (Fe) is essential for a wide range of physiological events in many organisms including plants since it serves as a central component of various types of metalloproteins. However, plants frequently suffer from Fe deficiency because the majority of Fe in natural soil is insoluble ferric (hydr)oxides, which is not bioavailable. To overcome this limitation, plants exudate plant-specialized metabolites capable of ferric Fe solubilization from roots in response to poor Fe environments. Fe-mobilizing coumarins (FMC), such as fraxetin, form a metabolite group contributing to this process in non-grass species. In Arabidopsis, FMC emission from cortex and epidermis into the rhizosphere relies on the the ABC transporter PDR9 (ABCG37). Interestingly, these cell layers were also shown to absorb coumarins exogenously supplied in Fe-limiting conditions. It raises the question of whether a cellular uptake of coumarins in these cells might play an important role in FMC secretion-based Fe acquisition. To answer this question, we aimed to identify membrane transporters involved in FMC uptake and characterized their physiological functions in Arabidopsis. We recently isolated Coumarin Import Transporter1 (CIT1), whose expression responds to Fe deficiency in Arabidopsis roots. Loss-of-function of CIT1 resulted in the disruption of coumarin secretion and seedling growth in Fe-limiting conditions (Figure). CIT1 protein accumulation was strongly induced in root epidermis encountering Fe deficiency, consistent with the major site of PDR9 localization. Transport assay using yeast cells demonstrated an uptake activity of CIT1 against fraxetin and its precursor scopoletin, but not against other compounds related to FMC biosynthesis. Given that coumarins are abundant in both cortex and epidermal cell layers, these results suggest that CIT1 reinforces PDR9-mediated FMC secretion from the epidermis by loading cortex-derived coumarins into the epidermis, leading to intense Fe acquisition.



SFBV PhD Award

Genetic and OMICS analysis of nectary development: identification of key traits for improvement of foraging activity

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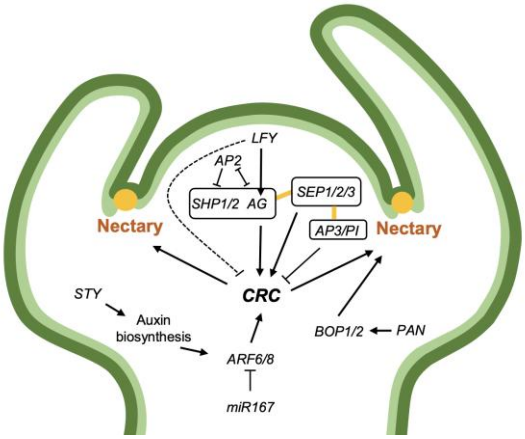
Nectar is the key reward that plants use to outsource pollination services and ensure reproductive success. The nectar-secreting organs – nectaries play the central role in maximizing seed set, however, the molecular mechanisms underlying their development remain understudied. This thesis addresses the challenge of improving the current understanding of nectary development and nectar secretion in relation to pollinator foraging. The project is structured around three scientific axes. The first axis is dedicated to phenotypic characterization of accessions of melon that differ in nectary development, nectar secretion and their effects on bee visitation. We implemented microcomputed tomography (µCT) which enabled a relatively easy and rapid generation of 3D volumetric data of the floral organs and bee bodies as well as 3D modeling of the nectar level which can be used to assess flower accessibility to pollinators in high resolution and perform comparative genotype analysis to identify pollination-related traits. To study the natural diversity of traits related to nectar production and foraging, we carried out an in-depth comparative analysis of nectar-related traits in the melon core collection. The second axis is dedicated to the characterization of the gene networks controlling nectary development in unisexual flowers using OMICS approaches. We characterized the nectar gland development in unisexual flowers. The analysis of gene expression from stage 9 of flower development until nectaries become functional is of major importance for the identification of key regulators controlling their development. The third axis is dedicated to the genetic validation of the concepts on the foraging activity and behaviour of bees. Finally, the objective of the project is to better understand the master genes controlling the nectar gland development and nectar secretion in relation to pollinators, as well as to identify phenotypes that could be targets for breeding.



14th International Conference of the French Society of Plant Biology SFBV

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Amphi Duguit, place Pey Berland, BORDEAUX

Plant and beneficial microorganism interactions, microbiome

Catching rhizobia to introduce high protein containing soybean for a sustainable agriculture in Europe

Goormachtig Sofie – **Speaker** ou poster presenter in bold

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To develop sustainable protein products, Europe would strongly benefit from soybean production at northern latitudes. However, soybean is not adapted to these environmental conditions and therefore the cultivation of protein-rich soybean is challenging. While several soybean varieties have been bred for optimal growth, to guarantee consistent high-protein beans, plants also need effective interaction with suitable soil bacteria that can fix nitrogen in root nodules. These nitrogen-fixing bacteria allow legumes to act as natural nitrogen fertilizers and green indicators of soil nutrition, and as such improve crop yield without the damaging effects of chemical nitrogen fertilization. Likewise, legumes contribute to solving major environmental challenges such as nitrogen pollution and declining soil quality. The current commercial inoculants are not adapted to cultivation under the local soil and environmental conditions of North-Western Europe. This hampers the interaction and leads to insufficient bean protein content for human food consumption. Local strains, adapted to our conditions may be more competitive and have a superior positive effect on soybean production. We set up a citizen science project to trap endogenous nitrogen-fixing bacteria that nodulate locally grown soybean. To have access to a large geographical gradient and different soil types in Flanders, over one thousand citizens across Flanders were recruited to grow and monitor soy plants, frequently entering data regarding plant phenotype into a platform specifically designed for the project. The outcome of the project in terms of microbial discovery but also correlations between soil parameters such as nutrient levels, soil texture, and nodulation performance will be discussed.

O25

Two bacterial apo-siderophores, pyoverdine and deferoxamine, impact plant iron homeostasis and promote the growth of *Arabidopsis thaliana* under iron-deficient conditions

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Key words: siderophores, *Arabidopsis thaliana*, iron-deficiency, growth, defense

Iron, an essential microelement for most organisms, is poorly bioavailable in soils. Bacteria acquire it by secreting siderophores that chelate it with high affinity. Our previous work showed that pyoverdine, a siderophore purified from the beneficial soil strain *Pseudomonas fluorescens* C7R12, stimulated plant growth when supplied in its iron-free form (apo-pyoverdine) to *Arabidopsis thaliana* seedlings grown under iron-deficient conditions. A transcriptomic analysis revealed that apo-pyoverdine strongly induced the expression of genes associated with the growth, import and redistribution of iron *in planta*. On the other hand, the expression of certain defense genes was reduced, and plants grown under iron-deficient conditions lost their resistance to a necrotrophic fungus when treated with apo-pyoverdine. Recently, we studied the impact of another apo-siderophore, deferoxamine (DFO), on the same model. Growth stimulation is linked to the ability of siderophores to chelate iron, as DFO also promotes *A. thaliana* growth in an iron-deficient environment. Growth stimulation depends on the amount of iron stored in the roots prior to DFO addition. It does not appear to depend on coumarin production, but is associated with increased expression of genes of the brassinosteroid signaling pathway that regulate the growth/defense balance. DFO stimulates lateral root elongation and induces a significant decrease in H_2O_2 and oxidized glutathione levels, as well as a decrease in the expression of stress-related genes in roots. In addition, DFO decreases expression of the *UPBEAT1* gene involved in H_2O_2-mediated root growth inhibition, and thus lifted inhibition of expression of its target genes, the class III peroxidases that stimulate root growth. Overall, our work indicates that growth promotion by apo-siderophores in an iron-poor environment is probably linked to their ability to induce a stronger response to iron deficiency while decreasing oxidative stress in roots, thus stimulating iron remobilization from root reserves to shoots and promoting secondary root growth.

O26

Understanding the mechanisms of interaction between vine rootstocks and soil microbiota, to move towards agro-ecological transition

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Soil is a reservoir of microorganisms playing important roles in biogeochemical cycles and interacting with plants either in the rhizosphere and in the root endosphere. Through rhizodeposition, plants regulate their associated microbiome composition depending on the environment and plant factors, including genotypes. Since the phylloxera crisis, *Vitis vinifera* cultivars are mainly grafted onto American Vitis hybrids. Rootstocks play a pivotal role in the grapevine development, as the interface between the scion and the soil. By interacting with the soil microbiota, the rootstock can contribute to the grapevine resilience to biotic and abiotic stresses. The ability of the rootstock to modify the structural and functional composition of the microbiota of the root system via rhizodeposition and exudation is therefore a potential key to viticultural adaptation.

Six rootstock genotypes were selected on the basis of their genetics (pure Vitis or hybrids), as well as their ability to control the scion growth and resistance to water stress. Samples of roots and rhizospheres were collected from the GreffAdapt plot in 2021 and 2022. The communities of bacteria, fungi, and arbuscular mycorrhizal fungi in the rhizosphere and the roots were analyzed by Illumina sequencing of 16S rRNA gene, ITS and 28S rRNA gene, respectively. The analysis of the metabolomic composition of the root and rhizosphere compartments is under progress and preliminary results will be presented. The metabarcoding results show dissimilarities in bacterial and fungal communities depending on rootstock or scion genotype, suggesting that both genotypes influence rhizosphere and root microbial composition, as well as putative microbiome functions (inferred using Picrust2 and FUNGuild). Finally, the roles of the microbiome in plant development and adaptation will be discussed by correlating its composition with plant phenotypic traits, as well as nutrient content of petioles and roots.

O27

An intracellular respiratory burst choreographs plant immunity independent of RBOH

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At pathogen attack, the plant cell recognizes conserved PAMPs by PRRs leading to a cascade of events to elicit PTI as a first layer of innate immunity. To investigate the extent to which RBOH activation contributes to cellular respiration, we measured oxygen consumption in leaves of Arabidopsis. We observed a sharp increase in oxygen consumption rate upon flg22. The increase was absent in the *bak1.5 bkk1.1* lacking functional PTI. Since elevated oxygen uptake coincided with the RBOH-mediated apoplastic ROS burst, we tested RBOH activation as a plausible cause. Elevated oxygen consumption was maintained, however, in the absence of the apoplastic ROS burst in *rbohd* and *rbohf* mutants, suggesting that RBOH activity had no impact on respiration. Accordingly, flg22 triggered oxidation of cytosolic H_2O_2 and glutathione redox potential as reporterd by the biosensors roGFP2-Orp1 (H_2O_2) and Grx1-roGFP2 (E_{GSH}) independently of RBOH activity. To test whether the mitochondria turn into a major source of intracellular oxidation upon flg22, we monitored *in vivo* NADH/NAD^+ dynamics using the cyto-nuclear biosensor Peredox-mCherry. We observed a pronounced and rapid transient in cytosolic NAD reduction in leaves in response to flg22. Deep characterization of NAD transient demonstrated it to be elicitor dose-dependent and a general PTI response not limited to Arabidopsis nor to elicitors. By using genetic and chemical interference, we observed that the NAD redox transient is governed by mitochondrial malate metabolism and oxygen availability while the activities of RBOHs are not required. Genetic impairment of elicitor-induced NAD transient through the ablation of mitochondrial *NAD-ME1* and *NAD-ME2* did not impair the induction of PTI-marker genes and apoplastic ROS burst while susceptibility to *Pseudomonas syringae* pv. tomato DC3000 and *Colletotrichum higginsianum* were observed. Our findings connect central metabolism of plants with their immune capabilities and place the intracellular respiratory burst as a new player in PTI signaling.

O28

Unveiling the mechanistic of plant immune activation by lipopeptides from beneficial bacteria

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Cyclic lipopeptides (CLPs) represent a prominent and structurally heterogeneous class of specialized metabolites synthesized by plant-associated bacteria that retain multiple ecological functions. Some CLPs from beneficial species of the genera *Pseudomonas* and *Bacillus* are potent inducers of immune responses in the host plant and confer systemic induced resistance (ISR) to infection by microbial pathogens, which is a key process for biocontrol of crop diseases. However, in contrast to the well-described pattern-triggered immunity (PTI), the molecular basis of CLP-triggered ISR remains poorly understood. We are investigating immune activation in *Arabidopsis thaliana* by the CLP surfactin from plant beneficial bacilli which is one of the bacterial compounds best described as an immune inducer in several plant species. Our data show that surfactin perception by plant cells is mediated by docking into specific sphingolipid-enriched domains of the plasma membrane and relies on host membrane deformation and subsequent activation of mechanosensitive ion channels. This mechanism leads to a specific immune activation signature regarding the type, timing, and amplitude of defense-related events associated with systemic resistance to the fungus *B. cinerea*. We will discuss the pro and cons of this peculiar mechanism in an applied perspective considering CLP-induced ISR as a biological alternative to the use of chemicals in sustainable agriculture.

Environmental stresses

How plants deal with heat and cold: Molecular mechanisms of auxin transport in response to temperature stress

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Our planet is warming, and extreme weather events such as sudden heat waves or cold spells will only become more frequent. Temperature stress can severely affect plant distribution, health, and productivity. While most studies to date have focused on the big-picture elements of plant responses to climate change (e.g., biomass), future research needs to focus on molecular and cellular responses to improve our mechanistic understanding of plant adaptation to heat and cold stress. To adjust and adapt, plants rely on hormones such as auxin, which plays an essential role in regulating plant growth and development. Its tightly regulated distribution throughout the plant body controls an impressive variety of developmental processes that tailor plant growth and morphology to environmental conditions. Auxin undergoes directional transport from one cell to another, which allows asymmetric distribution of this hormone in different cells and tissues. This system creates local auxin maxima, minima, and gradients that are instrumental in both organ initiation and shape determination. However, the molecular mechanisms by which auxin transport is modulated/regulated at the cellular level upon temperature stress are not well explored. Hence, we investigate the molecular mechanisms involved in auxin transport upon temperature stress, down to the tissue and cell-type-specific level, focusing on the root of the model organism Arabidopsis thaliana. Taken together, the fundamental knowledge obtained through our research will contribute to the mechanistic understanding of plant responses to the temperature variability that will accompany climate change.

O29

Elucidate the reality and functionality of Natural Deep Eutectic Solvents (NaDES) formation in desiccation tolerant seeds

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To ensure their continuity on earth, plant species must transfer their genetic heritage to the next generation via the reproductive propagules, i.e., pollen grains and seeds. As part of their development, these tissues undergo programmed desiccation and enter into dormancy to cope with various stresses during dispersal. Although the complete process of acquiring desiccation tolerance is not fully understood, the accumulation of compatible metabolite solutes and molecular chaperones is a key mechanism for stabilizing macromolecules during dehydration. Recently, some of these solutes have been highly coveted by chemists for preparing new classes of green solvents called natural deep eutectic solvents (NaDES). These solvents formed from the deep lowering of the melting point in mixture between biobased hydrogen bond donor and acceptor compounds, are much studied for their various applications such as solvation or biocatalysis, as well as for their putative occurrence as a third liquid phase in living dehydrated tissues. However, NaDES formation in cells remains unproven. The aim of this work is to provide tangible arguments regarding NaDES formation in oilseed rape (*Brassica napus L.*) and their role in seed desiccation tolerance. We first confirmed the presence of reliable NaDES ingredients in seeds using metabolic profiling techniques. Their colocalization and molecular interaction was also established *in vivo* by mass spectrometry imaging (DESI). Some of these ingredients have been highly predicted to form NaDES by cosmo-RS simulation software. In addition, their affinity to each other was assessed by cold spray ionization mass spectrometry (CSI-MS). Based on prediction and affinity results, some NaDES were prepared *in vitro* and their physicochemical properties studied. These properties then served as the basis for selecting some seed bioinspired NaDES in order to test their ability to protect macromolecules such as enzymes and physiological processes including embryo viability and germination performance.

O31

A unique *O*-methyltransferase catalyzes the biosynthesis of two methylated *C*-glucosyl flavones specifically accumulated in winter flax varieties

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Linum usitatissimum (flax) is a plant cultivated both for its fibers and seeds that are rich in omega-3 essential fatty acid. Over the last few years in France, fiber yields have been significantly reduced as a result of drought stress, occurring during the first stage of spring flax development. As a consequence of climate change, these accidents may occur more and more frequently. In this context, one solution is to avoid the stress by using winter varieties that are less exposed to drought period during their growth. The current lack of knowledge regarding flax cold tolerance hampers the effective development of winter varieties. Recently, two C-glucosylated flavones, *i.e*. swertisin and swertiajaponin, were found to be specifically accumulated in winter varieties and, thus, considered as interesting biomarker candidates for cold tolerance. To test this hypothesis, we attempt to identify the molecular determinants responsible for their synthesis. Through a quantitative trait loci (QTL) analysis, associated with the 2 flavones accumulation, a candidate gene was identified, which encodes a type I OMT protein (*LusOMT1*). *In vitro*, recombinant LusOMT1 protein turned out to specifically methylate the *C*-glycosyl flavones isovitexin and isoorientin at the 7-hydroxyl position to form swertisin and swertiajaponin, respectively. Moreover, transient overexpression of *LusOMT1* in *Nicotiana benthamiana*, a species that does not naturally accumulate these flavones, resulted in the production of swertisin and swertiajaponin when the substrates isovitexin and isoorientin were co-infiltrated. To assess the role of these molecules in cold tolerance, experiments are currently in progress, which consist in blocking their synthesis in winter flax varieties or activating it in spring varieties. These data should pave the way to the improvement of winter flax selection by breeders, which will help reducing the negative impact of climate change on flax fiber production in the future.

Keywords: Flax, Cold stress, *C*-glycosyl flavones, biosynthesis

O32

Unveiling the role of FOCL1: Modulating Guard Cell Structure and Plant Defense Against Drought and Pathogens

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Stomata, consisting of a pair of guard cells, are critical for regulating gas exchange and water loss in plants. To cope with the large increases in turgor pressure required to open the stomata, the guard cells require thick, elastic cell walls, especially around the outer cuticular edge. To identify novel key components involved in stomatal regulation, we conducted a screen for genetic suppressors of *ost2-2D*, an Arabidopsis mutant severely impaired in stomatal closure, using infrared thermal imaging. Through this screen, we identified an extragenic suppressor of *ost2-2D* intense transpiration named *hot4 (hotter ost2-2D temperature)*, which exhibited significantly higher temperature in young central leaves, despite showing no morphological differences compared to *ost2-2D* and wild-type leaves. Map-based cloning of *hot4* identified a stop codon mutation in the *FUSED OUTER CUTICULAR LEDGE1* (*FOCL1*) gene, which encodes a secreted protein with homology to hydroxyproline-rich cell wall proteins. When separated from the *ost2-2D* mutation, *focl1* mutants showed delayed rupture of the outer cuticular ledge (OCL) in stomata during leaf development leading to lower transpiration rates when compared to wild-type leaves. The delay in OCL removal in *focl1* plants coupled with reduced stomatal aperture flexibility conferred resistance to drought and *Pseudomonas syringae* (DC3000). This study highlights the crucial role of the OCL in stomatal function and its importance in plant physiology. Understanding these mechanisms provide new opportunities for developing strategies to protect crops against water stress and diseases.

O33

Understanding primary metabolism trade-offs of sub-Antarctic aquatic plants to water temperature increase in the context of plant-plant interactions

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All climate scenarios predict that temperatures will increase over the next decades, with effects on plant growth and phenology that could disrupt species interactions. The effects of climate change are intense in polar region and in aquatic systems harbouring plants (i.e. macrophytes), which constitute a resource and a habitat for many organisms. These aquatic plants are organised in communities, with interactions that could affect individual response to environmental changes. However, these responses to thermal stress in a context of plant-plant interactions remains so far poorly studied.
Plant response to new environment through morphological changes is likely to be based on plant metabolome rearrangements. The literature showed that individual plants exhibit opposing leaf response traits to heat stress compared to plant-plant interactions, relying on metabolic trade-offs. It thus raises the question of how plants can simultaneously cope with plant-plant interactions and increasing temperature, based on metabolic responses, and how this affects their performance.
In this context, we studied the metabolic responses to water temperature increase in two macrophyte species living in plant communities of the Iles Kerguelen (sub-Antarctic region). To understand metabolome changes, targeted metabolic analyses were performed on plant grown at 13°C, 18°C and 23°C, with different plant species as neighbours. We obtained metabolic fingerprints of each species and described the changes in primary metabolites due to heat stress. Then, we investigated neighbouring plant effects on the metabolic response to thermal stress.
We highlighted that each species had different metabolic responses to heat stress, while they were both less influenced by surrounding plants at all temperatures (Figure 1). However, deeper result analyses showed that neighbouring plants modified the metabolic responses to heat stress revealing metabolic trade-offs.
Characterising plant responses to heat stress is an essential prerequisite to understanding plant adaptation to climate change; however, further studies should integrate plant-plant interactions.

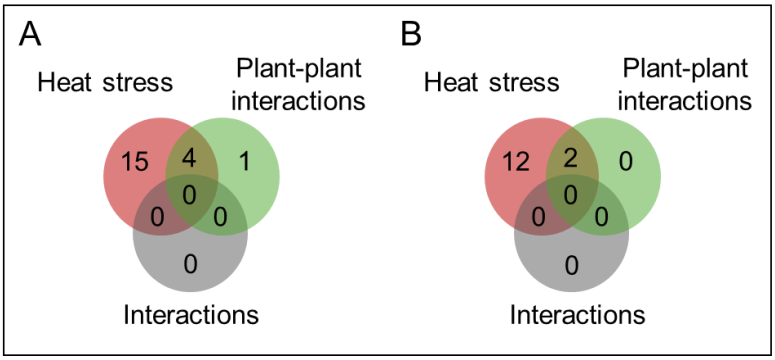

Figure 1: Venn diagrams summarising the two-way ANOVA results on 35 primary metabolites for the two species studied, *Callitriche antarctica* (A) and *Limosella australis* (B).

Genomics, genetics and breeding

Plant genetic resources: value for research, breeding and society

Alain Charcosset

Since the emergence of modern breeding in the mid-1800s, traditional cultivars (landraces) of most regions of the world have been replaced by improved varieties adapted to the objectives of new farming systems. This replacement has been wisely accompanied by the conservation of landraces in genetic resource collections (genebanks) and, to some extent, complementary initiatives such as in situ conservation and participatory breeding. Millions of accessions are currently stored in numerous genebanks throughout the world. They long have been utilised to a very limited extent, to the point that genebanks were called “seed morgues” in the 1980s. I will review how i) the emergence of association genetics gave them considerable value for genetic studies since the early 2000s and how (ii) advanced genomic prediction methods now make it possible to incorporate them efficiently in breeding programs to sustain their diversity and long-term efficiency. I will then discuss how climate change and agroecological transition call for more diversified varieties, for which genetic resources are expected to be key.

O34

BREEDIT : a multiplex genome editing strategy to improve complex quantitative traits in maize

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Growth and yield are complex quantitative traits that imply multiple molecular pathways of which the interplay is highly complex. To decipher the molecular networks and gene combinations that contribute to these relevant agronomic traits, we developed a flexible pipeline called BREEDIT (an acronym of breeding and gene editing) that combines multiplex gene editing with directed crossing schemes to integrate higher order edits per generation (**Figure 1**) (Lorenzo *et al.*, 2023). Using a Cas9-expressing maize line called EDITOR, super-transformed with multiple guide RNAs (gRNAs) bearing constructs called SCRIPTs, multiple candidate genes can be simultaneously mutated. In addition, higher order combinations can be achieved by crossing plants carrying the different SCRIPTs. This strategy allows to target entire gene families or multiple components of regulatory pathways, bypassing redundancy and enabling the detection of novel additive or synergistic gene combinations with the aim to improve agronomically relevant traits. In our plant population, a complex mosaic of INDELs or SNPs was found that are homozygous, heterozygous or biallelic with almost every plant proving to be unique, making statistical analysis impossible. Using high-throughput phenotyping in seedling and mature plants, we generated quantitative and qualitative data and used AI models to detect intricate patterns in highly complex datasets, to decipher the underlying relationships and determine the causative gene combinations. In parallel, to identify gene-edit combinations responsible for a given phenotype, we generated low-order mutants from our segregating lines using haploid inducer lines (Impens et al., 2023). Thus, using multiplex genome editing, double haploid technology and AI models, we identified gene-edit combinations responsible for an increase in leaf and yield-related growth parameters.

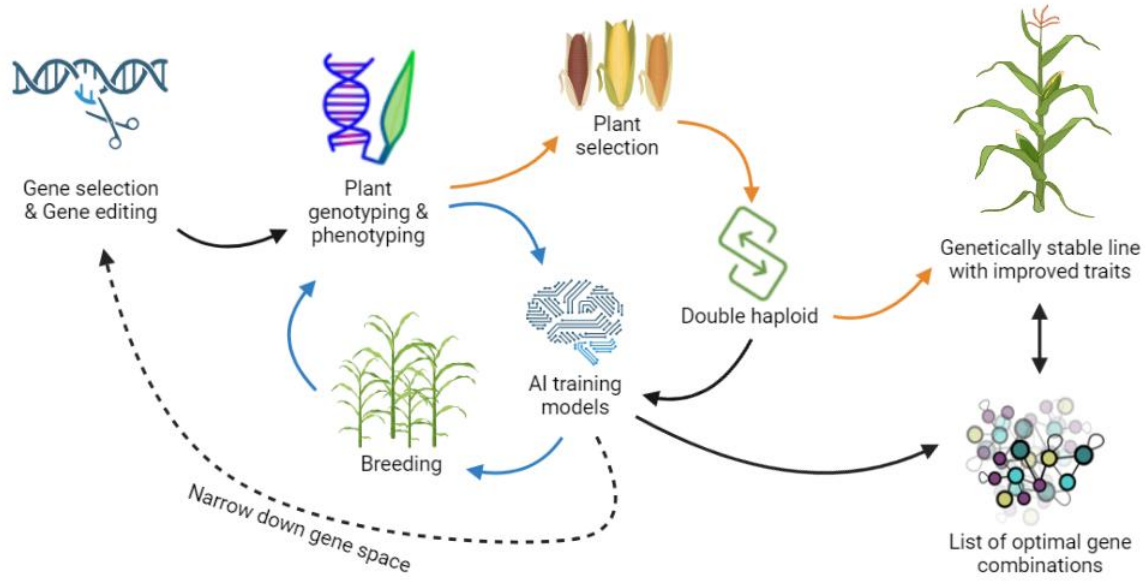

Figure 1. Concept of BREEDIT.

O35

The GATA4 transcription factor is a potential integrator of C/N signaling under elevated CO_2 in Arabidopsis thaliana

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The regulation of C/N signaling is essential to ensure proper plant growth according to the environmental conditions. Because of the rise in atmospheric CO_2 concentration, C/N balance in plants is currently disrupted, and this leads to major modifications in plant growth and physiology (Gojon et al., 2023). We recently followed a Genome-Wide Association approach that identified several genes associated with the alteration of N status under elevated CO_2 (Cassan et al., 2023). Among these genes, we report here the characterization of the transcription factor *GATA4*. We notably show that the natural variation of N content under high CO_2 is due to the haplotype-dependent expression of *GATA4*. Moreover, the analysis of *gata4* knock-out mutant, along with transcriptomic analyses, reveal that GATA4 is a potential integrator of C/N signaling. Indeed, the *gata4* mutant has reduced growth, biomass and N content compared to wild-type plants under elevated CO_2, and several major actors of C and N metabolism were identified as direct targets of GATA4, including *GLN2*, *NLPs* and cell wall remodeling genes. This work positions GATA4 as a major regulator of C/N signaling, and unveils novel molecular mechanisms influencing C and N metabolisms under elevated CO_2 in Arabidopsis.

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Gojon, A., Cassan, O., Bach, L., Lejay, L., and Martin, A. (2023). The decline of plant mineral nutrition under rising CO2: physiological and molecular aspects of a bad deal. Trends in Plant Science **28**:185-198. 10.1016/j.tplants.2022.09.002.

O36

Understanding graft union formation and graft incompatibility in grapevine

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Plants can naturally graft their trunks, branches and roots together, this mechanism is exploited by humans to graft together different genotypes for agricultural purposes. Graft union formation requires scion/rootstock adhesion, the construction of a common and functional cell wall at the graft interface, and the formation of vascular connections between the two partners. In some cases, the scion and rootstock do not form a successful graft and the graft interface is associated with problems of necrosis, which affects plant survival, even several years after grafting. Our research goal is to understand graft union formation and genetic regulation of graft incompatibility in grapevine, an economically important grafted crop. We aim to understand both the morphological and molecular changes occurring during graft union formation over time. We are using micro-computed tomography to study morphological changes occurring during graft union formation and the connection of functional xylem vessels between the scion and rootstock. We have used metabolome and transcriptome analysis to understand the molecular changes occurring during graft union formation; we show that changes in gene expression are associated with corresponding changes in tissue metabolite concentrations. We found that stilbenes accumulate in the wounded xylem parenchyma tissues, whereas naringenin accumulates in the newly formed callus tissues (Figure 1). This pattern of stilbene accumulation suggests that stilbenes have a role in plant defense. Whereas, metabolites accumulated in the newly formed callus tissues presumably have a wider range of roles such as forming new vascular tissues, signaling, defense, and developing a functional graft union. Empirical knowledge from grapevine nurseries suggests that graft compatibility is genetically controlled; we are currently characterizing the genetic architecture of grafting success in a biparental rootstock population. Ultimately, we want to transfer this knowledge to industry to improve grafting protocols and develop markers for selection of future rootstocks.

Figure 1. Spatial distribution of resveratrol (red), catechin/epicatechin (green) and naringenin (blue) at the graft interface of grapevine 15 d after grafting. Dotted line indicates the graft interface (from Loupit et al., 2023, DOI: 10.1111/pce.14693)

Plant and algae metabolism

New Insight into Starch Biosynthesis: How Plants Control Starch Granule Initiation and its Crystallization.

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ABSTRACT

Starch is a vital plant product and accumulates in plastids of green algae and plants. The major component of starch, amylopectin is a branched glucan polymer with similarities to glycogen made in animals, fungi and prokaryotes. However, unlike glycogen, starch occurs as insoluble, semi-crystalline granules which, in Arabidopsis leaf chloroplasts, always form between the thylakoid membranes. Using complementary approaches (electron tomography, isotope labelling and NanoSIMS imaging), we visualized how starch granules initiate and subsequently grow. Further, using a combination of biochemistry and genetics we identified a set of proteins involved in the initiation process. Modulation of these proteins can greatly influence the number of starch granules that form. One protein, MFP1 (MAR BINDING FILAMENT-LIKE PROTEIN 1) is anchored to the thylakoid membranes and assembles into discrete patches within the membrane system. Remarkably, MFP1 directs where the starch granules will form, and re-localising it is sufficient to change the site of starch formation. Many proteins involved in starch biosynthesis become embedded within the starch granules as they grow. Two granule-bound proteins, LIKE EARLY STARVATION 1 (LESV) and EARLY STARVATION 1 (ESV1), identified using proteomic analyses, both possess an unusual, conserved carbohydrate-binding surface. Functional studies in yeast and in plants suggest that LESV and ESV1 help promote the transition of amylopectin from a soluble phase to a semi-crystalline phase, and then stabilize the resultant granules. This is remarkable since crystallization was long assumed to occur spontaneously. Collectively, these studies into the cell biological aspects of starch biosynthesis offer a range of new gene targets with which to influence starch biosynthesis, to improve the amount of starch and/or starch properties in crops.

O37

Inactivation of LIKE EARLY STARVATION 1 in potato (*Solanum tuberosum* cv. Désirée) affects starch granule initiation in tubers

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Potato, because of the high amount of starch contained in its tuber and its unique thickening and pasting properties, finds various applications in industries such as paper, food, textiles, or pharmaceuticals. Starch properties are directly influenced by its molecular structure and organization. Potato tubers accumulate large amount of starch with granules displaying an average diameter of 25 µm. However, the starch market is on growing demand for smaller starch granules suitable for several applications, including biodegradable films. Understanding the starch initiation process, which governs granule size and morphology, is pivotal for controlling these parameters *in planta*. This process still remains unraveled in potato. Investigation of the potato tuber starch granule proteome has led to the discovery of novel non-catalytic proteins potentially involved in starch metabolism. Among them, LESV (LIKE EARLY STARVATION 1) was suspected to regulate starch metabolism and, interestingly, to be involved in controlling the priming of starch granule formation by interacting with other proteins/enzymes of the starch pathway, notably ISA1, a starch debranching enzyme suspected to control the formation of primer glucans in rice seeds and potato tubers. Here, using a CRISPR/Cas9 approach, the expression of gene LESV was knocked out. In nine independent KO lines produced, a strong size reduction of starch granules was obtained, and granule morphology was strongly impaired compared to the wild-type reference, without being affected for starch content, tubers number and plant development. Except a significant increase of starch phosphorylation in C6 position, starch ultrastructure was unaffected in *lesv* mutants. Our results, placed in the context of the recent description of rice *lesv* mutants, argue for the involvement of LESV in controlling the starch granule priming in potato tubers.

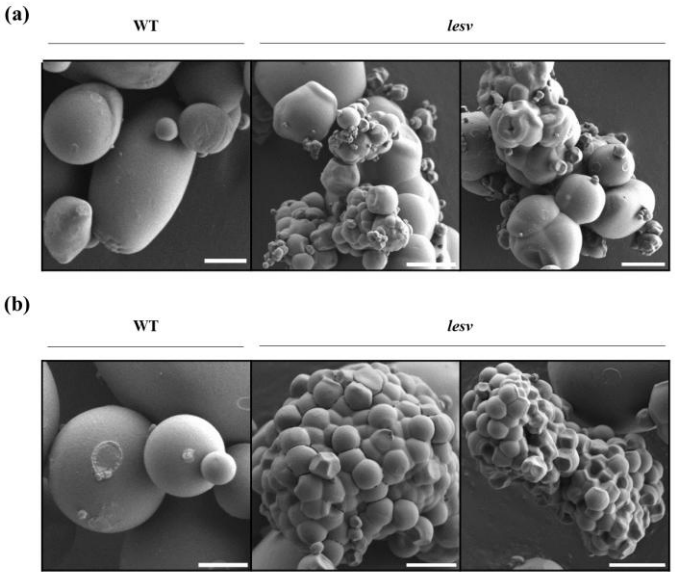

Figure: Scanning Electron Microscopy of purified starch granules of wild type (WT) and *lesv* mutants. Scale bar in (a) = 10µm and in (b) = 5µm

O38

A SCPL acyltransferase promotes chicoric acid and chlorogenic acid derivatives synthesis in chicory.

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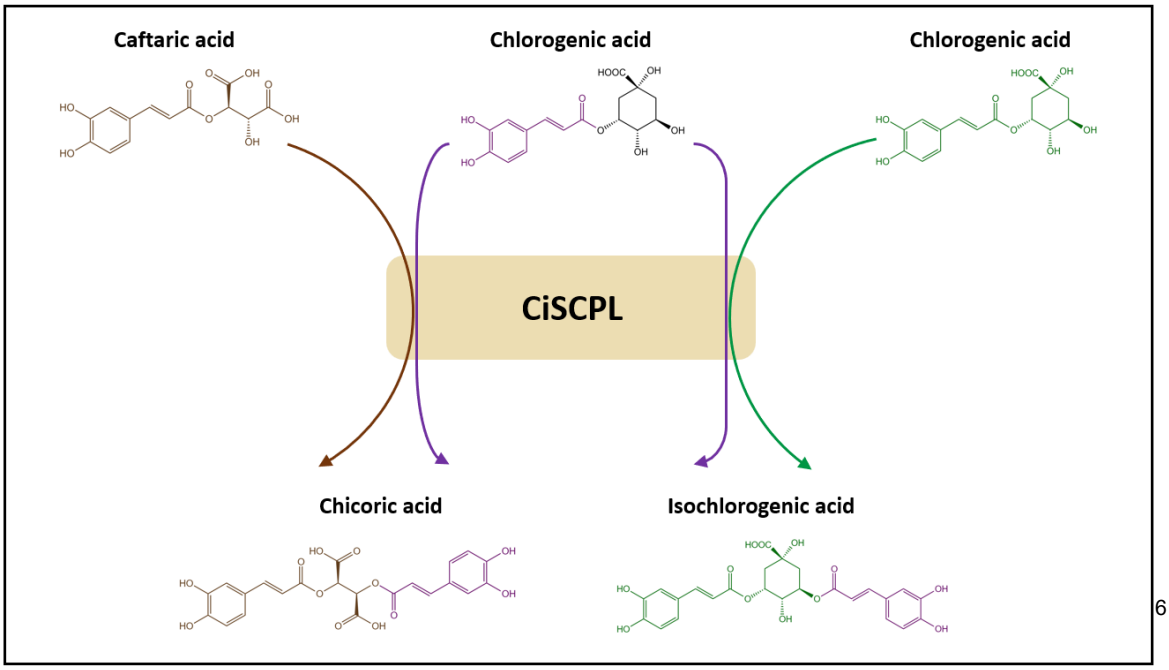
Caffeic acid esters are phenolic compounds widely distributed in the plant kingdom. They arise from the conjugation of caffeoyl moieties with various acids, including quinate, shikimate, tartrate and malate. Due to the presence of multiple hydroxyl groups, these molecules possess numerous biological properties namely antioxidant, anticancer, antiviral, and neuroprotective properties. Within plants, they play crucial roles in defense against pathogens and abiotic stresses such as UV exposure. Industrial chicory (*Cichorium intybus* var. *sativum*), a member of the *Asteraceae* family, has been historically used in northern France to produce a coffee substitute but also in traditional medicine. Its biological properties are partly due to the accumulation of four major caffeic acid esters: chlorogenic, isochlorogenic, caftaric and chicoric acids. While isochlorogenic acid is predominantly accumulated in roots, chicoric and caftaric acids are mainly located in shoots. Chlorogenic acid is equally accumulated in both plant parts. If the biosynthesis of chlorogenic acid is well documented in chicory, the metabolic pathways of the others remain to be described. In the present work, a gene encoding a protein belonging to the SCPL family, CiSCPL, was identified and characterized. *In vitro*, recombinant proteins catalyze the transfer of the caffeoyl moiety of chlorogenic acid to caftaric acid leading to the production of chicoric acid. Interestingly, when caftaric acid was omitted isochlorogenic acid could also be produced. These *in vitro* data were also supported by *in planta* experiments using *Nicotiana benthamiana* as a host platform. Additional experiments are under progress to definitively link the synthesis of these compounds to the activity of this protein. Elucidation of the molecular determinants of these pathways will help breeders to select plants with high nutritional values or improved fitness and could be of interest to initiate genetic engineering strategies of microbial hosts for the bioproduction of these high value molecules.

Putative role of CiSCPL protein in chicory



O39

Highlighting the plant responses against multi-stresses: Focus on heat induced grape berries tolerance to Botrytis

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Vineyards are facing an increase in heat waves due to climate change which can interfere with wine quality and production. In addition, common practices such as leaf thinning expose bunches to higher temperatures, particularly during fruit ripening. These temperature rises modify berry metabolism – *i.e.* sugar boost in detriment to organoleptic compounds – and potentially their response to diseases, such as gray mold caused by *Botrytis cinerea*. This fungus infects post-veraison fruit during the summer, and its uncontrolled spreading can severely reduce yield. Our research on fruiting cuttings endeavors to elucidate the intricate interplay between heat stress (HS, 6 days at + 10°C: 25°C vs. 35°C) and tolerance to subsequent Botrytis infection. In order to accurately reproduce vineyard microclimate, HS was applied only at the bunches levels of two prominent varieties, the cabernet-sauvignon and merlot, with contrasting tolerances to *B. cinerea*. *In vitro* test on detached berries inoculated 22 days post-HS revealed an improvement in tolerance against *B. cinerea* only for cabernet sauvignon bunches stressed at herbaceous stage, underscoring sensitivity of stress responses depending on both genetic background and exposure timing. We also investigated the accumulation of chemical and physical barriers in the fruits that with a focus on cuticle – *i.e.* waxes and cutin – and antimicrobials tannins. The heat-induced tolerance found in our study seems to be without any trade-off for the maturity or yield.

Interplay between carbon allocation and water flows under elevated atmospheric CO2: the role of SUC transporters and of aquaporins

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The transport of photoassimilates in higher plants plays a crucial role in their growth and storage processes, primarily facilitated by the phloem system. This transport occurs through a mass flow mechanism driven by hydrostatic pressure differentials, which are influenced by osmotic water movement generated by solute transport across membranes. Among these solutes, sucrose is crucial in generating osmotic potential within sieve tubes. Reverse genetics has demonstrated the critical role of sucrose/proton symporters from the SUC/SUT family in phloem loading and transport processes. Additionally, aquaporins from the PIP2 subfamily have been detected along the phloem pathway. This suggests their involvement in facilitating water exchanges between sieve tubes and adjacent cells, potentially aiding in radial water exchanges between the xylem and phloem.

In this study, we cultivated both *PIP2* knock-out quintuple mutant and transgenic lines with defective *SUC2* expression under two atmospheric CO_2 conditions (ambient or elevated). This approach allowed us to observe the effects of elevated CO_2 (eCO_2) on photosynthesis, carbon fixation, and carbohydrate storage, and how these factors influence carbon allocation for plant growth and root development. The results, which showed increased root and shoot biomass under eCO_2, were as expected. However, the observation of elevated leaf temperature in both *PIP2* and *SUC2*-defective lines, regardless of the CO_2 conditions, was not anticipated, indicating a defect in evapotranspiration. Despite this, under eCO_2, both *PIP2* mutant and *SUC2*-defective lines demonstrated more efficient translocation of carbon to the roots compared to wild-type plants. However, the consequences on root biomass and sugar accumulation in the roots varied between the lines. This study provides a new perspective on the complex interplay between carbon allocation and water flow regulation in plants, particularly under different atmospheric CO_2 conditions. Further investigations are warranted to fully understand the integration of the processes coupling carbon allocation and water flows.

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POSTERS
Session 1

P1

Striking similarities and divergences between HIGH CHLOROPHYLL FLUORESCENCE 101 proteins in plants and apicomplexan parasites Fe-S cluster assembly machineries

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Iron-sulfur (Fe-S) clusters are universal co-factors embedded in proteins within which they promote electron transfer, catalytic activities and protein folding [(1)]. **Proteins carrying such cofactors** (Fe-S client apoproteins) **support a large panel of fundamental biological functions**. To supply them with Fe-S clusters, cells have evolved towards the use of multicomponent Fe-S assembly and delivery machineries in various cellular compartments. They include the ISC (Iron-Sulfur Cluster) machinery in proteobacteria and in eukaryotic mitochondria, the SUF (Sulfur Utilization Factor) machinery in plastids, and the eukaryotic CIA (Cytosolic Iron–sulfur Assembly) machinery dedicated to cytosolic and nuclear client apoproteins [(2-3)]. Because of the importance of Fe-S client proteins in many cellular processes, our collaborative project aims at identifying essential components of these machineries and their specific roles in these pathways.

Here we focus on HCF101 proteins, a small class of atypical P-Loop NTPases belonging to restricted and divergent eukaryotic phyla, including plant, algae and apicomplexan parasites. The Arabidopsis HCF101 was the first identified as a late-acting Fe-S carrier protein in the SUF machinery [(4-5)] and shown to be essential for photosynthesis by targeting the [4Fe-4S] PsaC photosystem I (PSI) subunit [(6)]. Here we show that AtHCF101 is also essential for the biogenesis of the NDH (NADP dehydrogenase) complex in plastid, by targeting two Fe-S proteins of the NDH subcomplex A [(7)]. The Apicomplex parasite *Toxoplasma gondii* (*Tg*) also possesses an ancestral plastid (apicoplast) but its photosynthetic function has been lost during evolution, questioning the function of TgHCF101. Contrary to plants, we found that TgHCF101 localizes in the cytosol where it interacts with ABCE1, a highly conserved [4Fe-4S] protein required for eukaryotic translation initiation and ribosome biogenesis [(8)]. In conclusion, although HCF101 proteins share a highly conserved three-domain structure, they have evolved specialized functions in distinct Fe-S assembly machineries in plants and Apicomplex parasites.

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P2

Meristem response to the heat stress in tomato

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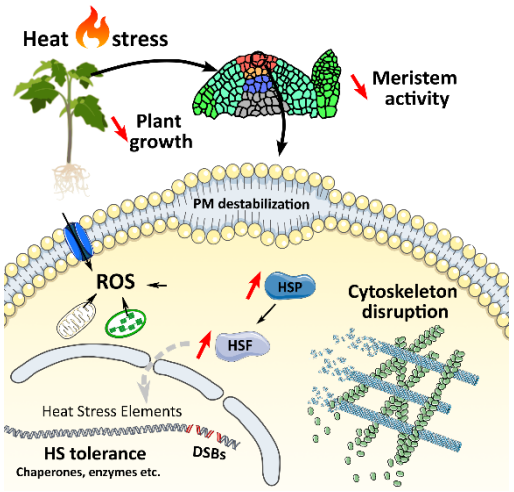

The shoot apical meristem (SAM) is the source of all cells from which the aerial part of the plant is built and the maintenance of its function is necessary for plant reproduction and survival. Given its presence throughout the plant lifecycle, the SAM is subject to various environmental stresses, including heat stress (HS). However, despite its importance in plant vegetative and reproductive development, the current knowledge about the responses of the SAM to HS remains very limited.

Our project aims at understanding the consequences of HS on various stages of tomato meristem development across different scales including organ, cell, and molecular responses in different tomato genotypes. We showed that at the seedling stage, different tomato genotypes display distinct tolerance to a brief but intense HS, corresponding to several hours at 45°C. The HS differentially affects leaf initiation and growth depending on the genotype suggesting a genotype-dependent meristem HS tolerance. Using confocal microscopy on immunofluorescently labeled slices of fixed meristems, and live-imaging with an on-stage heating system, we detailed the succession of cellular events occurring during the HS. We first observed an arrest of the nuclei movement, concomitant with microtubule cytoskeleton dismantlement. Next, we observed plasma membrane permeabilization starting from the cells at the edge of the leaf primordia, using propidium iodide, a hallmark of cell death. These observations suggest that the different cell types in the meristem react differently to the HS. We recently obtained RNA-seq data from the SAM of four tomato genotypes during and after HS to evaluate transcriptional reprograming and identify key molecular players of HS tolerance in tomato meristems.

P3

Plant lipid droplets in post-stress recovery

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During environmental stresses, such as nutrient deficiency or high temperature, plants trigger different survival mechanisms. Among these mechanisms, the formation of lipid droplets (LDs) in leaves has been described but its role is not well understood. LDs are the storage site for neutral lipids, predominantly triacylglycerols (TAGs), one of the most energy-dense molecules, and sterol esters. LDs are mainly known for their function as storage organelles in seeds to provide the energy needed for seedling growth after germination. However, several studies over the past decade have revealed diverse functions of LDs in non-seed tissues, mainly leaves and pollen. Notably, some Arabidopsis mutants affected in leaf LD biogenesis are less resistant to stress, suggesting a role of LDs in the plant response to environmental stress. Stress-induced LDs disappear when plants are back to normal growing conditions, but the mechanisms responsible for their disappearance (remobilization) remain unclear.

By lipidomics analysis we observed that TAGs and sterol esters accumulated in response to 10 days of nitrogen starvation in both leaves and roots. During the 24-48h of post-stress recovery phase, TAGs then rapidly disappeared, while sterol ester remobilization is slower. In addition, our analysis of different autophagy mutants, suggested that autophagy might be involved in neutral lipid accumulation in response to nitrogen starvation, but also in LD remobilization during the recovery phase. Further experiments will be conducted to confirm the involvement of autophagy in TAG degradation during post-stress recovery phase, and also to appreciate the role of the lipolysis pathway in this remobilization.

Keywords: lipid droplets, environmental stresses, triacylglycerol, Arabidopsis, leaves, post-stress recovery, autophagy, lipolysis

P4

Enhancing multigene editing in *Arabidopsis* through versatile cloning strategy and powerful screening method

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CRISPR-Cas9 technology has become an essential tool for plant genome editing. Recent advancements have significantly improved the ability to target multiple genes simultaneously within the same genetic background through various strategies. This approach addresses challenges such as gene redundancy and enhancing editing efficiency, eliminating the need for time-consuming multiple crossings. Additionally, there has been significant progress in developing methods for inducible or tissue-specific editing. These advancements offer numerous possibilities for fine-tuned genome modifications. Building upon existing research, and based on the previously described endogenous tRNA processing system, we have developed an optimized and modular strategy allowing the targeting of several genes simultaneously in combination with the synchronized expression of the Cas9 endonuclease in the egg cell. This system allows significant editing efficiency while avoiding mosaicism. In addition, the versatile system based on MultiSite Gateway® and Golden Gate technologies we propose, allows also to adapt to inducible and/or tissue specific edition according to the promoter chosen to drive the expression of the Cas9 gene.

To facilitate the screening of edited plants, we implemented our process with a rapid and cost-effective genotyping method based on primer-induced heteroduplex mobility assay (PRIMA). This method allows to easily detect heterozygous or homozygous 1-base pair (bp) insertion or deletion (indel) mutations.

Combining these cloning and screening strategies, we propose a versatile and powerful process to perform fine-tuned multigene editing in *Arabidopsis*.

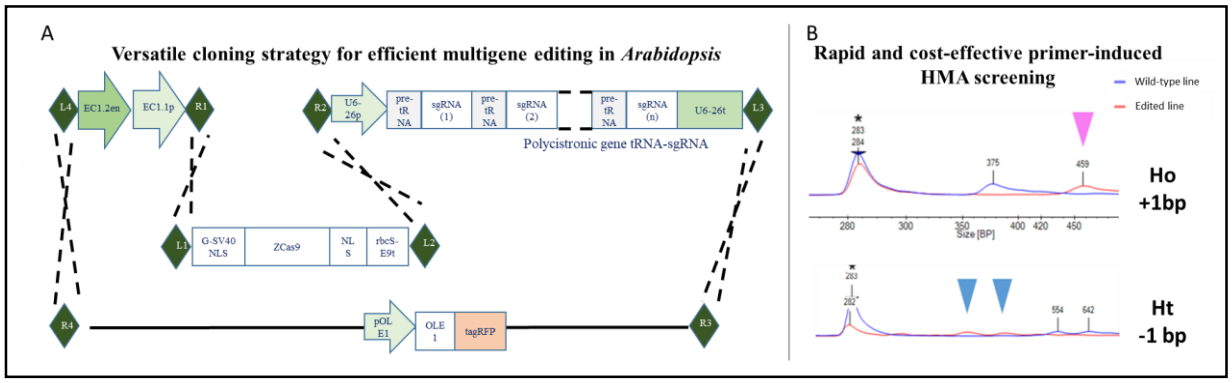

Figure 1. Cloning and screening strategies for multigene editing in *Arabidopsis*.

(A) Cloning strategy to express zCas9 under the control of egg cell promoter (EC1.1p) fused to enhancer sequence (EC1.2en) and to target DNA using several sgRNA cloned in the form of a polycistronic gene according to the endogenous tRNA processing system (Xie et al. 2015). (R1, R2, R3, R4, L1, L2, L3 and L4 represent recombination sites).

(B) Primer-induced heteroduplex mobility assay (PRIMA) for detection of a 1-bp homozygous or heterozygous mutation. Mobility profiles of primer-induced heteroduplexes after analysis by capillary electrophoresis are shown for wild-type (blue) and edited (red) lines. Arrows represent the signature of editing.

P5

Genome-wide identification of genes involved in ethylene biosynthesis and signaling in the legume plant *Medicago truncatula*

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When grown on nitrogen-deprived soils, legumes can establish a symbiotic association with soil nitrogen fixing bacteria collectively called rhizobia. This interaction leads to the formation of new organs on the roots of the host plants, termed nodules, in which the bacteria find a favorable environment for fixing atmospheric nitrogen for the benefit of its host. Ethylene is known for a long time as being a negative regulator of nodulation in many legumes. The components of the ethylene biosynthesis and signaling pathways modulating nodulation remain mostly unknown. Ethylene is produced by two enzymes. The first produces the direct precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC) and is named ACC synthase. The second, ACC oxidase (ACO), oxidizes ACC, releasing ethylene. Ethylene signaling in *Arabidopsis thaliana* involves, in the absence of ethylene, histidine kinase type ethylene receptors that phosphorylate CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) kinases, which itself phosphorylates the ETHYLENE INSENSITIVE 2 (EIN2) protein, blocking its cleavage and consequently the ethylene response. In the presence of ethylene, these receptors are inactivated, and EIN2 is thus no longer phosphorylated and is then cleaved. The released EIN2 C-terminal part subsequently promotes the degradation of E3 ligases EIN3-BINDING F-BOX Proteins (EBFs) encoding transcripts, which controls the stability of ETHYLENE INSENSITIVE 3 (EIN3s) transcription factors (TFs). In response to ethylene, EIN3 TFs are no longer degraded and can thus regulate ethylene target expression.
In the *Medicago truncatula* genome, we identified 9 and 6 genes encoding respectively ACS and ACO ethylene biosynthesis genes. Regarding ethylene signaling, we identified 2 genes encoding CTR1 proteins, 2 genes encoding EIN2 proteins, including one atypical lacking the usually conserved C-terminal end, and therefore likely non-functional, 4 EBF genes, and 12 genes encoding EIN3-like TFs. Mining of publicly available transcriptomic RNAseq allowed defining a subset of genes ethylene regulated during the interaction with rhizobia.

P6

Synergistic interactions of mycorrhiza and rhizobacteria with emphasis on plant nutrition and salt stress tolerance in rice

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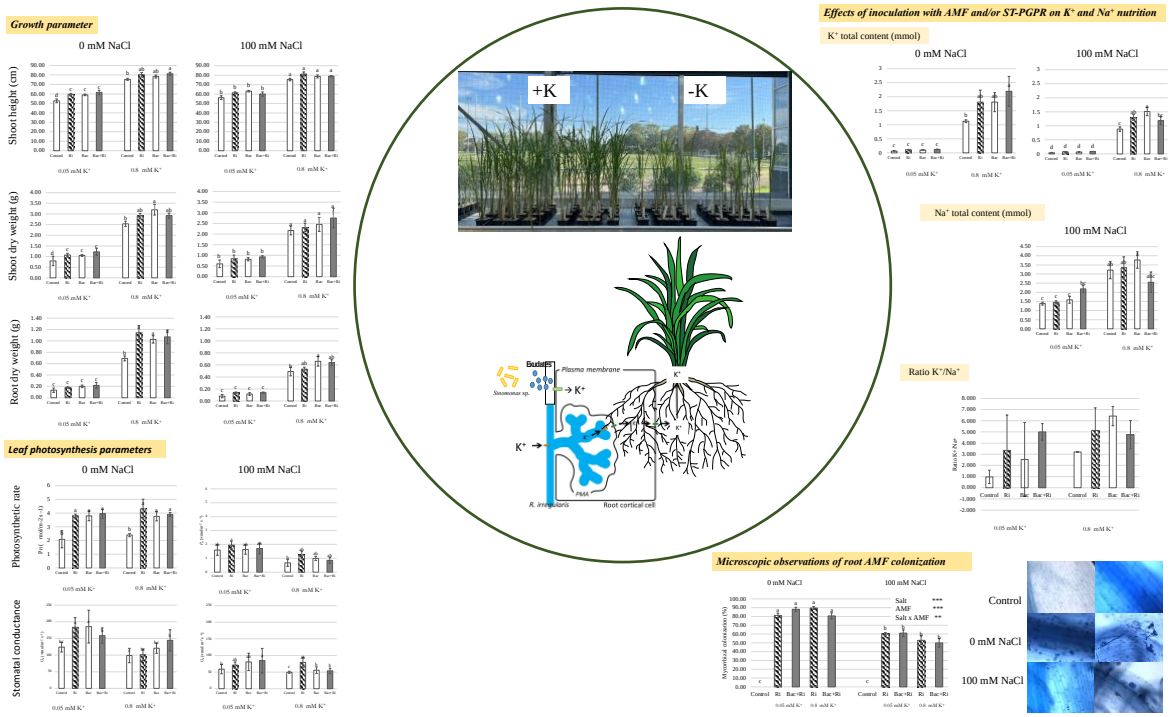
Abstract

Arbuscular mycorrhizal fungi (AMF) and salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) have developed interdependent connections with the roots and have been proved to have the ability to alleviate salt stress in rice (*Oryza sativa* L.) plants. These interactions increase the availability as well as the translocation of nutrients, and thus improve plant nutrition and growth. In the present study, ST-PGPR strains in combination with AMF were inoculated to KDML105 rice cultivar and subjected to a salt stress (100 mM NaCl). The application of ST-PGPR strains clearly provided a significant positive effect on growth parameters and K^+ total content of the rice both under normal and saline conditions when compared to their respective controls. Our results provide supporting evidence that AMF *R. irregularis* and *Sinomonas* sp. ORF15-23 synergistically insert beneficial effects on K^+ nutrition in rice, and their combined inoculation is a promising strategy for alleviating the harmful effects of soil salinity.

Keywords: Arbuscular mycorrhizal fungi, Rhizobacterial, Rice, Salt stress tolerance, K^+ transport systems



P7

Tolerance to water stress, root functioning and carbon flux optimized to improve yield in pea

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Pea crop (*Pisum sativum*, Fabaceae) is threatened by increasing drought, which can reduce yield due to carbon competition between roots and seeds. Pea crop is usually exposed to water stress during flowering. This can lead to flower and seed abortions, reducing crop productivity. However, drought episodes are now occurring earlier, affecting pea crop in its vegetative development and impacting root system establishment. To improve drought tolerance, it's now crucial to focus on root plasticity, especially at early stages of development and explore how carbon fluxes can be modulated in legume breeding programs.

The aim of our study is twofold. Firstly, to study the role of root development in plant tolerance to water stress. Secondly, to characterise the beneficial effects of biostimulant products on plant responses to water stress.

To this end, 11 spring protein pea cultivars were grown in Rhizobox systems, enabling direct observation of the root system architecture to identify contrasting responses to water deficit at early developmental stages. In some cultivars, a developmental delay to reach the 2-leaf stage was observed under water deficit. Using the SmartRoot software, we performed dynamic root system monitoring over a developmental time-course on five cultivars showing contrasting responses to water deficit. Our results suggest that, at early developmental stages, the shape of the root system is remodelled at fine scale under water deficit, without change in biomass output.

Then, three biostimulant protocols were tested and one of them showed significant results in mitigating the impacts of water stress during flowering. These included a reduction in the growth arrest during water stress and an increase resilience in seed production during the period of water recovery. These findings can support the development of new agroecological strategies to increase pea yield by focusing on carbon fluxes in the context of climate change.

Key words: Pea; Water deficit; Source-sink relationships; Carbon Flux; Root architecture; Biostimulants.

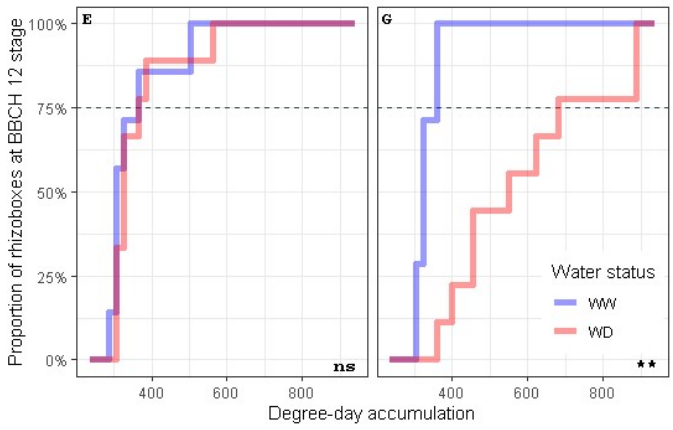

Figure 1: Developmental time - course describing the percentage of plants that have reached BBCH 12 stage for 2 varieties (E and G) under water deficit (WD 40% field capacity in, red) and well-watered conditions (WW 80% field capacity in, blue), based on degree-days accumulation since sowing. *The dashed line represents the threshold at which 75% of the plants have reached the two-leaf stage (BBCH 12), a threshold above which biological variability tends to increase. The statistics (Wilcoxon tests) at the bottom right of each quadrant (ns = not significant; ** = p < 0.01) indicate if the mean of degree-days accumulation at BBCH 12 stage is different in WD compared to WW control conditions.*

P8

Identification and functional responses of *STP13* genes in *Pisum sativum* under water deficit and fungal infection

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The culture of legumes, such as pea crop, is crucial for promoting protein independence for both animal feed and human consumption. In the context of climate change, plants face more frequent dry and wet periods, leading eventually to more severe water stress and fungal infection. These two major environmental threats significantly impact on plant carbon fluxes, resulting in reduced productivity and yield quality.
In plants, carbon flux is a key determinant of plant development, stress resilience and ultimately crop yield. Such carbon fluxes are controlled by sugar transport proteins (STP), mediating influx of sugars across the plasma membrane of plant cells. Sugar transporter genes have been shaped through natural selection and agricultural domestication for their specificity to supply carbon and therefore STP are candidates for harnessing multi-stress resistance.
We are investigating the function of *AtSTP13* homologs from pea*, PsSTP13.1* and *PsSTP13.2* genes, encoding putative plasma membrane H+/hexose symporters from the monosaccharide transporter family. In *Arabidopsis thaliana*, the expression profile of *AtSTP13* emerges as a discernible marker for biotic and abiotic stress responses. In a previous work, we also reported that the overexpression of *AtSTP13* enhanced Arabidopsis basal resistance to the necrotrophic fungus *Botrytis cinerea*. Conversely, *AtSTP13*-deficiency resulted in increased susceptibility, suggesting that AtSTP13 may enhance resistance by depriving the pathogen from resources and fueling the plant defense responses.
Here, we present the first results regarding the characterization of *PsSTP13.1* and *PsSTP13.2* genes. We are conducting a comprehensive analysis of PsSTP13 functionality, including their evolutionary conservation within the Fabaceae family and their expression patterns through a pea transcriptomics atlas. Notably, our investigation is centered on their response to water deficit and fungal infection. Altogether, our study of carbon fluxes in crops will help stabilize yields under changing climatic conditions by selecting genotypes more resistant to stress.

Key words: Pea crop (*Pisum sativum*), Carbon fluxes, Sugar Transport Protein 13 (STP13), Water deficit, Necrotrophic fungus (*Botrytis cinerea*).

P9

How does metabolism adjust to changes in photosynthetic rate? A multi-omics study in sunflower leaves

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Photosynthesis is the cornerstone of plant carbon primary metabolism, providing source carbon for many metabolic pathways, including nucleic acids and protein synthesis. Photosynthesis is prone to variations through several factors like CO_2 mole fraction, with reciprocal changes in photorespiration. It is currently unclear whether CO_2-driven changes in photosynthesis impact on other metabolic pathways in the short term. Recent works have examined how metabolism reacts to variations in photosynthesis and photorespiration activity in response to CO_2 and O_2 changes. It has been shown that the initiation of protein translation is positively correlated to photosynthetic activity. Nevertheless, metabolic pathways involved to sustain increased protein synthesis –such as amino acid provision– are still unclear. Moreover, it is unlikely that all proteins are up-regulated similarly when photosynthesis activity increases. Here, sunflower leaves have been subjected to different CO_2 mole fractions to modify the photosynthetic rate. Metabolomic analysis by GC-MS has been performed and indicates that the increase in photosynthetic rate up-regulated Calvin cycle, Krebs cycle and pentose phosphate pathway (OPPP) activity but also amino acids metabolism, probably sustaining higher translation activity. To check that out, sunflower leaves have been labelled with $^{13}CO_2$ and the incorporation of ^{13}C in proteins has been studied by shotgun proteomics. Our first results showed that the protein turn-over is relatively slow in sunflower mature leaves regardless of photosynthetic activity and thus the isotopic pattern is hardly detectable. To gain more sensitivity in ^{13}C analysis of peptides, the experiment has been repeated with ^{13}C-lysine labelling. Then, the partitioning of ^{13}C in metabolism has been studied by ^{13}C-NMR, GC-MS and EA-IRMS. As expected, our results seem to confirm the previous findings since a strong labelling in metabolites like sugars has been detected. Advances in studying metabolic adjustments in response to photosynthetic rate changes will be useful to improve metabolic models of plant net assimilation.

P10

From disorder to order: Biomolecular condensation orchestrates clathrin-mediated endocytosis

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Clathrin-mediated endocytosis is an essential cellular internalisation pathway involving the dynamic assembly of clathrin and accessory proteins to form membrane-bound vesicles. The evolutionarily ancient TSET/TPLATE complex (TPC) plays an essential, but not well-defined role in endocytosis in plants. Here, we show that two highly disordered TPC subunits, AtEH1 and AtEH2 function as scaffolds to drive biomolecular condensation of the complex. These condensates specifically nucleate on the plasma membrane through interactions with anionic phospholipids, and facilitate the dynamic recruitment and assembly of clathrin, early-, and late-stage endocytic accessory proteins. CLEM-ET experiments reveal that condensation promotes ordered clathrin assemblies. TPC-driven biomolecular condensation thereby facilitates dynamic protein assemblies throughout clathrin-mediated endocytosis. Furthermore, we show that a disordered region of AtEH1 controls the material properties of endocytic condensates in vivo. Alteration of these material properties disturbs the recruitment of accessory proteins, influences endocytosis dynamics, and impairs plant responsiveness. Our findings reveal how collective interactions shape endocytosis.

P11

Regulation of iron homeostasis in *Arabidopsis thaliana* by bHLH121 and clade IVc bHLH transcription factors

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Iron is an essential micronutrient for plant growth and development. In *Arabidopsis thaliana*, an intricate regulatory network involving several bHLH transcription factors (TFs) controls the homeostasis of iron. Among these TFs, bHLH121 plays a crucial role. bHLH121 interacts *in vivo* with clade IVc bHLH TFs and activates the expression of *FIT* and clade Ib bHLH TFs to stimulate the uptake of iron. How bHLH121 and clade IVc bHLH TFs function collectively and efficiently to maintain iron homeostasis is still unclear. Herein, we found that double loss-of-function mutants involving *bhlh121* and one of the clade IVc bHLH displayed more severe iron deficiency associated growth defects than each of the single ones. We also found that among the four clade IVc bHLH TFs, solely *bHLH34* and *bHLH105* could partially complement the iron-associated growth defects of *bhlh121* when overexpressed. These data, together with protein localization analysis, support that bHLH121 and clade IVc bHLH TFs act synergistically to regulate iron homeostasis and that different bHLH121/clade IVc and clade IVc/clade IVc protein complexes are involved in this process.

P12

Role of type III Glutaredoxin like proteins in regulating NO_3^- root responses

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Glutaredoxins (GRXs) are small oxidoreductases involved in numerous cellular processes, notably through their ability to reduce disulfide bond and cystein-gluthatione bond of target proteins. Often considered as antioxidants helping to minimize oxidative damage to proteins, class III GRXs now appear as major regulators of signaling pathways involved in response to several environmental signals and certain abiotic stresses including nitrate (NO_3^-) depletion. Morover, these GRXs can interact with transcription factor of the bzip family TGA, regulators of the nitrate root transporters of the NRT2 family involved in nitrate root transport and development of the root system archtecture. Our project aims to understand the role of GRXs in the molecular mechanisms that regulate root development and the absorption/assimilation of NO_3^-. This will involve studying in particular the function of GRXs in the NO_3^- signaling pathways in the model plant *Arabidopsis thaliana* in order to determine if they play a role in nitrate responses.

P13

Studies of vacuolar transporters controlling nitrate storage in *Arabidopsis thaliana*

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Nitrogen (N) is a major macronutrient in the functioning of living organisms, as it is an essential component of nucleic and amino acids. In plants, nitrogen is also found in key proteins in photosynthesis. Vascular plants primarily acquire their nitrogen through the absorption of nitrate (NO_3^-) via their root in well-aerated soils. Once inside the plant, nitrate is transported from the roots to the aerial parts where it is assimilated to promote plant growth. To meet the variability of its needs and maintain optimal metabolic conditions in the cytosol, the plant stores most of the available nitrate in an intracellular compartment, the vacuole (Martinoia et al., 1981). This anion can then be remobilized in case of deficiency. The main known player responsible for this accumulation in Arabidopsis is the nitrate/proton antiporter, AtCLCa (De Angeli et al., 2006). For its activity, it utilizes the proton motive force of the proton gradient generated by proton pumps, also located in the vacuolar membrane, the tonoplast. Disruption of two V-ATPases results in a specific decrease in nitrate content (Krebs et al., 2010), suggesting a defect in the efficiency of vacuolar nitrate accumulation. Althrough V-ATPases and vacuolar CLCs may interact, the analysis of plants deficient in both V-ATPases showed that the inactivation of these proton pumps do not affect the activity of CLCa but the regulation of vacuolar CLCs expressions. These studies demonstrate genetic regulation between these transporter families, with the signaling pathway yet to be determined.

Key words : nitrate, vacuole, chloride channel, photoperiod transcriptional regulation

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P14

Impact of agroforestry systems on crop disease: a case study on Fusarium Head Blight in wheat

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The general issue of crop diversification and the coexistence of plant species in the same area has been widely studied from biophysical, geochemical and ecological perspectives. However, the issue of biological regulation, particularly of pests and diseases in temperate agroforestry systems is poorly documented. It is essential to gain knowledge of the benefits and potential risks associated with the deployment of such a practice applied to field crops.

The aim of the study is to investigate the impact of the agroforestry system associated with cereal crops on the populations of fungal pathogens responsible for Fusarium Head blight (FHB). FHB is one of the major diseases affecting cereals including wheat or barley. A cohort of several *Fusarium* spp. are associated with FHB symptoms. While *F. graminearum* is often prevalent, the representativeness of the others is strongly influenced by the environment.

In this context, the presence of hedges or trees near wheat fields may modify pathogen populations, by acting as pathogen reservoirs, or by modifying the microclimatic conditions favorable to their development. These two hypotheses will be explored through population genomics approaches. At the interspecific level, the presence of *Fusarium* species in each compartment will be assessed using a metabarcoding approach targeting EF1-alpha gene (Boutigny et al 2019). Regarding *F. graminearum*, the dynamics of the pathogen populations between compartments and at the field scale will be studied. To do this, a PenSeq (for Pathogen enrichment Sequencing, Thilliez et al 2018) approach will be developed to free from the current limitation of strains isolation step in such a population study.

These data will be analyzed in the light of microclimatic data available to identify the key environmental factors that shape pathogen population structure and disease development. Our study will contribute to fuel scientific knowledge on the epidemiology of FHB in agroforestry landscape context.

P15

MAPKinase signaling pathway components are locked in stress granules upon heat stress : a bright future for plants pathogens ?

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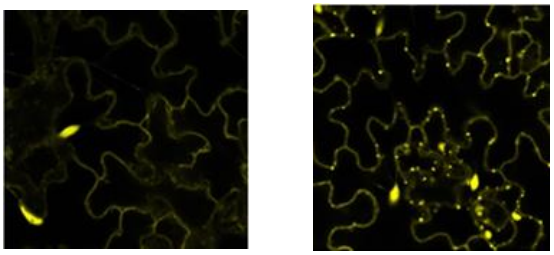
MAPKinase modules are major components of plant signaling cascades. They regulate many developmental processes as well as control responses to environmental stimuli. Noticeably, the crucial implication of two MAPK cascades (MKKK3/5-MKK4/5-MPK3/6 and MEKK1-MKK1/2-MPK4) in plant defense mechanisms against biotic stresses has been extensively described [1]. Moreover, they also are likely involved in plant responses to abiotic stresses (such as high temperatures) since few studies identified compounds of MAPK modules in ribonucleoproteic complexes upon heat stress [2,3]. These heat stress granules (HSGs) were shown to favor heat stress resistance, probably through translational regulations mechanisms [2,3,4,5]. Interestingly, using microscopy, we confirmed the presence of MKK4, MKK5 and MPK3 in those HSGs. My project aims to understand the role of MAPK localization to HSGs for plants thermotolerance and pathogen resistance. The long-term objective is to understand how climate change will affect immune signaling pathways and responses.



20°C 40°C

Heat shock (2h at 40°C) applied to mpk3 :: MPK3-YFP transgenic lines (A.thaliana in soil pots)

I focused my attention on two MAPKs involved in immune responses : MPK3 and MPK6. Despite their strong homology *(72,5 % aa identity*) and extensive functional redundancy, MPK3 localizes in HSGs upon heat stress while MPK6 does not. In order to understand the mechanisms leading to this differential localization, as well as its functional roles, I would like to create a plant carrying an MPK3 which no longer localizes in HSGs upon heat. Therefore, using a structure-function analysis from MPK3/MPK6 homology, my current objective is to determine domains in MPK3 that control its localization in the HSGs. My preliminary results also suggest that MPK3 phosphorylation capacities and substrates interactions are modified when accumulated inside HSGs. Considering the dramatic temperatures rises which will be triggered by climate change, the driving force of our work is the prediction of possible incompatibilities between the implication of MAPKs in heat response and their involvement in other processes, such as triggering the immune response.

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P16

Molecular and Cellular control of Seed Dormancy and Germination by PhosphatidylEthanolamine Binding Proteins (PEBPs)

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Seed germination is a crucial process that enables seedling establishment under favourable conditions. Seed dormancy is an adaptive trait that enables plants to survive adverse conditions and restart growth in suitable season. These two processes are tightly controlled by both environmental and internal factors. Several actors have been identified to regulate seed dormancy and germination, including the phytohormones balance between abscisic acid (ABA) and gibberellic acid (GA). Previous results suggest that PhosphatidylEthanolamine Binding Proteins (PEBPs) may also play a critical role in these regulatory processes. Here, we provide further evidence confirming the involvement of PEBPs in the control of seed dormancy and seed germination. We revealed that PEBPs are expressed early during seed development, when dormancy establishment occurs; and characterized additional elements that might interfere with its expression. PEBPs have previously reported as regulating seed dormancy and germination in response to temperature, thus our findings may help to expand our comprehension of the crucial role of PEBPs under challenging environmental conditions.

P17

Study of the mode of action of miPEPs in *Arabidopsis thaliana*

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MicroRNAs (miRNAs) are negative regulators of gene expression via an interfering RNA mechanism. Through this mechanism miRNAs regulate a very wide range of processes in plant life, such as vegetative growth, development and response to various biotic and abiotic stresses. The biosynthesis of miRNAs is well documented in the literature: a miR gene is transcribed into a precursor called pri-miRNAs, which in parallel to its transcription is cleaved by the multi-protein complex DICER into microRNAs. In 2015 it was shown that short open reading frames (ORFs) are present on pri-miRNAs and can be translated into peptides called miPEPs (miRNA encoded peptides). miPEPs act as positive regulators of their own pri-miRNA expression. However, how this activation is achieved still remains unclear.

We hypothesized that the mode of action of miPEPs is based on protein-protein interactions. To test this hypothesis, we developed phenotypic tests with two *Arabidopsis thaliana* miPEPs (miPEP397a and miPEP165a) on different mutants of the DICER multiprotein complex. Thus, we were able to identify four proteins potentially involved in the action of miPEPs: hyponastic leave1 (HYL1), dawdle (DDL), serrate (SE) and cap-binding protein 20 (CBP20). The interaction between a miPEP and these four proteins, already known to be involved in pri-miRNA biosynthesis, is currently being studied *in planta* using a FRET-FLIM approach.

In parallel, we plan to characterize the interactome of *A.thaliana* miPEPs, with the aim of identifying all protein partners in an unbiased manner. For that we are developing a proximity labelling approach using a Turbo-ID biotin ligase-fused miPEP expressed in *A. thaliana.* The biotinylated partner proteins will then be purified and identified by mass spectrometry. This approach will allow us to detect weak and transient interactions between a miPEP and its associated proteins.

P18

Launch of the project CABiosE: Comparative Analyses of Biostimulant Effects on Tomato Crop

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The aim of the project is to study the response mechanisms induced by biostimulants on tomato plants under controlled conditions and production conditions. In particular, the objective is to determine the direct contribution of biostimulants on plants and their indirect contribution, through the variations in rhizosphere and phyllosphere microbiota.

We planned a three-stage approach: Product –Soil – Species. The product approach aims at screening the most effective products for biostimulation in tomato under water stress. Biosolutions of various natures are tested: algae extract, vegetal extract, mineral. The soil approach is designed to assess whether selected biostimulants at the end of the screening influence the microbiota of various types of soil (natural, artificial). The species approach will enable us to conclude if the biostimulants effects can be generalized on different crops.

In 2024, we set up an experiment on tomato crop under water stress conditions (n=500). Seven biostimulants are tested with two foliar applications, from the 4-6 leaf stage and at 7 days of interval. The stress is applied two weeks after the first treatment. We are carrying out observations and phenotypic measurements at ten time points until fruit production. We also take samples of leaves and roots for metabolomic analysis. This trial is conducted in semi-controlled condition, in a greenhouse. Then, at the end of 2024, the study of the biostimulant effects on plants and plant microbiota will be performed in controlled condition (*in vitro* and in a growth chamber). We are considering metabolomic investigations combined with metagenomic to assess the influence of selected biostimulants on the phyllosphere and rhizosphere microbiota communities and their functions.

Thanks to this project we hope to gain insights into the mechanisms by which plants develop resilience to abiotic stress in response to biostimulant applications directly and on the indirect contribution via the microbiota.

P19

Lipid droplets : New actors of the plant virus infection

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Lipid droplets (LDs) are organelles dedicated primarily to the storage of neutral lipids, and are found in most of organisms from archaea to eukaryotes. Besides their energy storing capacity, they are also involved in inter-organelle transport of lipids and proteins, stress signalling, and lipid homeostasis. LDs have been shown to be hijacked by positive-sense single-stranded RNA for their replication in animal cells. Like animal viruses, plant positive-strand RNA viruses have to reroute host proteins, intracellular membranes, and lipids to create an optimized lipid/membrane microenvironment for their efficient viral replication compartment (VRC) assembly. However, the possible involvement of LDs in this plant virus infection process has not been explored.

In this study, we monitored LD biogenesis upon infection by the positive single-strand RNA turnip mosaic virus (TuMV, potyvirus) in *Arabidopsis thaliana* and *Nicotiana benthamiana*. Using confocal microscopy, we revealed that infection by TuMV leads to a significant proliferation of LDs compared to mock-inoculated leaves, both in Arabidopsis and *Nicotiana benthamiana*. Both confocal and transmission electron microscopy data also showed that LDs are recruited to TuMV-induced VRCs in infected leaf cells. Consistently, a significant accumulation of neutral lipids was observed in TuMV-infected leaves in *Nicotiana benthamiana*, supporting the premise that TuMV-infection induces LD biogenesis. We also demonstrated that the TuMV propagation is significantly reduced in Arabidopsis *ldap* (*lipid droplet-associated protein*) knock-out mutants and increased in *LDAP1* overexpressing Arabidopsis plants. Taken together, our results indicate that LD biogenesis could play an essential role in a plant virus infection.

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POSTERS
Session 2

P20

Exploring agroforestry effects on grapevine root microbiota: Investigating tree species and age influences on microbial richness and diversity

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Agroecology integrates environmental and social principles into farming systems. Agroforestry, the combination of tree plantations with other agricultural systems, is a promising mean of improving the sustainability by offering environmental and economic benefits. In viticulture, the integration of trees into vineyards has gained attention for its potential to improve soil health. The study of the vineyard microbiome is an emerging field of science and harnessing the microbiome has the potential to increase the resilience of grapevines (*Vitis vinifera*) to climate change. Understanding the grapevine root microbiome and its dynamics is crucial for optimizing these systems. The objective of this study is to characterize the effects of tree species and age on the richness and diversity of grapevine root-associated microorganisms. Through field experimentation, we will assess the microbial communities inhabiting the soil and roots of grapevines in an agroforestry system. The microbiota composition of grapevine roots near three tree species will be described: plum tree (*Prunus domestica*), quince tree (*Cydonia oblonga*), and maple tree (*Acer campestre*). Additionally, the tree root microbiota will be investigated to analyze the interaction between underground fungi and bacterial communities in such systems. Microbial diversity and taxonomic composition will be studied using high-throughput sequencing techniques. It is hypothesized that different microbial assemblages may be observed in the vicinity of different tree species, reflecting the unique soil environment created by different tree species compositions. Furthermore, this study should help to elucidate whether tree age plays a crucial role in shaping microbial community structure, with notable shifts in microbial richness and diversity observed at different stages of tree development. This study contributes to our understanding of the complex interactions between perennial species in agroforestry systems.

P21

Getting new insights into the control of plant mineral nutrition: The transcriptional regulation of Iron Homeostasis

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Iron (Fe) is an essential micronutrient for plant productivity and plays a major role during plant growth and development. It is a cofactor for numerous reactions involving electron transfer/oxidation-reduction such as photosynthesis, respiration, nitrogen assimilation etc. Iron homeostasis is tightly regulated in order to avoid deficiency or excess that could be detrimental to the plant. The regulation of the plant response to fluctuations in Fe availability has been extensively studied in many species, highlighting the importance of the transcriptional regulation in this process. In this context, we aim to further characterize the molecular mechanisms that control iron homeostasis in plants, focusing on three main transcription factors URI/ BHLH121, ILR3/BHLH105 and PYE in the model plant *Arabidopsis thaliana* (non-grass species) and comparing the degree of conservation of this regulatory network in a grass species, *Brachypodium distachyon* since both type of plants use similar but different strategies to maintain the homeostasis of this micronutrient.

P22

Exploring the role of grapevine sugar transporters in plant defense against pathogens: Insights from VvHT5 overexpression

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Regulation of sugar allocation is essential for plants interacting with their environment, including interactions with pathogens. However, despite increasing evidence indicating that sugar transporters and invertases play important roles in plant resistance to pathogens [1], the underlying mechanisms remain poorly understood. In a previous work, we reported that the overexpression of PM-localized H^+/ hexose symporter AtSTP13 enhanced Arabidopsis basal resistance to the necrotrophic fungus *Botrytis cinerea*. Conversely, AtSTP13-deficiency resulted in increased susceptibility, suggesting that AtSTP13 may enhance resistance by depriving the pathogen from resources and fueling the plant defense responses [2,3]. By contrast, STPs could also act as susceptibility factors by promoting the proliferation of biotrophic fungi during infection, as previously demonstrated in wheat and barley [4,5]. Building upon these studies, we are now focusing our research on plants of agricultural importance, such as grapevine. To investigate the functional role of sugar transporter genes in response to *Botrytis cinerea* infection, we used a gain-of-function approach. We have obtained transformed lines in both Arabidopsis and grapevine overexpressing VvHT5, the AtSTP13 closest homolog from grapevine, and are currently studying their phenotype in the context of defense against pathogenic fungi. Our results support that the control of apoplastic sugars by the activity of PM-localized hexose transporters is an important component of host plant defense.

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P23

Silicon Transporter Genes in Vitis ssp.: Implications for Plant Resistance to Biotic and Abiotic Stresses

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In this study, we uncover the crucial influence of silicon. Silicon plays a pivotal role as an essential nutrient for the optimal growth and sustainable production of higher plants. Its accumulation in plant shoots acts as a protective mechanism against various abiotic and biotic stresses, especially when levels exceed 10%. Our first observations on grapevines revealed the positive effects of silicon in the fertilization of young grafted-welded plants. Treated plants exhibited notable improvements in growth, resistance, structural integrity, and leaf pigmentation compared to untreated plants. Based on these findings, we hypothesized a positive impact of silicon on overall plant physiology. According to existing literature, two genes involved in silicon transport have been identified in grapevines VviLSI1 (NIP 2;1 Aquaporin) and VviLSI2 (Lsi2-like transporter), localized in stems and roots. We present here our work and our first results on the characterization of Lsi genes in grapevine.

We employed gain and loss of function strategies to investigate LSI functions exploiting vectors for the overexpression and knock-out (with CRISPR/Cas9 system) of the candidate gene. Our preliminary results indicate that optimizing the plant's ability to assimilate and utilize silicon could strengthen its defenses against both biotic and abiotic stresses, such as pathogenic fungi and water deficit.

P24

Gel-less tomato fruits reveal the implication of the Zinc Finger protein *Sl*ZFP2 in locular tissue morphogenesis

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The locular tissue (LT) is a jelly tissue that surrounds the seeds in tomato fruit and differentiates from its central axis after fruit set. This tissue is proposed to be essential for seed development and dissemination, due to its inhibitory action on early germination and its involvement in initiating fruit ripening.

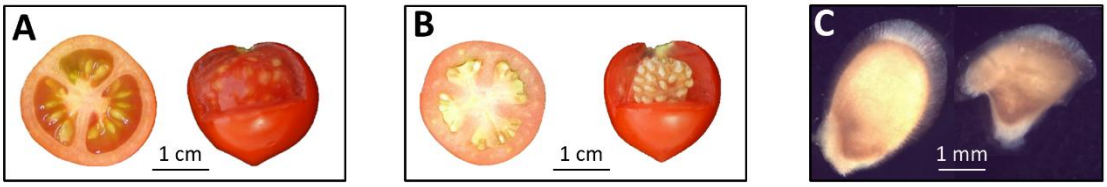

Figure 1. Gel-less mutant phenotype. A) Equatorial section and partial dissection of the pericarp of a red-ripe (RR) fruit from a WT plant and B) a *gel-less* plant. C) Seed from a WT (left) and a *gel-less* plant (right).

In this work, we characterised a tomato mutant deficient for LT formation, presenting an atrophied LT and sharp seeds (Fig. 1). By classic mapping combined with mapping-by-sequencing strategy, we identified the mutation that causes this "*gel-less"* tomato phenotype as the insertion of a transposable element in the coding sequence of *SlZFP2*, a C2H2 zinc finger transcription factor (TF). We produced several altered versions of *Sl*ZFP2 by CRISPR/Cas9 that induced incremental *gel-less* phenotypes. Histological and cytological analyses of *SlZFP2* knockout lines demonstrated the early and significant effects of *SlZFP2* disruption on cell division and endoreduplication in the LT. Model-based analysis of cellular data further proposed that cell division is the preponderant process altered by *zfp2* mutations. RNA-Seq analysis of LT suggested a large reprogramming of LT metabolism in *zfp2* mutants compared to the WT, and yielded a first list of potential direct targets of the SlZFP2 TF.

Altogether, this work revealed a new function for *Sl*ZFP2 TF as a crucial regulator of LT morphogenesis and lays the groundwork for further investigations into the complex regulatory networks controlling the development of tomato fruit tissues.

P25

Leafamine® biostimulant promotes cell division and alleviates heat stress effects in tomato roots

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Biostimulant products enhance plant development through improved nutrient assimilation or increased tolerance to abiotic stresses. However, their utilization is hindered by the lack of available knowledge regarding optimal usage and the mechanisms underlying their actions. Among the different classes of biostimulants, Leafamine® (LA) is a free amino acid-based biostimulant produced by BCF Life Sciences, and studies have shown its effectiveness in mitigating drought stress in lettuce grown in pots or in fields where the influence of microorganisms can contribute to the effect of Leafamine®. To expand on this research, we are now focusing on tomatoes, which are increasingly subjected to heat stress during various developmental stages, including the fruiting stage. In this study, we are focusing on the direct response triggered by Leafamine® in tomato plants; *in vitro* conditions were selected to avoid potential indirect effects of Leafamine® on beneficial microorganisms. Initially, our analyses focus on root growth at the cellular and molecular levels under both normal (CT) and high ambient temperatures (HS). Results indicated that Leafamine® improved root development by 15% under both normal and HS conditions, resulting in increased meristem size and cell numbers. To determine the plant responses induced by Leafamine® treatments, genome-wide expression analyses were conducted 5 days after Leafamine® treatment. The results demonstrated that under normal conditions, Leafamine® upregulated the expression of genes involved in cell division. Additionally, application of Leafamine® under HS conditions reduced the expression of HS marker genes, while increasing the expression of genes associated with growth promotion. Furthermore, gene ontology analyses of genes consistently impacted by Leafamine® treatment show that Leafamine® stimulates plant responses to nutritional factors, reduces responses to stresses, modifies secondary metabolic processes, and alters hormone signaling. To demonstrate the significance of such genes in the Leafamine®-induced tomato response, further experiments including functional analyses will be performed.

P26

Regulation of intracellular free zinc concentration in *Arabidopsis thaliana* roots

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Zinc (Zn) is an essential micronutrient for all living organisms. It acts as a structural or catalytic cofactor in proteins, with Zn-binding proteins representing nearly 10% of the proteome in eukaryotes. The risk of Zn malnutrition affects one third of the global human population and plants represent the main entry point for Zn into the food chain (Assunção *et al.*, 2022; Stanton *et al.*, 2022). Enhancing Zn accumulation and Zn-use-efficiency in crops is required to improve plant nutritional value and production in nutrient-deficient soils. Recent findings indicate that Zn uptake in plant root is regulated by F-bZIP transcription factors that directly perceive intracellular free Zn concentrations (Lilay *et al.*, 2021). Here, we use eCALWY, a genetically encoded fluorescent Zn sensor, expressed in the cytosol of Arabidopsis cells to investigate the regulation of intracellular Zn concentration in roots (Vinkenborg *et al.*, 2009, Lanquar et al. 2014). We find that cytosolic free zinc concentration lies in the 100 pM range and increases transiently in response to an elevation of Zn concentration in the medium. We analyze the regulation of cytosolic Zn concentration in mutant backgrounds lacking MTP1 or MTP3, the transporters involved in Zn storage inside the vacuole, or HMA4, the plasma membrane Zn efflux pump. This research will highlight the mechanisms regulating intracellular Zn concentration and in turn the activation of Zn deficiency responses or toxicity due to Zn excess.

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Identification of novel regulators of the metal transporter IRT1

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Protein ubiquitination is a post-translational modification playing a major role in living organisms. While the role and mechanisms of ubiquitination are relatively well described for soluble proteins present in the cytoplasm or nucleus in plants, the situation is much less clear for membrane proteins such as receptors and transporters. We recently found that the metal transporter IRT1, present on the cell surface of Arabidopsis root epidermis, is ubiquitinated, phosphorylated, endocyted and then degraded when is subjected to a non-iron metal excess (Barberon et al, 2011; Barberon et al, 2014 ; Dubeaux et al, 2018 ; Spielmann 2022). Such regulation optimizes soil nutrient uptake by limiting the risk of over-accumulation of metals within plant tissues. Our goal is to better understand the mechanisms leading to ubiquitination and endocytosis of IRT1 by discovering new regulators upon non-iron metals excess. We have developed a bio-ID protocol consisting of a proximity labeling technique coupled with mass spectrometry allowing the identification of weak and transient protein-protein interactions. This approach is based on the fusion of IRT1 in its first cytosolic loop and a biotin ligase (TurboID) and allowed us to identify a total of over 3,000 proteins after mass spectrometry analysis. Among these candidates, we selected around twenty proteins for further tests. To validate their interaction with IRT1, we first carried out interaction tests such as Split-Luciferase, BiFC and Co-IP in *Nicotiana benthamiana* as well as the very first phenotyping analyses of Arabidopsis mutants on metal media. These preliminary results have enabled us to identify 3 candidates that interact with IRT1 and whose role in its regulation is currently being investigated.

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Tomato Fruit Response to Chronic Heat Stress

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Human society faces two major challenges, the world demographic growth and global climate change inducing a global warming. To address these issues, it is crucial to develop new strategies to enhance plant resistance to chronic heat stress. Tomatoes, one of the most consumed fruit worldwide, have been extensively studied for their flower response to heat stress, characterized by short durations at very high temperatures (40°C). However, less research has been conducted on fruit response to heat stress. Global warming typically results in prolonged heat waves lasting a week or more, with an average temperature of over 30°C per 24 hours, and a nighttime temperature exceeding 25°C. This study focuses on describing the response of five tomato genotypes (*S. lycopersicum cv.* Microtom, West Virginia 106, Ailsa Craig, Moneymaker, and *S. pimpinellifolium*) to a chronic heat stress of 36°C for one week during fruit development. Specifically, two stages are targeted: 5 days post-anthesis (DPA) during the cell division phase and 15 DPA during the cell expansion phase. Preliminary findings suggest that fruits are rather affected in seed development, with a complete stop in development after heat treatment. Additionally, locular tissue/gel formation is impacted, with some genotypes producing completely gel-free fruits, as observed in *S. pimpinellifolium*, or exhibiting desynchronized tissue development between gel and pericarp, as seen in Moneymaker. This characterization serves as a first step towards a comprehensive understanding of tomato fruit response to chronic heat stress, ultimately contributing to the development of new strategies to enhance plant resistance to abiotic stress.

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TCP8 transcription factor: a new negative regulator of seed germination in Arabidopsis

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Teosinte Branched1/Cycloidea/Proliferating cell factor (TCPs) are plant-specific transcription factors that control development and response to environment. Among the 23 Arabidopsis TCPs, TCP14 and TCP15 promote seed germination, in relation with gibberellin signaling. We now evidence that TCP8, a close relative of TCP14/15, functions as a negative regulator of seed germination and promotes seed dormancy. At harvest, *tcp8* mutant seeds exhibited low dormancy when *TCP8*-overexpression led to exacerbated dormancy. ABA and paclobutrazol sensitivities were also lowered and enhanced in *tcp8* and TCP8-overexpressing seeds, respectively. Transcriptomic analyses identified 206 and 64 genes that were respectively up- and down-regulated in *tcp8* seeds compare to WT after 24h imbibition. Deregulated genes relate to numerous processes including development, cell wall organization, hormone response and lipid biosynthetic process. In agreement, out of the 206 up-regulated genes, 84 and 46 are known to be ABA-repressed and GA-induced in seeds, respectively. Interestingly, 42% of up-regulated genes were enriched or specifically expressed in the endosperm and *TCP8* transcripts were detected in endosperm and in embryo. Among deregulated genes expressed specifically in the endosperm, *CYP714A1,* a monooxygenase involved in GA inactivation, presented a TCP-binding motif in its promoter and was hypothesized as a direct target for TCP8. Transactivation experiments evidenced that *CYP714A1* transcription was directly activated by TCP8. Moreover TCP8 interacted with a MAP kinase, MPK8, in the nucleus as we showed earlier for TCP14 and MPK8 significantly enhanced TCP8 transcriptional activity. As a whole, our data indicate that TCP8 functions oppositely to TCP14/15 in regulating seed germination and its activity is control by the same MAP kinase than TCP14. The future works should unravel if and how TCP8/14/15 functions coordinate during seed germination.

P30

Study of the role of complex lipids in plant responses to stress

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Plants are subjected to various biotic and abiotic stresses during their development, including wounding, pathogen attack, insects, viruses, temperature variations, drought, and many others. Among the plant responses to these stresses, the production of phytohormones, reactive oxygen species and cytosolic calcium are notably induced. Phytohormones in particular play a regulatory role in plant development, reproduction and death.

Nevertheless, plants are able to resist certain external attacks more effectively through interactions with non-pathogenic micro-organisms such as plant growth-promoting rhizobacteria (PGPR) i.e, the *Bacillus* genus. This is called microbial priming, which is part of the plant induced systemic resistance.
Among the secondary metabolites produced by these non-pathogenic microorganisms, *Bacillus* lipopeptides, and more precisely surfactins, are well known to be microbial elicitors that enable priming in plants.

In the present poster, we first wish to present the current knowledge about microbial priming in plants by surfactin. Then we will present the strategy that will be developed by our lab to understand the impact of surfactin in the context of priming on the metabolome of *A. thaliana*. To address this issue, we will use a system composed of the plant, the surfactin and chitin to mimic a fungal attack. Then, the metabolome of those plants will be analysed by LC-MS/MS in a targeted and in non-targeted way. All in all, this will allow a better understanding of the mode of action of surfactin as an elicitor and its impact on the plant metabolome.

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Variability of symbiotic efficiency on growth of pea and lentil inoculated with different rhizobial genotypes originating from subhumid and semi-arid regions of eastern Algeria

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ABSTRACT

Nitrogen nutrition of legume relies on both soil mineral N absorption and symbiotic nitrogen fixation by rhizobia, the symbiotic interaction depends on each of the partners. This study evaluates the variability of symbiotic efficiency on growth of two cultivars of pea and lentil inoculated with 16 different rhizobial genotypes originating from sub-humid and semi-arid regions of eastern Algeria. The genotypes being defined by the combination of haplotypes characterized by rRNA intergenic spacer (IGS) and a nodulation gene marker *(nodD* region). Analysis of shoots is an effective method to examine plants growth. The results revealed that efficiency is linked to the rhizobial genotype, effect of the strains and host plant species. Strains showing genotypes originating from sub-humid zones are more effective than those originating from semi-arid zones on the development of tested plants. This functional variability is highly linked to the genetic variability of the chromosomal ribosomal IGS. Strains isolated of lentil are more effective on the lentil growth than those isolated from pea. Nitrogen content correlate with the plant growth. In conclusion, the inoculation of plants cultivated in a semi arid soil with effective strains from a sub humid origin might be more beneficial under severe environmental.

P32

News prospects on the role of the *Arabidopsis* BOLA4 protein in iron-sulfur (Fe-S) assembly machineries

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Iron-sulfur (Fe-S) clusters are universal cofactors embedded in proteins that support numerous fundamental biological processes such as photosynthesis and respiration [(1)]. Assembly and delivery of Fe-S centres to client proteins take place within specialised Fe-S assembly machineries involving numerous proteins and cofactors, some of which are still poorly characterised in plants. Considering that more than 200 key client proteins in *Arabidopsis* require Fe-S clusters to perform their function [(2)], we are developing research on BOLA proteins known in few model organisms as interactors of two essential classes of Fe-S shuttle proteins within Fe-S assembly machineries [(3-5)], but their importance and exact role in plants remain to be assessed. Focusing on *Arabidopsis thaliana* BOLA paralogs, our recent work showed that the cumulative depletion of the plastidial/mitochondrial BOLA1 and BOLA4 proteins leads to a severe dwarf phenotype with early primary root arrest, suggesting important role of these proteins for plant development [(6)]. To go further in understanding the function of these proteins, we describe here selected strategies based on the use of *bola1bola4*/proBOLA::gBOLA-GFP transgenic lines to unravel their interactomes with a highlight on BOLA4, with the aim to position its function within the mitochondrial and platidial Fe-S assembly machineries.

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P33

Exploiting lipid metabolism: Interaction of barley yellow dwarf virus with phosphatidylinositol transfer proteins in plants

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Abstract: Phosphoinositols (PtdIns) are minor but essential phospholipids in plant cells and play key roles in cellular membrane identity and signaling pathways against biotic and abiotic stresses [1]. Phosphatidylinositol transfer proteins (PITPs), which transfer PtdIns and other lipids from the endoplasmic reticulum (ER) to various cellular membranes, are pivotal in lipid metabolism, membrane trafficking, and PtdIns-dependent signaling under environmental stress [2]. Viruses exploit host subcellular membranes and lipid metabolism machinery to assemble viral replication compartments, thereby facilitating their replication and spread within the cell [3].

Using TurboID-mediated proximity labeling in *Nicotiana benthamiana*, we identified a CRAL-TRIO domain-containing PITP (NbCRAL) that interacts with the barley yellow dwarf virus (BYDV; positive-stranded RNA luteovirus) read-through domain (RTD). The CRAL-TRIO domain is crucial for lipid binding, with specific amino acid residues identified for binding to PtdIns and phosphatidylcholine (PtdCho). A sequence blast of NbCRAL against the wheat genome revealed three orthologs—TaCRAL1, TaCRAL2, and TaCRAL3—with protein sequence identities of 53%, 65%, and 67%, respectively. A yeast two-hybrid indicated direct interactions between BYDV RTD and NbCRAL, TaCRAL2, and TaCRAL3, but not TaCRAL1 (Figure 1A). These interactions were further confirmed by bimolecular fluorescence complementation assays (Figure 1C). Structural analysis using AlphaFold2 predicted interaction domains at the C-termini of TaCRAL2 and TaCRAL3, differing significantly from TaCRAL1, potentially explaining the lack of interaction (Figure 1B). The CRAL proteins from both *N. benthamiana* and wheat co-localize with the viral RTD in the nucleus and cytoplasm, and presence of RTD does not alter their localization, indicating a stable intracellular position during viral interaction (Figure 1D). Virus-induced gene silencing of NbCRAL in *N. benthamiana* led to reduced expression of pathogen-associated molecular pattern-triggered immunity (PTI) related genes, implying a role in plant defense. We hypothesize that BYDV may manipulate CRAL-TRIO domain-containing PITPs to evade plant immune defenses and alter host phosphoinositide metabolism. This manipulation likely facilitates viral replication complex assembly and viral movement across cellular compartments, highlighting a strategic viral exploitation of host lipid metabolism pathways.

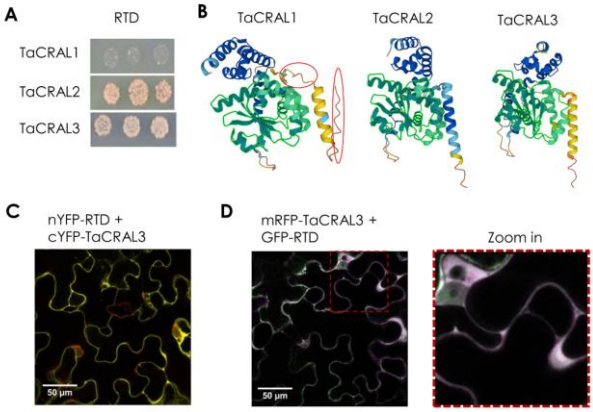

Figure 1. Barley yellow dwarf virus (BYDV) protein, read through domain (RTD), interacts with CRAL-TRIO domain containing phosphatidylinositol transfer proteins. *(A) Wheat CRAL-TRIO domain containing proteins TaCRAL2 and TaCRAL3, but not TaCRAL1, interact with virus RTD protein in yeast two-hybrid assay. (B) Structural analysis using AlphaFold shows varying motifs at C-terminal of TaCRAL1 (red circles) compared to TaCRAL2 and TaCRAL3. (C) Independent validation of RTD-TaCRAL interaction using bimolecular fluorescence complementation assay (BiFC). Only TaCRAL3 is shown here. (D) TaCRAL3 shows nucleocytoplasmic localization, and its subcellular localization does not change in the presence of RTD.*

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P34

Development of a qPCR assay to evaluate the quality of ATAC-seq libraries prior to next-generation sequencing

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Polyploid cells support the development and function of various tissues in eukaryotes. These cells often arise from endoreduplication, a modified cell cycle involving genome replication without mitosis. In vascular plants, polyploid cells are frequent and play an important role in regulating cell and organ size. In tomato fruit pericarp, the fleshy part of the fruit, up to 7 successive rounds of endoreduplication can generate cells with a total DNA content of up to 256C. We showed that resulting cells differ greatly in size and display ploidy-specific gene expression patterns. To investigate the mechanisms underlying the changes in gene expression during endoreduplication, we thought to investigate changes in chromatin accessibility using the Assay for Transposase Accessible Chromatin (ATAC-seq). This technique relies on the ability of the Tn5 transposase to cut and insert known adapters in regions of chromatin that are devoid of nucleosomes and thus “accessible”. To measure chromatin accessibility in homogeneous populations, nuclei of different ploidy levels are sorted by fluorescence-activated nuclei sorting (FANS). We evaluated a number of experimental factors and found that the ATAC-seq profiles we generated showed variable levels of signal enrichment in the TSS region. We used the TSS enrichment score, a signal to noise calculation, to evaluate the quality of profiles generated from our samples. In order to assess the quality of ATAC-seq libraries prior to sequencing, we developed a qPCR assay targeting regions of expected signal enrichment and regions of background signal. We used this data to build an index of library quality prior to sequencing. Using sequenced ATAC-seq libraries, we show that our qPCR-generated score is able to discriminate with good confidence samples with high, moderate or low TSS enrichment score. This will allow us to streamline the optimization of our ATAC-seq protocol for the different experimental conditions without systematically sequencing every library.

P35

Impact of the environmental influence on the transcriptional variability of genes involved in nitrogen nutrition

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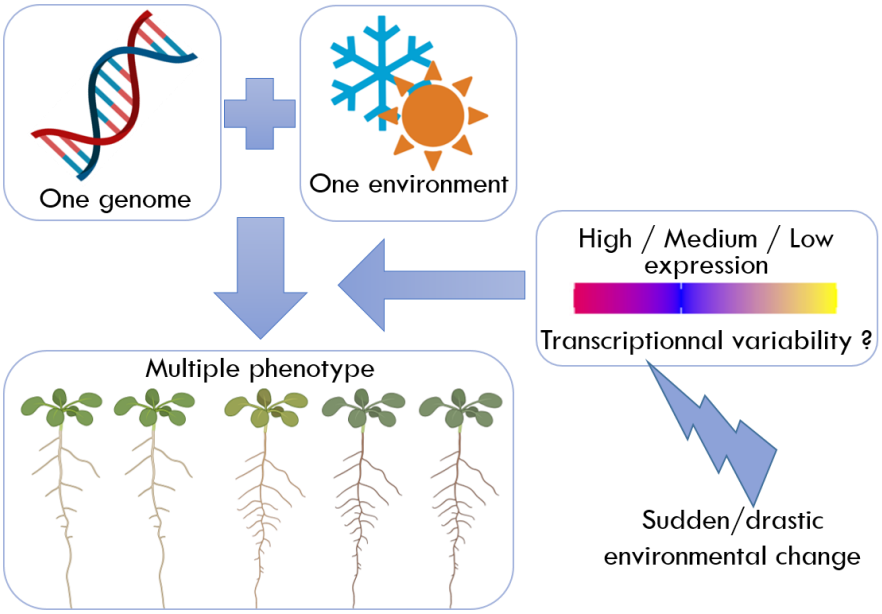
A key question in biology is to understanding how a phenotype is generated by a given genotype. However, it exists some situations where we observe multiple phenotypes for genetically identical plants in the same environment. This observation cannot be explained by plasticity (response to environmental changes) or genetic variation (differences between mutant or ecotypes). This phenotypic variability might be partly caused by variability in gene expression between plants.

We are interested in understanding if this transcriptional variability might be of use for plants in their natural environment. For this, we study the impact of mild as well as strong environmental changes on this inter-individual transcriptional variability.

We are focusing on genes involved in nitrate nutrition as several ones have a high variability. Moreover, nitrate is one of the most determining factors in the growth, survival and reproduction of plants, but fluctuates a lot in the soil. A sensitive regulation of the expression for genes involved in its uptake and its transport is therefore decisive.

We show that the high affinity nitrate transporter *NRT2.1* is a highly variable gene and that this transcriptional variability as phenotypic consequences. We confirm also that these differences in expression between seedlings for *NRT2.1* are stable and maintained over time but not transmitted to the next generation. We are trying to understand at what point in the plant's life these differences are established and why.

Finally, we highlight that variability for *NRT2.1* is only affect by a sudden and drastic environmental change like nitrate starvation. These results will be essential to define if this mechanism could be beneficial for a population of plants facing a sudden and drastic environmental change.



P36

An *Arabidopsis* TRAPPII phosphorylation code mediates adaptation to abiotic stress.

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Plant adaptation to stress conditions is often achieved via differential growth. We have recently shown that a module consisting of shaggy-like kinases (GSK3/AtSKs) and the Transport Protein Particle II (TRAPPII) complex is essential for differential growth decisions. AtSKs phosphorylate a TRAPPII-specific subunit at three different sites, and the TRAPPII phosphostatus mediates adaptive growth responses at the seedling stage. AtSKs are multifunctional kinases that integrate a broad range of signals at the cell surface, and TRAPPII coordinates resource allocation at the trans-Golgi network (TGN). How environmental conditions are encoded to elicit diverse plant adaptation responses via the AtSK-TRAPPII interaction remains unclear. The three phosphorylation sites of TRAPPII give rise to at least eight different phosphorylation states. This raises the question as to whether each combination of phosphorylation states conveys a distinct information; or whether the phosphorylation sites instead cooperate as a gradient with additive effects. To address this question, we tested different phosphovariants of the three identified GSK3/AtSK phosphorylation sites in response to a variety of abiotic stress, applied singly or additively. Different physiological assays were used to assess plant adaptive responses. A phosphovariant with a single phosphomimetic site showed the most sensitive phenotype to osmotic stress, and this could partially be rescued by mutating also the second or even third phosphorylation site to phosphomimetic. Under future climate scenarios including heat and/or drought stress, the phosphovariants showed different resilience levels than under osmotic stress. We discuss how the emerging pattern could point to a TRAPPII phosphocode that regulates plant adaptation to different environmental cues.

P37

A combined lipidomic and proteomic profiling of Arabidopsis thaliana plasma membrane

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The plant plasma membrane (PM) plays a key role in nutrition, cell homeostasis, perception of environmental signals, and set-up of appropriate adaptive responses. An exhaustive and quantitative description of the whole set of lipids and proteins constituting the PM is thus necessary to understand how the way these components, are organized and interact together, allow to fulfill such essential physiological functions. Here we provide by state-of-the-art approaches the first combined reference of the plant PM lipidome and proteome from *Arabidopsis thaliana* suspension cell culture. We identified a reproducible core set of 2,165 proteins which is by far the largest set of available data concerning the plant PM proteome. We combined lipidomic approaches, allowing the identification and quantification of an unprecedented repertoire of 405 molecular species of lipids. We showed that the different classes of lipids (sterols, phospholipids, and sphingolipids) are present in similar proportions in the plant PM. Within each lipid class, the precise amount of each lipid family and the relative proportion of each molecular species were further determined, allowing us to establish the complete lipidome of Arabidopsis PM, and highlighting specific characteristics of the different molecular species of lipids. Results obtained are consistent with the plant PM being an ordered mosaic of domains and point to a finely tuned adjustment of the molecular characteristics of lipids and proteins. More than a hundred proteins related to lipid metabolism, transport or signaling have been identified and put in perspective of the lipids with which they are associated. All these results provide an overall view of both the organization and the functioning of the PM.

P38

Characterization of a new potential transporter of iron-mobilizing coumarins in *Arabidopsis thaliana*

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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. To cope with this poor bioavailability, plants have evolved a sophisticated mechanisms to mine Fe from the soil. Recently, it has emerged that non-grass species secrete Fe-mobilizing coumarins (specialized metabolites) by the plant root system via the PDR9 transporter to improve Fe uptake. Here we will present recent findings on the characterization of a potential novel transporter of Fe-mobilizing coumarins, PDR9-like.