"XX National Plant Biochemistry and Molecular Biology Congress, 3rd Meeting of the Mexico Section of the American Society of Plant Biologists, 13th Mexico-USA Plant Biology Symposium"

Abstract Book

October 16 – 20 / 2023
Oaxaca, Oax. México

Index

Plant Biology in Oaxaca!

Welcome to the 20th National Plant Biochemistry and Molecular Biology Congress of the Mexican Biochemistry Society (SMB), 13th Joint Plant Biology Symposium between Mexico and the USA, and the 3rd Mexico Section meeting of the American Society for Plant Biologists (ASPB).

This meeting is a forum for interaction between Plant Biologists from Mexico, the United States, Canada, Europe, and the rest of the world. We will have Keynote, Plenary, Oral, and Poster presentations from scientists working in the fields of Abiotic Stress, Ecology and Evolution, Biotic Interactions, Metabolism, Development, and Novel Technologies. When selecting Keynote and Plenary speakers, the organizing committee strove for gender equality as well as representation from different disciplines and geographic areas.

We see this meeting as an outstanding opportunity to bring cutting-edge Plant Biology researchers to Mexico. Our goal was to provide a forum for interaction of Mexican students and postdocs with top international researchers, without the costs and long-distance travel normally associated with an international meeting. It is our hope that this meeting will facilitate the emergence of new synergies and collaborations between plant biologists working in different disciplines and different places.

Organizing Committee

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Keynote Lecture

Biodiversity and the autonomous pest control in coffee agroforestry systems

Ivette Perfecto

Bunyan Bryant Collegiate Professor of Environmental Justice, School for Environment and Sustainability, University of Michigan, USA

Coffee can be produced in a variety of systems, from monocultures to very diverse agroforestry systems with a high diversity of planned (agrobiodiversity) and associated (wildlife) biodiversity. In this presentation, we will look at how the management of shade trees affects coffee yield and how biodiversity can help control pests such as the coffee berry borer, green coffee scale, and coffee rust. Additionally, we will introduce an ecological game that demonstrates the complexity of ecological networks, including predator-prey interactions and trait-mediated indirect interactions.

Keynote Lecture

How interorganellar communication regulates plant adaptive responses

Katayoon Dehesh

Institute of Integrative Genome Biology and Department of Botany and Plant Sciences
UC Riverside, USA

Interorganellar communication is an evolutionary necessity for maintenance of cellular homoeostasis in response to prevailing environment that, in part, is exquisitely controlled via retrograde-signaling pathways.

We have identified a novel stress-specific plastidial retrograde signalling metabolite, methylerythritol cyclodiphosphate (MEcPP), previously known solely as an intermediate in the isoprenoid biosynthetic pathway. The additional function of MEcPP as a stress sensor and a coordinator of transcriptional and post transcriptional regulation of key stress-responsive nuclear genes, has unraveled the central role of this metabolite in cellular functions in response to a wide range of environmental and developmental cues.

To identify the underlying molecular mechanism of the MEcPP-mediated stress responses, we have performed a multi-omics approach. These studies have led to the identification of a transcriptional hub activated by MEcPP and have further established a previously unrecognized link between this plastidial retrograde signal and the transcriptional reprogramming of endoplasmic reticulum genes critical for readjustment of protein-folding capacity in stressed cells. Moreover, we have gained an insight into the molecular mechanism by which MEcPP alters subcellular structures and contributes to phytochemical diversity.

In brief we have advanced our understanding of how MEcPP reprograms a repertoire of intricate networks crucial for coordinating the physiological and metabolic processes required for stress-induced developmental and adaptive responses.

Keynote Lecture

Root system diversity in Mexican native maize

Ruairidh J. H. Sawers

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The domestication of maize (*Zea mays* ssp. *mays*) about 9000 years ago in Mexico was one of the most significant events in the history of agriculture. Within Mexican territory alone, the tireless efforts of generations of farmers have produced almost 60 different recognized native varieties (*razas*), adapted to elevations from sea level to well over 3,000m, and environments ranging from semi-desert to the hot, humid tropics. As maize dispersed, it was the roots that most directly faced many of the challenges posed by new environments. While the diversity of ear morphology readily illustrates the richness of Mexican maize, it is what is happening below ground that may be of greatest importance with regard to resilience in the face of climate change. Here, I will present high-throughput root phenotyping approaches that are beginning to provide a picture of the variation in root system architecture, root anatomy, and root-microbe to be found in Mexican native maize. I will discuss the challenges of determining the functional implication of such observed variation and of relating this to potential adaptive strategies in different environments. I will introduce a novel multi-parent genetic mapping resource for Mexican native maize and illustrate its application in testing adaptive hypotheses.

Keynote Lecture

Reprogramming ABA receptors for new plant functions

Sean Cutler

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Plants sense the hormone abscisic acid (ABA) using chemical-induced dimerization (CID) modules, including the receptor PYR1 and HAB1, a protein phosphatase inhibited by ligand-activated PYR1. This system is unique because of the relative ease with which ligand recognition can be reprogrammed. To expand the PYR1 system, we designed an orthogonal response module that harbors a dimer-interface salt bridge that prevents cross-activation of the wild-type module, as supported by X-ray crystallographic, biochemical, and in vivo analyses. We used this module to create new CID modules, PYR1*MANDI/HAB1* and PYR1*AZIN/HAB1*, that possess nM sensitivities to their activating ligands mandipropamid and azinphos-ethyl. Experiments in A. thaliana and S. cerevisiae demonstrate the sensitive detection of banned organophosphate contaminants using living biosensors and the construction of multi-input/output genetic circuits. Our new modules enable ligand-programmable multichannel CID systems for plant and eukaryotic synthetic biology that can empower new plant- and microbe-based sensing modalities. We also highlight engineered plant hormone sensors for creating inducible phenotypes in Arabidopsis, diverse Citrus varieties, and Setaria. Lastly, by developing a high throughput screening platform to conduct unbiased screens, we demonstrate that PYR1-derived sensors can recognize a substantial fraction of drug-like chemical space, which suggests that designing sensors for user-specified molecules and, thus, ligand-controlled plant traits is feasible. The biochemical features that make PYR1 an excellent scaffold for designing orthogonal receptor-ligand interactions are shared by GA and strigolactone receptors, which suggest that other plant hormone-sensing systems can be harnessed to reprogram hormonal and transcription responses so that ligands of choice control them.

Plenary Talk

Engineering synthetic CAM and tissue succulence into C_3 photosynthesis plants to improve water-use efficiency and drought tolerance

Arely V. López Pérez[1], Johnathan S. Lomas[1], Mia L. Swain[1], Lisa M. Petrusa[1], Won Cheol Yim[1], Sung Don Lim[2], and John C. Cushman[1,*]

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The global climate crisis is rapidly increasing major crop losses around the world due to increased intensity and duration of drought events. Therefore, novel strategies are needed to create more climate-resilient crops. Remarkable progress has been made in advancing our understanding of the functional genomics of crassulacean acid metabolism (CAM) in the last decade. Dozens of CAM plant genomes have now been sequenced including obligate CAM species such as *Kalanchoë fedtschenkoi*, facultative CAM species such as *Mesembryanthemum crystallinum*, and important crops such as *Agave*, *Ananas*, *Hylocereus*, and *Opuntia*. Well-annotated genomes along with extensive transcriptomic, proteomic, and metabolomic datasets and associated co-expression and transcriptional regulatory networks provide a strong foundation for understanding the biochemical and regulatory frameworks that underpin the diel and circadian operation of CAM. Detailed time-resolved transcriptome profiling analysis in the facultative CAM plant *M. crystallinum* has revealed hundreds of genes with putative CAM-associated functions that provide the building blocks for creating synthetic versions of CAM in the C_3 photosynthesis model *Arabidopsis thaliana*. We have built different gene circuits to recreate synthetic versions of the carboxylation, decarboxylation, and core diel carboxylation + decarboxylation modules of CAM. The carboxylation module increased CO_2 assimilation, nocturnal malate accumulation, and plant biomass whereas the decarboxylation module improved water-use efficiency. Combining the carboxylation and decarboxylation modules resulted in increased CO_2 assimilation, nocturnal malate accumulation, plant biomass, and improved water-use efficiency. Design and implementation of new iterations of the SynCAM design cycle (SynCAM 2.0) also target soybean, a major crop that suffers large economic losses due to damage from drought events. CAM functions optimally within succulent leaves so the benefits of tissue succulence engineering will also be discussed.

Plenary Talk

Salt stress resilience; think global, act local

Christa Testerink

Wageningen University & Research
Netherlands

Salinity of the soil is highly detrimental to most plant species. Recently we have identified several molecular and cellular pathways in the model species Arabidopsis that contribute to finetuning of root development to better deal with salt stress. I will highlight what we can learn from these pathways and their regulation at the tissue-specific level in our new approaches to improve salt tolerance of plants. Yet while we are sometimes successful in translating our findings from Arabidopsis to crops, we must also broaden our investigations to acquire knowledge on the molecular basis of stress-induced developmental responses in crops and in naturally salt-adapted plant species.

Plenary Talk

Two pathways for post-transcriptional regulation of gene expression during stress responses in plants

José Luis Reyes Taboada

Instituto de Biotecnología, UNAM, México

Plants are sessile organisms exposed to constant changes in their environment. Therefore, they have developed diverse and efficient mechanisms to contend with different external factors, including abiotic factors such as extreme temperatures, light intensity and quality, air and soil quality, or the availability of water for growth. My research group has focused on the study of responses to water deficit, considered an important factor for plant growth and development. One of the stages of gene expression that is finely regulated in plants occurs at the post-transcriptional level, by regulating the half-life, fate, or translation of messenger RNAs. In this context, we are studying two pathways to regulate the metabolism of mRNAs that are relevant in different biological systems: the pathway mediated by microRNAs and that mediated by the methylation of adenosines in the mRNA.

The discovery of small RNAs as regulators of gene expression led us to explore the participation of microRNAs in the regulation of the response of *Phaseolus vulgaris* (common bean) and, in general, of legumes, to conditions of water limitation. Our studies have revealed their involvement and some interesting properties of legume microRNAs during their biogenesis and mechanisms of action, such as miR2119. More recently, we are exploring the functions of miR396 in *P. vulgaris* and miR2199 in *Medicago truncatula* under water-limiting conditions.

A second theme in our research is focused on the methylation of adenosine residues at position N6 (m6A) as the most abundant internal post-transcriptional modification in mRNAs and lncRNAs in both plants and animals. This modification can alter the secondary structure of RNA or can affect its subcellular localization, translation, stability and other processes. In plants little is known about the consequences of the addition of this modification and in general the processes that are regulated by this pathway, thus we are exploring different participating factors using several plant models, including the model plant *Arabidopsis thaliana*, where we study the m6A-binding protein ECT8 and its contribution to stress responses.

Plenary Talk

Genomics for conservation of Mexican Agave and cacti

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Mexico has a tremendous legacy and management responsibilities in hands. The country is one of the few biodiversity hotspots for both wild and domesticated plants in the world. Many species, varieties and landraces can only be found in the region; due to geographical, biological and cultural reasons. Two of the most important plant groups with endemic species in Mexico are the Agave genus and the cactus family. Approximately 75% of species of Agave are endemic and 84% of the cactus found in Mexico are found only here. The most recent assessments from conservationist groups reveal that both Agave and cactus species are the most endangered, threatened of extinction mostly from illegal poaching, change in land use and global warming. During this talk, I will explain some of the research initiatives of Desert Botanical Garden in collaboration with several institutions in Mexico and the community to study and preserve both cactus and Agave biodiversity using genomic tools. In particular, I'll explain our study of the columnar saguaro cactus and the efforts we are making to involve and actively engage the community to set awareness of climate change and their effects in cactus biodiversity. I will also talk about our efforts to document and preserve the almost lost mezcal and tequila traditional Agave landraces, threatened by the enormous demand for only two cultivars that produce the spirits that are highly sought-after in the USA and Europe.

Plenary Talk

Aethionema arabicum; a novel model to study the light control of seed germination

Zsuzsanna Merai

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The timing of seed germination is crucial for seed plants and is coordinated by internal and external cues, reflecting adaptations to different habitats. Light is a major environmental factor regulating seed germination, which provides information about the position in the soil, the presence of competitors, day length, and the season. Seeds can be categorized based on their response to white light during germination; light-requiring seeds germinate only after light exposure, light-inhibited seeds germinate only in the dark while light-neutral seeds germinate in both light and darkness. Seeds of *Arabidopsis thaliana* and lettuce (*Lactuca sativa*), the model plants for research in this field, require a minimal light exposure for complete germination. Therefore, most insight into the role of light for seed germination originates from the light-requiring seed type. We discovered a natural variation in seed germination responses in *Aethionema arabicum* (*Brassicaceae*) accessions, a plant originates from arid and semi-arid habitats. One accession from Turkey has light neutral seeds while the seed germination of an accession from Cyprus is strongly inhibited by white, red, far-red and blue light. The germination inhibition increases with light intensity and duration, possibly evolved as an adaptive trait to response to day length to limit the germination time in early spring with short days. In CYP seeds light induces gene expression changes of key regulators converse to Arabidopsis, resulting in antipodal regulation of hormone balace. These findings illustrate that similar modular components of a pathway in light-inhibited, light-neutral, and light-requiring germination among the Brassicaceae have been assembled in the course of evolution to produce divergent pathways. Screening the first mutant collection of *A. arabicum*, we identified *koy-1*, a mutant that lost light inhibition of germination, due to a deletion in the promoter of HEME OXYGENASE 1, the gene for a key enzyme in the biosynthesis of the phytochrome chromophore. Hormone and gene expression comparison between wild type and *koy-1* under different light intensities revealed that very low fluence stimulates germination, while high irradiance is inhibitory, indicating a dual role of phytochromes in light-regulated seed germination. The mutation also affects the ratio between the two fruit morphs of *A. arabicum*, suggesting that light perception via phytochromes can fine-tune several parameters of propagation in adaptation to conditions in the habitat.

Plenary Talk

Photosynthetic cell identity is triggered by a root transcription factor

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Photosynthesis is one of the most common biochemical processes on earth and is responsible for fueling practically the entire biosphere. However, we know remarkably little about how plant tissues differentiate to acquire photosynthetic functions. For example, early in development most leaf cells contain proplastids and are exposed to the same environmental cues, however only a specific cell type, the mesophyll, acquires photosynthetic identity. What makes some tissues uniquely primed to become photosynthetic in response to light is a fundamental question in plant biology. Moreover, the relocation of photosynthetic functions across leaf tissues can greatly impact CO2 fixation efficiency, as seen in C4 plants where the bundle sheath and not the mesophyll acquires photosynthetic identity. In this work, we have generated several CRSIPR mutant lines showing defects in photosynthetic tissues in the C4 model plant *Setaria viridis*. Through single-cell RNAseq profiling of mutant leaves, we identified a transcription factor previously associated with root patterning that drives the expression of the photosynthetic machinery in leaves, as well as chloroplast differentiation genes in a cell-type specific fashion. Moreover, we leveraged expression data of hundreds of cell-type specific genes to quantify and track how cell identity changes in mutant lines. We discovered that mutant mesophyll and bundle sheath cells not only lose photosynthetic identity, but also acquire identity of non-photosynthetic cells like epidermis. Finally, we show this has important consequences for photosynthetic efficiency and plant productivity.

Plenary Talk

Mining plant-pathogen interactions: Insights from genomics, transcriptomics, and machine learning

Alejandra Rougon-Cardoso

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One of the major threats to food security are pests and diseases, which account for an estimated yield loss of 35% worldwide, and up to 50% in developing countries. The plant immune system is based on a sophisticated complex of interactions at two main levels. The first one makes use of pattern recognition receptors to survey for pathogen associated molecular patterns (PAMPs). This first level of recognition termed PTI or PAMP-triggered immunity triggers responses that can stop microbial growth. However, successful pathogens that can surpass PTI deliver an arsenal of proteins, called effectors, which are able to manipulate or suppress plant defenses. To survive, plants have evolved a second level of intracellular receptors system which can monitor effectors and activate the downstream response cascade quickly and with a higher amplitude. This second level of resistance is called ETI or Effector-triggered immunity. Genomics research on the interplay between parasite effectors and host receptors can provide a relatively fast and comprehensive understanding of the disease and resistance processes, allowing the development of novel biotechnological approaches to reduce crop losses and provide a more sustainable agriculture production. One of our goals is to generate genomic data of important parasitic nematodes, to understand how specific pathogen effector proteins have evolved, and search for genes involved in host resistance. We aim to develop computational pipelines to better predict effector proteins and their interactors using a combination of traditional alignment tools and machine learning. Another area we are focusing on is the exploitation of expression data already available in databases using unsupervised learning to predict phenotypes and find pathogen-specific patterns. Additionally, we have been looking for bacterial resistance hints in wild host species. Lastly, we have analyzed plant sciences papers of the last 20 years to identify and quantify social and taxonomy disparities across the field. Our goal is to visualize these disparities and, hopefully, encourage people to work towards reducing them.

Plenary Talk

Untangling biological complexity to reveal root metabolites the drive microbiome assembly

Sarah Lebeis

Dept. of Plant, Soil and Microbial Sciences
Michigan State University, USA

Plant microbiomes are assembled and modified through a complex milieu of biotic and abiotic factors. Despite dynamic and fluctuating contributing variables, specific host metabolites and immune responses are consistently identified as important mediators of microbial interactions. Mechanistic understanding of how these interactions combine to dictate microbiome establishment remains largely enigmatic. We aim to identify specific plant and microbial factors mediating plant-microbe and microbe-microbe interactions at the root interface. We use a range of experimental biotic complexities, from single strains to natural microbiomes, and host genetic mutants to gain both mechanistic and holistic understanding of how individual phenotypes scale-up to higher-order community dynamics. We combine information from a large-scale metatranscriptomic dataset from natural poplar trees and experimental genetic manipulation assays in seedlings of the model plant *Arabidopsis thaliana* to converge on a conserved role for transport of the plant metabolite *myo*-inositol in mediating host-microbe interactions. Our data suggests host control of this compound and resulting microbial behavior are important mechanisms at play surrounding the host metabolite *myo*-inositol. We then combine bacterial strains into synthetic communities and assess the impact of different salicylic acid on microbiome establishment and maintenance. We find that individual bacterial strains have distinct phenotypes in the context of plant roots and these behaviors can be explained by salicylic acid presence. Together, these studies reveal that two common root exudatesp play distinct roles in root microbiome assembly and subsequently influence varying microbiome functioning and biodiversity.

Plenary Talk

New and old tricks used by a protist to manipulate plant immunity

Edel Pérez López

Université Laval
Québec, Canada

Plasmodiophora brassicae is the causal agent of clubroot, a devastating disease affecting cruciferous crops worldwide and responsible for more than $500 millions in lost for the canola industry in Canada in the last ten years. Although several breakthroughs have happened recently, clubroot research is at least a decade behind and our understanding of the molecular strategies used by the clubroot pathogen to escape plant immunity are mostly unknown. Since 2020 our lab is focusing on the functional characterization of putative effectors and their role in the plant. Among the results generated in these three years, we will present in this concurrent the story behind three main findings: (i) the characterization of SSPbP53, a cysteine protein inhibitor that seems to be key for the clubroot pathogen pathogenicity, (ii) the characterization of PbChiB2 and PbChiB4, two chitin-binding proteins that stop chitin-triggered immunity during the clubroot pathogen infection, and (iii) the characterization of PbPK1, a pseudo-kinase that induce tolerance to the clubroot pathogen. Altogether, the new mechanisms presented here are advancing our understanding of a less typical vascular pathogen putting at risk the economy of several countries and our food security.

Plenary Talk

Evolution of CAM photosynthesis in the Agavoideae

Karolina Heyduk

Ecology and Evolutionary Biology Department
University of Connecticut, USA

CAM photosynthesis is an important elaboration on the typical C3 photosynthetic pathway, allowing numerous plants to succeed in environments limited by water. The Agavoideae — which includes iconic desert plants like *Yucca* and *Agave* — has multiple origins of CAM photosynthesis, providing a unique opportunity to examine the mechanisms underlying repeated evolution of CAM. Further, the Agavoideae species that use CAM do so to varying degrees, including constitutive CAM, intermediate C_3+CAM, and facultative CAM (where plants can upregulate CAM under abiotic stress). Here I will explore how the independent origins of CAM are in some ways not convergent, recruiting different gene families to accomplish the same enzymatic functions. I'll further discuss how new genomic data is highlighting the difficulty of classifying plants as CAM or C_3, and what implications this has for the evolution of CAM in the Agavoideae.

Plenary Talk

Harnessing plant metabolism for new catalysts, medicines, and materials

Jing-Ke Wang

Institute for Plant-Human Interface
Northeastern University, Boston, MA, USA

Plants contain diverse specialized metabolites, many of which are of significant pharmaceutical and industrial importance to humans. Nevertheless, exploration of specialized metabolic pathways underlying specific chemical traits in nonmodel plants has been technically challenging and historically lagged behind that of the bacterial systems. Recent advances in genomics, metabolomics, phylogenomics, and synthetic biology now enable a new workflow for interrogating unknown specialized metabolic systems in nonmodel plant hosts with greater efficiency and mechanistic depth. In this talk, I will discuss our current effort in elucidating a number of specialized metabolic pathways in various medicinal plants using such workflow. Facilitated by this newly learnt knowledge, we engineer chassis organisms to produce valuable plant natural products and their new-to-nature analogs with broad industrial, agricultural, and pharmaceutical utilities. In addition to small-molecule natural products, plants also produce a wide range of macromolecular biopolymers which are key to plants' adaptation to the terrestrial environments. I will also discuss our recent effort in studying the chemistry, biochemistry and evolution of sporopollenin, an extremely inert biopolymer that coats the outer wall of all land plant spores and pollen grains. Engineering sporopollenin-like synthetic polymers and sporopollenin biosynthesis in crop plants may open new avenues for new materials and scalable strategies for mitigating climate change.

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Plenary Talk

Discovery and engineering of plant metabolic pathways for plant and human health

Elizabeth Sattely

Department of Chemical Engineering and HHMI
Stanford University, USA

Abstract: Humans are extraordinarily reliant on plants and plant-derived molecules for food, medicine, and energy. However, remarkably little is known about how plants perform the chemistry responsible for making these molecules. New plant genome sequences and synthetic biology tools have opened the door to three research areas that inspire the work we do in my lab: 1) Identifying and exploiting the enzymes responsible for synthesizing known plant-derived chemicals, and 2) discovering new molecules from plants, and 3) developing new strategies for sustainably enhancing plant fitness. This talk will describe efforts in my lab to use a combination of biochemistry, synthetic biology, bioinformatics, transcriptomics, and metabolomics to accelerate the discovery and engineering of plant metabolism. We use both a candidate gene approach to uncover novel pathways and new molecules, as well as a candidate molecule approach for targeted elucidation of metabolic enzymes. Our vision is to build metabolic pathways from newly discovered enzyme catalysts that can enhance human health, plant health, and the production of sustainable chemicals.

Plenary Talk

"To self or not to self": Pollen rejection in *Nicotiana*

Felipe Cruz García

Depto. de Bioquímica, Facultad de química, UNAM, México

In Solanaceae, self-incompatibility (SI) is under the *S*-locus control, which encodes both the female (S-RNase) and male determinants (a suite of SLF proteins). However, other non-*S*-locus linked genes, called modifiers genes (MG), such as *HT-B*, *120K*, *NaStEP*, *NaTrxh*, and *NaSIPP,* are essential to correctly perform the pollen rejection response in S-RNase base SI systems. *NaTrxh* encodes a thioredoxin h, an extracellular localized protein in the pistil transmitting tract. NaTrxh physically interacts with the female determinant, the S-RNase. As a result of this interaction, NaTrxh reduces a specific disulfide bound in the S-RNases, provoking a seven-fold increase in its ribonuclease activity. Gain of function assays in *Nicotiana* transgenic plants, expressing a pistil mutated NaTrxh variant, which is not able to reduce any disulfide bound in the S-RNases, generate a negative dominant phenotype in which the ability to recognize the self-pollen is disrupted. *NaStEP* encodes a stigma-specific protein that exhibits a dual activity as a protease inhibitor and voltage channel blocker. NaStEP is taken up by pollen tubes (PT). Loss of function assays show that NaStEP suppression disrupts pollen rejection in an *S*-specific manner. Another crucial pistil protein for SI is called HT-B. It is degraded in PTs from compatible crosses but not in SI ones. However, in the absence of NaStEP, HT-B is degraded inside PTs no matter if the cross is compatible or incompatible. It suggests that with its proteinase inhibitor activity, NaStEP protects HT-B from degradation.

We looked for NaStEP PT protein interactors by Y2H. By this approach, we recovered a mitochondrial phosphate carrier called NaSIPP, specific and highly expressed in mature pollen of SI *Nicotiana* species.

A loss of function approach shows that NaSIPP is essential to SI, and BiFC assays demonstrate that the interaction of NaStEP with NaSIPP occurs in the PT mitochondria.

In this talk, I propose a new model for the pollen rejection response in *Nicotiana*, in which all products of these modifier genes integrate the genetics and biochemistry pathway that underlies the pollen rejection response *S*-locus depending on *Nicotiana*.

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Plenary Talk

Morphogenetic determinants of plant female germ cell precursors specification and plasticity

Inès Ouedraogo[1], Gabriella Mosca[2], Luciana Delgado[3], Olivier Leblanc[1], Elvira Hernandez-Lagana[1], Ethel Mendocilla Sato[4], Olivier Hamant[5], Arezki Boudaoud[6], Christophe Godin[5], Ueli Grossniklaus[4], Marc Lartaud[6], Geneviève Conéjéro[6,7], Célia Baroux*[4] and Daphné Autran*[1]

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In higher plants, female gametes formation is a crucial step in the plant reproductive cycle and determines seed formation, hence participating in crop yields. The plant female germline initiates in the ovule primordium, with the specification of the Megaspore Mother Cell (MMC), the only cell which will undergo meiosis to produce gametes. However, germ cell fate in the early ovule appears flexible. Genetic variants and apomictic species show that somatic cells neighboring the MMC can enter the MMC identity program or even directly produce female gametophytes without meiosis. By combining 3D morphometrics, growth modelling, gene markers and genetic analyses, we have shown in Arabidopsis that this developmental plasticity is also part of MMC ontology in wild-type, before channeling toward MMC singleness, a process controlled by ovule tissue growth. Recently, we developed new routes further exploring Arabidopsis MMC growth using time-lapse (3D+t), and establishing a 3D morphometric atlas of ovule primordia in Maize, a sexual grass model, to understand the genericity of MMC formation and its plasticity.

Plenary Talk

Mechanisms of Imprinting Heterogeneity in *Arabidopsis*

Mary Gehring

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Dept. of Biology, Massachusetts Institute of Technology, Cambridge MA USA

Seeds are the end product of reproduction in flowering plants and represent the unit by which information is passed from one generation to the next. Plant reproduction is accompanied by distinct alterations to DNA methylation and repressive chromatin modifications in male and female gametes. These alterations are important for establishing gene expression programs after fertilization, including gene imprinting. Imprinting refers to genes that are preferentially expressed from either the maternally or paternally inherited allele. Our recent work using single-nuclei and cell-type specific profiling suggests that additional epigenetic reprogramming occurs in specific cell types after fertilization in a seed nutritive tissue called the endosperm, where imprinting occurs. Although the endosperm is an ephemeral tissue that does not pass on genetic material to the next generation, it is essential for the development of viable seeds and is the source of much of the calories we consume. I will present our recent findings on epigenetic dynamics and gene imprinting in the endosperm, including the role of the 5-methylcytosine DNA glycosylases ROS1, and discuss how these data fit within a consideration of potential genetic conflicts within seeds.

Plenary Talk

Rational search of hypoglycemic natural products from cloud forest plants: application of a computational-chemotaxonomic selection approach*

Cecilia Isabel Mayo-Montor

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Diabetes mellitus type 2 (DM2) is a disease considered a worldwide public health problem. In order to discover new alternatives for its treatment, we explored the potential of the cloud forest (CF), an ecosystem with wide botanical diversity but scarcely studied in the pharmacological and phytochemical context. Due to the lack of ethnopharmacological information, it is necessary to establish strategies that allow a rational selection of plant species for being included in experimental studies. With this aim, a library of chemical compounds (LCC) produced by species belonging to botanical families described for the Veracruz CF was elaborated. A ligand-based virtual screening (LBVS) was performed on the LCC to identify metabolites with appropriate physicochemical properties for oral administration (Lipinski and Jorgensen rules) and high similarity to drugs (Tanimoto coefficient) used in the DM2 treatment. The botanical families with the most molecules identified by the LBVS were selected and considered the best candidates. The selected plants were collected, the methanolic extracts were prepared, and the evaluation of their hypoglycemic activity was carried out by *in vitro* enzyme inhibition assays on α-amylase (αA), α-glucosidase (αG), and dipeptidyl peptidase IV (DPP4). The potential hypoglycemic activity of selected plant species was experimentally confirmed since out of the 16 plant species evaluated, belonging to ten genera and four families, 25%, 69%, and 75% of them inhibited more than 50% of the activity of DPP4, αG, and αA, respectively, at 1 mg/mL. Of the total species evaluated, *Sida rhombifolia* (SR, Malvaceae) showed a selective inhibition profile towards DPP4 (inhibition percentages: DPP4= 39.2 ± 2.41, αA= 1.8 ± 1.49 and αG= 6.9 ± 0.60) in contrast to *Sida glabra* (SG, Malvaceae) (inhibition percentages: DPP4= 47.4 ± 2.07, αA= 50.0 ± 1.29 and αG= 62.5 ± 1.32). In order to identify metabolites, untargeted and phenolics-targeted metabolomic analyses were performed. The untargeted metabolomic analysis performed in SR and SG methanolic extracts allowed the identification of mainly phenolic and terpenoid compounds, while through targeted metabolomics, 29 phenolic compounds were quantified. The SR/SG comparison of the chemical profiles of their methanolic extracts obtained by the different metabolomic approaches led to the tentative identification of around 40 metabolites accumulated in SR (fold change≥2), which are now being studied as potential selective inhibitors of DPP4, αG, and αA by *in silico* and *in vitro* methods. In conclusion, the computational-chemotaxonomic selection approach allowed us to rationally select candidate plant species for their experimental study based on their predicted hypoglycemic effect and focus on identifying new potential selective enzymatic inhibitors.

*Taken in part from PhD thesis of C. I. Mayo-Montor

Plenary Talk

Mapping biomolecules using AI/ML: Protein Structural and Functional Insights

Annabel Romero Hernández

SFL Scientific / Deloitte
New York, USA

In recent years, there has been a proliferation of algorithms dedicated to protein structure prediction; fundamentally, they have modified our comprehension of protein function. At the same time, using protein Large Language Models (pLMs) has expanded the horizons of understanding amino acid sequences beyond alignment. In this talk, I'll present our most recent development for protein function insights.
Firstly, I will show how pLMs are harnessed to discover unique connections among proteins across diverse species characterized by similar structures and, consequently, comparable functions. Then, I will discuss our utilization of protein prediction models for determining protein coordinates and define the relevant surface area for protein-protein interactions. Lastly, I will illustrate the efficacy of our methodology using a small example of allergen cross-reactivity in fruits.

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Plenary Talk

From Vine to Wine: Innovative Approaches to Enhance Grape Breeding

Luis Diaz-Garcia

Department of Viticulture and Enology
University of California, Davis, USA

Grapes, owing to their economic, historical, and cultural significance, hold a paramount position in agriculture worldwide. With a cultivation history that spans millennia, grapes have been used for diverse purposes, including wine making, fresh consumption, raisin production, and a variety of derived products. Consequently, a multitude of grape cultivars has emerged over the centuries, which underscore the relevance of this crop to humanity. Remarkably, a mere fraction of this genetic diversity, approximately x/x, currently accounts for over 44% of global grape production. While present climatic conditions and agricultural practices ensure stable grape production, a narrow genetic base can leave vineyards vulnerable to emerging pests, diseases, and adverse climatic conditions—particularly concerning the context of climate change. In this presentation, we will discuss why new, diverse, and more resilient grape cultivars are needed, and how novel, multidisciplinary approaches can increase breeding program efficiencies. For context, we will delve into the technical challenges that have limited the progress of grape genetic improvement and how cutting-edge genomics and phenomics technologies have the potential to overcome such limitations. Specifically, we will shed light on innovative approaches, encompassing robotics, hyperspectral imaging, AI-computer vision, genomics, and vine physiology, that enable the screening of breeding germplasm at large scale, both at the greenhouse and vineyard level. We will discuss, for example, how these techniques are facilitating the rapid screening of salt and drought tolerance traits in thousands of *Vitis* germplasm with potential for rootstock breeding. I will also provide an overview of the ongoing restructuring of the grape breeding program at the University of California, Davis, and our current efforts to increase the rate of genetic gain in wine grapes and grape rootstock materials.

Short Talk Selected from Abstract

A long non-coding RNA involved in shade avoidance

Irving J. García-López[1], Aaron Vélez-Ramírez[2], Stewart Gillmor[1], Selene Fernández Valverde[1,3]

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Long non-coding RNAs (lncRNAs) are non-coding RNA molecules greater than 200 nt that can interact with RNA, DNA and proteins. Their regulatory importance has been highlighted at epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels. Thousands of lncRNAs have been identified in plants through RNA-seq and bioinformatic analyses. So far these lncRNAs have been shown to function in processes like vernalization, photomorphogenesis, phosphorus starvation and male fertility.

When the plants grow under a tree canopy or at high plant densities, changes in the Red:Far Red light ratio trigger a phenotypic response known as Shade Avoidance Syndrome (SAS). These responses are characterized by petiole and hypocotyl elongation, chlorophylls reduction and cotyledon closure. In a previousd work, we assembled transcriptomes from cotyledon and hypocotyl tissues of Arabidopsis seedlings exposed to high Red/Far Red (high R/FR; normal condition) and low Red/Far Red (low R/FR; shade condition) conditions. Through bioinformatics, we identified 103 lncRNAs differentially expressed in cotyledons and 530 lncRNAs differentially expressed in hypocotyls. By employing co-expression networks, we inferred possible biological functions of lncRNAs in the SAS.

We selected 18 Arabidopsis lncRNA genes for functional characterization using T-DNA insertional mutants and phenotyping under high and low R/FR ratio. Loss of 7 lncRNA genes resulted in abnormalities in the petioles and hypocotyls under low R/FR ratios. We are currently focusing on a gene whose loss of function shows a petiole hyper elongation response in seedlings under low R/FR conditions. We will produce promoter:GUS fusion of the lncRNA to see patterns expression in the plant and silence the gene using amiRNAs. These experiments will allow us to determine whether these lncRNAs do indeed play a role in shade avoidance and will broaden our knowledge of lncRNA biology.

Short Talk Selected from Abstract

Determinate root growth in Cactaceae: new data on the incidence and unveiling of genetic regulation.

Svetlana Shishkova[1*], Gustavo Rodriguez-Alonso[1], J. Pablo Villa Nuñez[1], Ramsés U. Albarrán Hernández[1], Kenia A. Galván Alcaraz[1,2], Joel Rodríguez Herrera [1,2], Sofia Esteban Hernández[1,3], Julieta Olvera Berruecos[1,3], Y. Corín Pacheco Blancas[1], Kelly Juárez Hernández[1], Alejandra Lara Vargas[1], Selene Napsucialy-Mendivil[1], Joseph G. Dubrovsky[1]

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To thrive in deserts, Cactaceae plants possess a jackknife of developmental adaptations. Determinate root growth, i.e. the root apical meristem (RAM) consumption, or exhaustion, and subsequent differentiation of all root-apex cells soon after germination, is one of these adaptations (Dubrovsky, 1997). Determinate growth of primary and lateral roots of seedlings of many Cactaceae species from the Cactoideae subfamily (Shishkova *et al.,* 2013) leads to the formation of a compact root system that might provide seedlings with an advantage for survival in arid and semiarid environments. We analyzed the primary-root growth pattern, determinate or indeterminate, for ca. 150 Cactoideae species and all but one species exhibited determinate growth. To explore the genetic regulation of the RAM exhaustion in Cactaceae, we previously sequenced and *de novo* assembled the *Pachycereus pringlei* (cardón) transcriptome of the primary-root apex; and inferred genetic regulatory network that operate in the root apex (Rodriguez-Alonso *et al*., 2018). Recently, new findings were added from the analysis of *de novo* assembled *Carnegiea gigantea* (saguaro) root apex transcriptome. These transcriptomes, as well as the highly fragmented draft genome of six Cactoideae species (Copetti *et al.*, 2017, Zhen et al., 2021) allowed us the exploration in Cactaceae of the PLT and WOX5 pathways of RAM maintenance described in *Arabidopsis thaliana*. Functional analysis of candidate genes would help to unravel the molecular mechanisms of RAM exhaustion in Cactaceae; therefore, we are also interested in identifying suitable model Cactaceae species. For a few species with short life cycle, which is a very important characteristic for being model species, we estimated the feasibility of the lab handling, as well as the indirect *in vitro* regeneration and transformation via *Agrobacterium tumefaciens*.

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Short Talk Selected from Abstract

Natural variation in Mexican maize leaf anatomical traits

José Luis Coyac-Rodríguez[1,2], Marcela Hernández-Coronado[1], Daniel Oswaldo Camo-Escobar[1], Armando Espinoza-Banda[2], Oralia Antuna-Grijalva[2], Carlos Humberto Ortiz-Ramírez[1*]

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The leaf vascular system provides tissue's support, as well as transport of water, nutrients and photosynthates. Although their direct role on photosynthetic efficiency is yet unclear, evidence from C_4 plants -where higher vein density is observed- suggests a strong link. In this work we explore the natural anatomic variation in leaves of Mexican maize landraces evolved under diverse environmental conditions. Based on the hypothesis that a higher leaf vein density is related to a higher photosynthetic performance, we investigate vein density among landraces as well as through developmental stages. To analyze the leaf anatomy of the eight selected landraces we generated a rapid imaging protocol using simple dyes and fluorescence microscopy, allowing us to identify transversal tissue elements and three types of veins. Our first results confirmed a wide variation in rank-2 vein density (R_2VD) of V5 stage leaves between landraces, with values ranging from 44.1±4.1 to 79.6±4.6. Studies sampling leaves at the later stage V9 showed a decrease in the variation, where a tendency to reach a R_2VD around 62.9±3.7 to 80.4±4.5 is observed. Altogether, indicating both variation and plasticity for this trait. The environmental effect on this attributes were further evaluated under two contrasting environments: cool (~22°C) and hot (~28°C), simulating the origin regions of landraces. Preliminary results in the hot environment revealed a significant increase in the R_2VD from V5 to V9 stages in several landraces, demonstrating high plasticity in response to this environment, but not in cool environment. Also, changes in rank-1/rank-2 vein ratio (R_1/R_2) were observed, constituting a better indicator of anatomical relationships. Current experiments are directed to investigate the relationship of the observed changes with photosynthesis efficiency and related parameters. This will allow us to establish a direct link to productivity, and to establish new guidelines for plant breeding and use of genetic resources.

Short Talk Selected from Abstract

Towards Associative Transcriptomics to Study Meristem Activation in Perennials

Hernández-Coronado, M[1*]., Alonso-Nieves, A.L.[1], Pérez-Limón, S. [2], Ortiz-Ramírez, C.H.[1]. & Sawers, R.J[2].

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Perennialism is a complex trait involving diverse aspects of the biology of plants, resulting in the ability to regrow for several cycles after senescence. The possible contribution of perennialism to sustainable agriculture has gained recognition in recent years (a, b). But although the advances in obtaining perennial crops, little is known about the underlying genetics controlling such characteristics. To date, GWAS and QTL studies using the perennial teosinte *Zea diploperennis* allowed the identification of three loci related to regrowth (reg1, reg2 and reg3) (c, d). However, fine mapping to identify the genes involved has been hindered by the loss of the phenotype in subsequent selfing generations. Emphasizing the multigenic nature of perennialism and urging for a different approach. In this work we propose the use of associative transcriptomics to study meristem activation in perennials, one of the key elements for regrowth. Using two distinct segregating populations involving *Z. diploperennis* and the maize inbreeds CML312 and P39, we tracked the presence of the reg loci while looking for novel associated ones. In parallel, transcriptomic profiles of apical, axillar and rhizome meristems both from annual and perennial teosintes, as well as from selected individuals of the mapping populations, were generated. Altogether, setting the basis to identify candidate genes associated with meristem indeterminacy and activation.

[a] Crews, T. E., & Cattani, D. J. (2018). Strategies, advances, and challenges in breeding perennial grain crops. *Sustain. Sci. Pract. Policy*. 10:2192.
[b] Zhang, S., et. al. (2023) Sustained productivity and agronomic potential of perennial rice. *Nature Sustain.* 6, 28.
[c] Ma A., et. al. (2019). The Genetics and Genome-Wide Screening of Regrowth Loci, a Key Component of Perennialism in *Zea diploperennis*. G3. 9(5):1393-1403.
[d] Swentowsky K.W., et. al. (2021). QTL Map of Early- and Late-Stage Perennial Regrowth in *Zea diploperennis*. *Front Plant Sci*. 12:707839.

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Short Talk Selected from Abstract

Tracking the poison: The subcellular pathway that S-RNases follow inside the pollen tube toward its death

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Several hermaphroditic species have genetic mechanisms to prevent self-fertilization to avoid inbreeding depression. These are the incompatibility systems based on rejecting the self-pollen. In gametophytic self-incompatibility systems (GSI), pollen rejection occurs by inhibiting pollen tube growth. In Solanaceae, the female determinant is an *S-RNase* expressed in mature pistils. At the same time, the male determinant is a cluster of genes called *SLF* expressed in mature pollen, both linked to the *S* locus. In addition, modifier genes (MG) independent of the *S* locus are also essential in the pollen rejection genetic pathway. Nevertheless, in interspecific incompatibility, the pollen rejection response exclusively relies on the S-RNases, in some species since MGs are not required. However, the cellular and molecular mechanisms underlying the pollen rejection dependent on S-RNases must be better understood. This study aims to track the subcellular pathway that follows the S-RNases after they are taken up by *N. tabacum* pollen tubes and determine if there are cellular changes that lead to pollen tubes to death in an S-RNase-dependent manner. We have *N. tabacum* transgenic plants expressing different subcellular compartment markers fused to GFP to evaluate it. These subcellular markers are specifically expressed in pollen because they are under the control of the *Lat52* promoter. Pollen from these transgenic plants was germinated *in vitro* and incubated with fluorescent labelled-S_{C10}-RNase. Confocal microscopy results revealed that when S_{C10}-RNase enter pollen tubes, they are sorted into vesicles since the label S-RNases colocalizes with the Rab11:GFP, with a higher accumulation in the pollen tube tip. When pollen tubes were challenged with exogenous S-RNases, pollen tube growth was inhibited, corroborating the S-RNase cytotoxic effect. We currently perform *in vitro* pollen tube assays with pollen from transgenic plants expressing different subcellular markers to track the subcellular changes that led to dead pollen tubes in an S-RNase-dependent manner.

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Short Talk Selected from Abstract

Long distance signaling in developmental plasticity for light.

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Light, in addition to powering photosynthesis, also holds a multitude of signals that inform plants about photoperiod, directionality and presence of competing vegetation. Dedicated photoreceptors sense specific wavebands of the light and act through signaling pathways to adjust plant physiology and development. One important example is the detection, via phytochrome B inactivation, of far-red (FR) light that is reflected by neighboring vegetation. FR light serves as signal of upcoming competition and shading neighbors. In response, plants typically optimize their architecture to facilitate light capture in dense stands of plants.
Here I report our latest insights into how organ-specific detection of FR light stimulates shoot elongation and leaf movements and suppresses lateral root development. These insights come from an integration of approaches such as RNA sequencing, confocal microscopy of biosensors, genetics and plant physiology. I will discuss how FR-induces *de novo* synthesis of auxin in the leaf tip to drives a remote differential growth response in the petiole base, how local detection of FR in this petiole itself initiates a different response, and how FR sensing in the leaves controls lateral root development through an independent pathway.

Short Talk Selected from Abstract

Effect of transposon mobilization on genomic imprinting in *Arabidopsis*

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Genomic imprinting is an epigenetic phenomenon that causes the differential expression of parental alleles. In plants, imprinting is mainly limited to the endosperm, a nursing tissue that supports embryo development and originates after fertilization of the central cell (CC) with one of the two sperm cells (SC) of the male gametophyte. Asymmetric epigenetic modifications deposited in the CC and SC results in parent-of-origin expression after fertilization in a group of genes referred as Paternally Expressed Genes (PEGs) and Maternally Expressed Genes (MEGs).
Conservation of genomic imprinting is limited among angiosperm species and only few genes have been identified to be imprinted across species. The phenomenon of genomic imprinting is connected to transposable elements (TEs) and is at least partly contributed to differential TE silencing in male and female gametes. Using a strategy to remobilize TEs in the genome, we have generated a line containing several new TE insertions and monitored their effect on genomic imprinting. The consequences of transposon mobilization on genomic imprinting and on the epigenetic landscape of the Arabidopsis endosperm will be discussed.

Short Talk Selected from Abstract

Transcription-directed membrane association organizes the chloroplast nucleoid structure.

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DNA is organized into chromatin-like structures, which support the maintenance and regulation of genomes. A unique and poorly understood form of DNA packaging exists in chloroplasts, which are endosymbiotic organelles responsible for photosynthesis. Chloroplast genomes, together with associated proteins, form membraneless structures known as nucleoids. The internal arrangement of the nucleoid, molecular mechanisms of DNA packaging, and connections between nucleoid structure and gene expression remain mostly unknown. We show that *Arabidopsis thaliana* chloroplast nucleoids have a unique organization driven by DNA binding to the thylakoid membranes. DNA associated with the membranes has high protein occupancy, reduced DNA accessibility, and is highly transcribed. In contrast, genes with low levels of transcription are further away from the membranes, have lower protein occupancy, and higher DNA accessibility. Disruption of transcription at specific genes in sigma factor mutants causes a corresponding reduction in membrane association, indicating that RNA polymerase activity causes DNA tethering to the membranes. We propose that transcription organizes the chloroplast nucleoid into a transcriptionally active membrane-associated core and a less active periphery.

Short Talk Selected from Abstract

High-throughput and high-precision phenotyping for studying plant stress responses

Nuria De Diego[1]*, Francisco Ignacio Jasso-Robles[1], Carlos Eduardo Aucique-Perez[1], Klimeš Pavel[1], Xenia Roviralta-Travieso[1], Markéta Ulbrichová[1], and Lukáš Spíchal[1].

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The aim of the research group Phenotyping is to develop and apply automated, non-invasive image-based phenotyping methods for monitoring the environmental interactions of plants, concretely the phenotype plasticity of model plants and crops under different growth conditions. We implement innovative hardware and software tools combined with multivariant statistical approaches and machine learning to efficiently select new technologies for agriculture and biotechnology.

High-throughput phenotyping methods using model plants offer complex testing in broad concentration ranges combined with different ways of application in multiple environmental conditions for selecting and identifying potential markers, helping to understand their mode of action. Our pipeline based on a Multi-Trait High-Throughput Screening of libraries of chemicals, biostimulants, or genotypes, has the capacity to test higher tens of variants in one experimental run (>25,000 plants) in controlled conditions, using simple RGB[1]. The variants represent combinations of concentration ranges of tested chemicals/products, genotypes, individual abiotic (water and nutrient limitation, salinity, heavy metals) or biotic (*Botrytis*, *Pseudomonas*) stresses, and their multiple combinations. The tested agents can be applied through seed/seedling priming or root absorption. The images are automatically processed by our software pipeline consisting of a neural network-based plant recognition algorithm, quantifying growth dynamics and stress response traits, and the result visualization.

This screening can be followed by large-scale plant-based bioassays using various crops in normal and abiotic stress conditions (water limitation and salinity) for seed application. The traits followed are seedling emergence, early seedling development, and stress response. Selected treatments or genotypes can be further tested in indoor high-precision phenotyping using different non-invasive sensors (RGB, FluorCam, IR, VNIR, SWIR) to describe their effect on plant morphology and physiology in normal and stress conditions[2,3].

[1]Ugena et al. (2018). Front. Plant Sci.
[2]Marchetti et al. (2019). Front. Plant Sci.
[3]Blicharz et al. (2021). Plant J.

Short Talk Selected from Abstract

Evaluation of SHORT-ROOT as a regulator of leaf development and its importance in C4 photosynthesis.

Daniel Oswaldo Camo-Escobar[1], Carlos Eduardo Alcalá-Rodríguez[1], Ernesto Palafox-Figueroa[1], Marcela Hernández-Coronado[1], Vincent Eloel Cerbantez-Bueno[1], Stefan de Folter[1], Carlos Humberto Ortiz-Ramírez[1*]

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C4 photosynthesis evolved from anatomical modifications of specific cell types in leaves that resulted in a unique tissue patterning known as Kranz anatomy. This structural modification allows plants to increase CO_2 concentration around RubisCO, decreasing photorespiration and improving productivity. Therefore, there is great interest in recreating this type of anatomy in agronomically important C3 plants, like rice and wheat, to improve their productivity. Nevertheless, our understanding of the genetic components that regulate the development of Kranz anatomy is extremely limited. In this work, we performed histological and physiological analyses in mutant plants of Setaria viridis (millet), a C4 model, of the SHORT-ROOT (SHR) gene to evaluate its possible role during leaf development in C4 plants. We observed that in the C4 monocot S. viridis, the Kranz patterning is regulated by SHR function. Shr2 mutants show an aberrant ratio between rank 2 and rank 1 veins with a general reduction in rank 2 vein number and many veins that are separated by three, rather than two mesophyll cells. Noticeably, a significant decrease in seed weight was observed in shr2 mutants. The anatomical changes were accompanied by the reduction of the leaf CO_2 compensation point, confirming a negative effect on photosynthetic efficiency. The results indicate a fundamental role of SHORT-ROOT as part of the Kranz anatomy modeling gene network.

Short Talk Selected from Abstract

Development of a biosensor based on an Arabidopsis intrinsically disordered protein for tracking the effects of osmotic stress in plants

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Plants are constantly subjected to water deficit conditions, which eventually leads to hyperosmotic stress. In response to hyperosmotic stress, plants accumulate a group of proteins known as late embryogenesis abundant (LEA) proteins. LEA proteins lack a well-defined 3D structure and are considered intrinsically disordered proteins (IDPs). Recently, our laboratory developed a genetically encoded fluorescent biosensor that is capable of reporting the effects of osmotic stress on different organisms, including yeast, bacteria, *Nicotiana benthamiana*, and human cells. The biosensor, named Sensor Expressing Disordered protein 1 (SED1) uses the *Arabidopsis thaliana* AtLEA4-5 as the sensor domain. AtLEA4-5 dynamically changes its structure depending on the osmolarity and the macromolecular crowding of the cellular environment. However, the major limitation that SED1 presents is the inability of reporting osmotic changes in *Arabidopsis*. Since AtLEA4-5 is an *Arabidopsis* protein, the lack of response could be the result of hyperphosphorylations that might prevent AtLEA4-5 compaction. Also, the donor (mCerulean3) fast photobleachingcould directly affect FRET efficiency measurements. Here, we aim to modify SED1 togenerate functional versions in *Arabidopsis.* We generated two variants: AtLEA4-5 protein incapable of being phosphorylated (SED1-phosphonull) and a construct with a different FRET pair (mTurquoise2 and mNeonGreen; SED1-mTq2-mNG). These variants were characterized in yeast cells and *Nicotiana* leaves under hyperosmotic stress conditions. We found that, while SED1-mTq2-mNG exhibited a FRET change comparable to the original SED1 version in yeast and in *Nicotiana* under stress, SED1-phosphonull exhibited a lower FRET change in yeast and did not accumulate in *Nicotiana* leaves. Our results suggest that SED1-mTq2-mNG could be a functional biosensor in *Arabidopsis.* This study will help to obtain a biosensor in *Arabidopsis* that allows us to dynamically track the effects of osmotic stress in this model organism.

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Short Talk Selected from Abstract

Comparative biochemistry beyond chemotaxonomy: Using evolutionary principles to predict biosynthetic pathways

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With sequencing technologies becoming more affordable, in the last years there has been an explosion of reports on genomes, transcriptomes, and other molecular data. The amount of information has made it impossible for humans to understand it in its entirety: "we are drowning in information, but thirsty for knowledge." Data-driven approaches offer unusual solutions to complex problems by processing staggering amounts of information. However, the scarcity and sparsity of data related to biosynthetic pathways does not allow big data applications: there are few routes, discovered in a handful of plants. Integrating data from diverse species becomes imperative to overcome this problem; nevertheless, phylogenetic models are, by the very nature of the field, bounded by observations from extant metabolism.

In this project, we developed a predictive model for the evolution of chemical diversity in the non-canonical monoterpenes, iridoid glucosides. We generated a set of biosynthetic pathways that best explain the extant iridoid chemical diversity in the Lamiaceae, by developing a pathway reconstruction algorithm that connects iridoid reports, constrained by phylogenetic relationships between genera. We trained this model by using a perfectly labeled expanded dataset, generated by emulating *in silico* the evolution of iridoid glucosides, and (re)constructing nature-like "alternative evolution" ("multiverse") pathways. This model allowed the integration of a sparse transcriptomics dataset from different Lamiaceae species, and was successfully applied to discover a cytochrome P450 enzyme that catalyzes the oxidation of bartsioside to aucubin, predicted by our model and previously unreported. We found active orthologues in *Callicarpa americana*, *Vitex agnus-castus*, and the outgroup *Paulownia tomentosa*, further strengthening the hypothesis, enabled by our model, that the reaction was present in the ancestral biosynthetic pathway. We hope that this proof-of-concept sets the basis to use evolution models as a tool to allow the integration and analysis of structural data, to facilitate hypothesis-driven gene discovery.

Short Talk Selected from Abstract

Dissecting the integration of immune response in maize by studying *Sympathy for the ligule 1* (*Sol1*)

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Sympathy for the ligule (*Sol1*) is a genetic modifier of *Liguleless narrow* (*Lgn1*). *Lgn1* encodes a plasma membrane-localized receptor-like kinase required for the proper formation of diverse tissues in maize development. The absence of *Lgn1* triggers a mitogen-activated protein (MAP) kinase signaling cascade due to a constitutive defense response that causes an autoimmunity phenotype. MAP-kinases are key signaling regulators of plant immunity. Although the intimately connected development and immunity have been studied, the underlying molecular mechanisms to balance plant growth and defense are still unknown. The existing studies indicate that *Sol1* is the maize ortholog of Arabidopsis *ENHANCE DISEASE RESISTANCE 4* (*EDR4*) whose activity possibly represses the severe developmental defects produced by *Lgn1* mutation. However, the restoration of this severe phenotype is dependent on temperature and maize background because the developmental defects are significantly attenuated by *Sol1*-Mo17 but not by the *Sol1*-B73 one. Based on previous genetic and biochemical analyses of two allelic versions of *Sol1* and its target, we propose studying the molecular mechanisms of *Sol1* activity. To investigate how *Sol1* mediates immune response modification, we are characterizing *sol1* mutants produced by CRISPR-Cas9, during the maize-pathogen interaction to dissect regulatory changes. Moreover, to test the activity of the allelic variants Sol1-B73 and Sol1-Mo17 we performed subcellular localization by transient expression of onion epidermal cells and *N. benthamiana* plants. Preliminary results suggest that the increased level of *Sol1* expression possibly drives the repression of MAP-kinase high activity triggered by *lgn1*. Besides, subcellular localization of the *Sol1*-B73 allele is affected by temperature, but *Sol1*-Mo17 is not. Our findings are providing insights of the *Sol1* role in maintaining the balance of development and immunity in maize, and possibly in other species. Future experiments will be aimed to testing whether the gene dosage is involved in *Sol1* activity and identifying interacting proteins.

MM

Short Talk Selected from Abstract

Functional genomics annotation insights of *Quercus macdougallii* (Fagaceae), an endemic oak from Oaxaca

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Candidate genes for local adaptation were identified from RAD-seq data of 79 individuals of *Quercus macdougallii*, an endemic oak in the Sierra Juárez of Oaxaca that is considered endangered (IUCN) and threatened (NOM-059). Nine sampling sites were identified, five in the northern zone of its known distribution and four in the southern zone. For the identification of single nucleotide polymorphisms (SNPs), ipyRAD assemblies were performed using *Quercus lobata* and *Quercus robur* transcriptomes as references. From these assemblies, a sequence homology search was performed from alignments with the UniProt database with Blastx to identify the genes where SNPs were found between the two study areas. A total of 1381 SNPs were identified from the transcriptome of *Q. lobata* and 1791 from that of *Q. robur.* Principal component analyses showed differences between the two groups. Candidate genes with a large number of SNPs were identified, including those encoding disease-resistance proteins or those related to developmental and regulatory processes. An analysis with PCAdapt identified outlier SNPs, nine with the transcriptome of *Q. lobata* and 18 with that of *Q. robur*. These results help to complement current conservation strategies for the species, as well as to explore functional genomics in an endemic oak of Mexico, considered one of the main centers of oak diversity worldwide.

Short Talk Selected from Abstract

microRNA function during zygote elongation and early embryogenesis in *Arabidopsis thaliana*

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In Arabidopsis, fertilization of the central cell (CC) and the egg cell (EC) produces the endosperm and the zygote, respectively. The zygote divides asymmetrically to form a small apical cell and a large vacuolated basal cell. The apical cell forms the embryo, and the basal cell forms the suspensor, a transient tissue that promotes nutrition of the embryo. microRNAs (miRNAs) are known to act during embryogenesis, and recently it was shown that loss of miRNA function causes a loss of cell polarity of the zygote.
The focus of this project is to determine if the loss of zygote polarity observed in miRNA mutants originates in the zygote or is inherited from the egg cell. In other words, do miRNAs promote the polarity of the egg cell? What other aspects of cell identity in early embryos depend on miRNAs? To answer these questions, we are characterizing egg cell and zygote phenotypes of *dcl1* and *se,* mutants that are globally defective for miRNA function. We are also testing the effect of loss of miRNA function on markers for the transcription factors *WOX2*, *WOX8*, *DRN.* So far, we have found ectopic expression for the apical cell marker *pDRN::GFP* in the suspensor. The results of our experiments will define the window of action of miRNAs during gametogenesis and in the zygote and will shed light on the role that miRNAs play in establishment of cell identity in the early embryo of Arabidopsis.

OO

Short Talk Selected from Abstract

Effects of engineering synthetic de/carboxylation modules on the photosynthetic performance in Arabidopsis

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Crassulacean acid metabolism (CAM) is an elaboration of C_3 photosynthesis present in 6-7% of vascular plant species that improves water-use efficiency and allows plants to occupy environments with seasonal or intermittent water supply. Our recent omics datesets from the common ice plant (*Mesembryanthemum crystallinum*), a facultative CAM plant, have revealed CAM-associated genes to create synthetic versions of CAM (SynCAM) in C_3 photosynthesis plants. We have built gene circuits to recreate versions of the carboxylation (CGC), decarboxylation (DCGC), and core C_4 metabolism modules of CAM to enhance photosynthetic performance and to improve water-use efficiency in *Arabidopsis thaliana*. We have evaluated the CAM-related traits in multiple independent transgenic lines expressing these modules using fifteen photosynthetic parameters, five phenotypic measurements, and three indicators of nocturnal acidification. Plants expressing the CGC module showed an ~83% increase in dawn/dusk ΔH^+, ~96% more malate accumulation at night, and a $\delta^{13}C$ change of ~0.8%. Twenty four hour gas exchange analysis showed a 1.86-fold decrease of nocturnal photorespiration in CGC module expressing plants. The DCGC module expressing plants showed a 1.75-fold improvement in WUE_i. These results will inform new iterations of the SynCAM design cycle and flux balance analysis (FBA) in *A. thaliana* and crop plants.

Keywords: Crassulacean acid metabolism, synthetic CAM, water-use efficiency, drought tolerance, *Arabidopsis thaliana*

Short Talk Selected from Abstract

The contribution of the root microbiome to local adaptation and plant performance in traditional Mexican maize

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The association between microorganisms and plants plays a remarkable role in plant development and performance, mainly under unfavorable environmental conditions. During domestication, plants have undergone radical transformations at phenotypic and genetic levels. Locally cultivated maize varieties (landraces) have been selected for many years in all types of environments resulting in location-specific adapted varieties. These maize varieties are a source of high genetic diversity since they are an intermediate step between wild relatives and improved plants. In this study, we aim to describe the contribution of root-associated microorganisms to plant adaptation and performance. We generated a multi-parent mapping population using 8 traditional Mexican maize varieties coming from different regions and diverse environments of Mexico (MEXI-MAGIC population). We conducted a two-year field experiment to identify microorganisms associated with the endosphere and the rhizosphere of 180 independent families of this MEXI-MAGIC population. In addition, we characterized plant development (plant height, ear height, flowering time) and measured ear traits (length, width, grain number, color) and yield. These data will allow us to compare the recruitment of microbial communities to the rhizosphere or endosphere, analyze the microbial contribution to plant adaptation and yield, and determine the effect of the plant genotype on the establishment of beneficial microbial communities.

Short Talk Selected from Abstract

Insights in polyamine transport under biotic stress: roles in local and systemic responses.

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Polyamine transport is the less-understood mechanism involved in plant polyamine homeostasis. Until now, all the characterized plant polyamine uptake transporters (PUTs) import polyamines (mainly spermidine), amino acids (i.e., leucine), and the herbicide paraquat. The *Arabidopsis thaliana* PUT family (*At*PUT) consists of five members with different intracellular localizations, which point to an important role in polyamine mobilization within the cell. Under biotic stress, significant changes in polyamine metabolism occur, like the accumulation of free and conjugated forms at the infection site. Hydrogen peroxide is generated by polyamine catabolism, it signals in plant defense and interconnects polyamines with other important stress-responsive pathways. Herein, we describe for the first time that polyamine transport is essential for plant defense. *Arabidopsis thaliana Atput* single mutants *(Atput1-1 to Atput5-1)* were screened in response to *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) and *Botrytis cinerea* (*Bc*) infection. Among the *Atput* family, two genes were identified to be differentially expressed in response to these pathogens. Under *Bc* infection, a bigger lesion size was found in the single and double mutant lines, and spermidine supplementation did not alleviate disease symptoms in the mutant lines. Deregulation in ROS levels, polyamine oxidase activity, and the expression of SA and JA/Et marker genes were found, which suggests that polyamine transport impacts ROS homeostasis and hormone signaling. In addition, under *Pst* infection, local and systemic responses to the pathogen were assessed, observing that one *Atput* mutant line abolishes systemic acquired resistance to *Pst* and *Bc*. RNA-seq data revealing changes in the systemic transcriptome of WT and *Atput* mutant line will be presented and discussed. Altogether our data suggest an important role for polyamine transport in the plant immune responses to pathogens of different lifestyles and point to the speculation that polyamines are or generate a signal for systemic responses in plants.

Short Talk Selected from Abstract

Identifying heat-tolerant bread wheat for the Yaqui Valley: from source-to-sink.

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In wheat, yield relies both on the efficiency of flag leaf photosynthesis and sugar export to grains. Under heat stress (HS), photosynthesis is mostly limited by biochemical factors, like chlorophyll degradation and/or reduced CO_2 fixation by Rubisco. Thus, the increased frequency of HS in the field is an issue for wheat producers and consumers. The aim of this work was to identify heat-tolerant bread wheat genotypes, based on flag leaf performance and photosynthate translocation to grains under HS. Six potentially heat-tolerant bread wheat genotypes (SOKOLL, WEEBL1.P, SOKWB.1, SOKWB.4, SOKWB.6, and BORLAUG100) were planted in the field conditions of the Yaqui Valley, a semi-arid zone, and the main wheat producer region in Mexico. Field temperatures during the reproductive stage of wheat plants were 26/10 °C and 32/11 °C in the control and HS planting, respectively. Chlorophyll content was not reduced by HS in any of the genotypes. Analysis of Rubisco activity showed that carboxylation was maintained in two genotypes and increased in four of them (WEEBL1.P, SOKWB.1, SOKWB.4, SOKWB.6) under HS, indicating that all genotypes sustained photosynthetic activity efficiently. HS induced a tendency to increase sucrose in most genotypes and reduced starch accumulation in the flag leaf of SOKOLL, SOKWB.1, and SOKWB.4, suggesting that the increased temperatures accelerated photosynthate export to the spike. Grains were harvested at maturity to analyze yield and quality. Grain weight per spike was significantly reduced in all genotypes, except in SOKWB.1. Grain quality analysis showed that HS did not affect starch content, which was increased in genotypes WEEBL1.P, SOKWB.4, and SOKWB.6. Although these results point to the identification of wheat genotypes apt for the Yaqui Valley producers, they also evidence the complexity of the source-sink relationship under HS conditions in wheat, as yield was not directly dependent on photosynthate accumulation in grains.

Short Talk Selected from Abstract

Transcriptomic analysis of the desiccation-tolerant moss *Pseudocrossidium replicatum* in response to multiple abiotic stressing factors

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Desiccation tolerance (DT) is the ability of cells to recover from an air-dried state. Recently, our group identified the moss *Pseudocrossidium replicatum* as a fully desiccation-tolerant (FDT) species (Ríos-Melendez et al., 2021). Its gametophores rapidly lost more than 90% of their water content when exposed to a low-humidity atmosphere (23% RH), but abscisic acid (ABA) pretreatment diminished the final water loss after equilibrium was reached. During slow dehydration, *P. replicatum* gametophores maintained good maximum PSII efficiency (Fv/Fm) for up to two hours; however, ABA pretreatment induced a faster decrease in the Fv/Fm. ABA also induced a faster recovery of the Fv/Fm after rehydration. Protein synthesis inhibitor treatment before dehydration hampered the recovery of the Fv/Fm when the gametophores were rehydrated after desiccation, suggesting an inducible protective mechanism activated in response to abiotic stress. This observation was also supported by accumulation of soluble sugars in gametophores exposed to ABA or NaCl. Exogenous ABA treatment delayed the germination of *P. replicatum* spores and induced morphological changes in protonemal cells that resembled brachycytes. ABA is also important in protecting and repairing *P. replicatum* protonema when exposed to high salinity (300 mM NaCl) or freezing (-80ºC). To study the molecular response of *P. replicatum* to abiotic stress, here we performed an RNAseq study using protonema cells subjected to different stressing conditions: Control, slow Dehydration (63 % HR), Rehydration, ABA 10 µM, NaCl 200 mM, Sorbitol 400 mM, and Glucose 300 mM. Our results suggest that *P. replicatum* is an FDT moss equipped with an inducible molecular response that prepares this species for severe abiotic stress and that ABA plays an essential role in this response.

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Short Talk Selected from Abstract

Transcriptional responses to *Fusarium* spp. infection in a Mexican avocado variety

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Pathogens cause transcriptional reprogramming in plants, activating different response pathways to avoid pathogen establishment. This reprogramming involves protein-coding genes and non-coding RNAs, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). There are few examples in which the role of miRNAs in pathogenesis has been studied, but there are even fewer examples in which a functional role of lncRNAs has been proved. Unlike miRNAs, there is a lack of conservation between plant species at both the sequence and the secondary structure levels in most of the lncRNAs. The above is due to the evolutionary processes that explain its origin; this is the neofunctionalization of duplicated protein-coding genes (either the product of whole genome duplications or gene duplications events), co-option of transposable elements in the genome, duplication followed by neofunctionalization from other lncRNAs, and *de novo* emergence. Based on this previous knowledge and using as a study model an avocado (*Persea americana*. *drymifolia* var.) - *Fusarium* spp. pathosystem, two types of RNAseq libraries (miRNAs and mRNAs) were generated at 1,7,14 and 21 days post-infection (dpi). As a result of performed analyses, we identified 13,700 protein-coding genes that are differentially expressed, 29 new potentially predicted miRNAs and 2,810 putative lncRNAs, all of them responsive to *Fusarium* infection and involved in different biological processes related to plant immune response. Besides, the potential target genes of miRNAs and the putative function of lncRNAs were also predicted by *in silico* analysis and/or the functional description of neighbouring genes. This is the first transcriptional study performed on avocado (one of Mexico's most important agricultural species) in which protein-coding genes, miRNAs and lncRNAs are holistically considered to describe the host responses to a pathogenic agent such as *Fusarium* spp.

Short Talk Selected from Abstract

Genomic and transcriptomic studies of the root hydrotropic response and their association with natural variation in drought tolerance of maize (*Zea mays* L.).

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Although water scarcity continues to be the single-most imperative factor controlling successful food production by agricultural practices, there are very limited studies on how roots of crop plants differentially grow in response to water potential, i.e., hydrotropism in the field. Hydrotropism help roots to obtain water from the soil and at the same time contribute to the establishment of the root system. Root hydrotropism in maize varies enormously in different hybrids, landraces and teocinte, and we have classified their root hydrotropic response in robust (>40^o angle of curvature) and weak (<39^o angle of curvature). The phenotyping of root hydrotropism in 285 Drought Tolerant Maize for Africa (DTMA) maize hybrids allowed us to perform a GWAS (Genome Wide Association Studies), which is a valuable tool for comprehending the genetic basis of trait variation. Second, we performed RNA sequencing (RNA-seq) to detect genes that were differentially expressed among those maize lines with robust hydrotropic response (RHR) versus weak hydrotropic response (WHR). Third, we examined the involvement of protein ubiquitination and protein degradation in the proteasome in root hydrotropism since several associated genes by GWAS and differentially expressed RNAs were identified with these biological processes. Our results suggest that the signal transduction pathways induced by hydrostimulation in maize are like those triggered by growth (protein synthesis), water stress (mainly intrinsically disordered proteins), and protein degradation. Our analysis implied that the robust hydrotropic response of maize roots was partly due to an increase in the accumulation of intrinsically disordered proteins and the activation of the ubiquitin-proteasome degradation pathway. These findings offer a novel prospect for modeling root systems in response to drought, which is important for crops as vital as maize under drought conditions caused by the current climate crisis.

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Short Talk Selected from Abstract

Time-course RNA-seq analysis unveils molecular mechanisms underlying natural variation of primary root penetrability in *Arabidopsis thaliana*

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Humanity is confronted with an unprecedented crisis resulting from global warming and climate change. The excessive emissions of atmospheric CO_2 are predominantly responsible for escalating temperatures worldwide, giving rise to natural catastrophes that pose an immediate threat to future agriculture and global food security. A potential solution for the net removal and reduction of CO_2 lies in the capture of organic carbon from the soil through the design of plants with larger, deeper root systems that produce complex carbohydrates, enabling the sequestration of carbon underground. Soil compaction emerges as a significant obstacle to the growth of plant roots, as it hampers their penetration into deeper soil layers. Mechanical impedance in compacted soils often serves as a major hindrance to root penetration, confining the development of root systems to shallow soil layers where essential resources are scarce. Consequently, soil compaction and high soil impedance severely impede root absorption, restricting access to water and nutrient resources from deeper soil layers and resulting in global crop yield losses ranging from 20% to 75%. In this study, we have undertaken a systematic, integrated, and multidisciplinary approach to identify the molecular and regulatory mechanisms governing root system penetrability (PSR) in *Arabidopsis thaliana*. Using genomics and transcriptomics, we have unraveled the mechanisms that determine root penetrability by comparing two accessions of *Arabidopsis thaliana*: one tolerant and one sensitive to soil compaction, at different stages of penetration. Through this research, we have discovered regulatory gene networks, metabolic pathways, and signaling pathways related to PSR, shedding new light on the natural variation in primary root penetrability in Arabidopsis and uncovering the genetic architecture underlying this agriculturally significant trait.

Abiotic Stress

Physiological and anatomical responses of *Agave fourcroydes* Lem. under drought stress

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Drought is one abiotic stress with a severe impact on crop productivity. Some plant species growing in semiarid and arid regions have developed physiological and anatomical adaptations to deal with drought stress. *Agave fourcroydes*, worldwide used as a fiber source, is an agave specie well-adapted to tropical dry forests. In this work, we studied the physiological shoot responses and anatomical root adaptations of *A. fourcroydes* under drought. Agave bulbils were grown under four different soil humidity levels (-0.02, -0.3, -2,and -80 Mpa). Leaf physiology and development and anatomy of the root system were evaluated during 90 days. After 60 days, significant changes in plant height and leaf numbers were observed between drought treatments and control plants, negatively impacting the shoot biomass accumulation. Surprisingly, bulbils growing at -0.3 Mpa, an stress level that reduced about 25% of shoot biomass, showed mild reductions of leaf water potential and maximum photosynthetic efficiency (Fv/Fw) compared to well-watered plants. Under drought stress, the root system developed was severely affected. After 90 d of treatment, root biomass was reduced at 30% and 8% when soil humidity levels were -2 MPa and 0.3 Mpa, respectively. After 30 days of stress, drought (-2 and -80 Mpa) triggered profound changes in endodermis anatomy (cell wall thickening) and lacunae formation; after 60 and 90 days of stress, flavonoid accumulation in endodermis cells was also visualized. Our results suggest that a buffering leaf water status and fast adaptation of root anatomy during drought may explain the success of colonization and survival of agave species in dry environments.

Abiotic Stress

Contribution of different sequence motifs on the structural sensitivity of Arabidopsis AtLEA4-5 disordered protein

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Late Embryogenesis Abundant (LEA) proteins are intrinsically disordered proteins (IDPs) that participate in the plant responses to periods of water deficit. Arabidopsis thaliana AtLEA4-5 remains in a disordered state during hydration conditions, however, during desiccation treatments it forms alpha-helical structures. This dual characteristic of AtLEA4-5 could be key to confer resistance to drought stress. The mechanism by which AtLEA4-5 is able to perceive changes in water levels and trigger a functional response in the plant is still not clear. However, the duality of a disordered state to form a stable three-dimensional structure seems to be necessary. Bioinformatic structural predictions show that AtLEA4-5 and its homologues have a similar topology of two helices linked by four amino acids that are conserved in different plant species. By designing mutants impacting the structure of AtLEA4-5, we sought to determine the contribution of sequence motifs in the three-dimensional conformation of AtLEA4-5. This work will contribute to understanding how changes in water levels impact the structure and function of plant IDPs.

Abiotic Stress

Regulation of SnRK1 activity not only depends on canonical threonine phosphorylation

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SnRK1 is an important energy-sensitive regulator involved in maintaining cell homeostasis. SnRK1 consists of two regulatory subunits (β and γ) and one catalytic subunit (α); the latter has two isoforms (SnRK1α1 and SnRK1α2). It has been postulated that for SnRK1 to be active, phosphorylation at threonine 175/176 of catalytic subunits by upstream kinases Grik1 and Grik2 is needed. Although it is accepted that phosphorylation in the Thr present at the T-loop reflects the metabolic status of the cell, this modification does not always correlate with kinase activity. We studied the activation and phosphorylation of the catalytic subunits using different mutants of the catalytic domain (DC-SnRK1α). We found that serine adjacent to the T-loop was also crucial for SnRK activity *in vitro*. To understand whether this serine participates in the activation of SnRK1 in plants, we generated transgenic lines of *A. thaliana* that overexpress the different phosphorylatable versions of the SnRK1α1 catalytic subunit. We will discuss the importance of this results in terms of SnRK1 activation under energetic stress.

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ATG8b is a positive modulator of autophagy in responses to water and endoplasmic reticulum stresses in *Arabidopsis thaliana*

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Autophagy is a conserved catabolic process in all eukaryotic cells, in which dysfunctional cytoplasmic material, is transported for degradation and recycling to the vacuole in plants. Interested in analyzing a specific response to water stress of the *ATG8b* gene, bioinformatic analysis was carried out searching for transcriptional factors (TFs) that hypothetically bind to a selected region from the starting codon to a 1 Kb promoter region of the *ATG8b* gene. Our analysis showed twenty-seven TFs had binding sites in the promoter of ATG8b. Related to the response to water deprivation or ER stress we found ATHB6/7/12 and bZIP60, respectively. A co-expression analysis indicates that ATG8b is co-expressed with ATHB7/12 and bZIP60 then these TFs may be regulating ATG8b expression.
Going further, autophagy induction was analyzed throw the observation of specific ATG8b autophagosomes by confocal microscopy in the root of the line pATG8b:GFP:ATG8b.
Three days old seedlings were treated and transferred from the control medium to Water Stress Medium (WSM). Results showed the formation of GFP-ATG8b fluorescent spots as early as 10 min with a maximum of 20 min decreasing after 30 min. ER stress was induced by transferring seedlings to Control medium supplemented with DDT (10 mM) (DTT). Results showed the formation of GFP-ATG8b fluorescent dots between 10 min and 30 min. We also analyzed the root growth of the *atg8b* mutant in DTT compared with *atg8i* mutant and wild-type plants in Control, WSM, and DTT. Results showed that *atg8b* mutant grew at a slower rate than the other lines. We suggest that ATG8b and autophagy are required for proper root growth in control conditions and during water and ER stress. These data support the bioinformatic results in relationship with the possible role of ATHB7/12 in the ATG8b promoter in response to water deficiency and bZip60 in the ER stress response.

Abiotic Stress

Impact of the sound of running water on jalapeño peppers under drought conditions.

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The effect of sound on plants is a field that is gaining more strength with the passage of time as more and more research is being done on the subject. Sound acts as a physical elicitor on the plant and depending on the time, frequencies and power (dB) with which it is applied, different results will be obtained, from generation of secondary metabolites related to defense against drought, increased yield, expression of certain genes, increased total sugars, among other reported effects. This research suggests that the jalapeño bell pepper, exposed to the sound of running water for 20 minutes at 80 dB for 7 days at random times probably helps to mitigate the damage caused by the lack of water by activating its defense mechanisms when stimulated by the sound of running water reflected in the expression of Superoxide dismutase enzyme (SOD), Peroxidase (POD) and Proline. Regarding yield the control group only obtained 10.79% more yield than the group with acoustic treatment without irrigation, with a total of four groups 1) Water Sound with Irrigation, 2) Water Sound without irrigation 3) White Noise and 4) Control. Sound as an elicitor in agriculture becomes a real option with scientific basis for its application in the field.

Key words: Sound, POD, SOD, Proline.

CHARACTERIZATION OF CHANGES IN THE STRUCTURAL CONFORMATION OF LEA PROTEINS IN DIFFERENT ENVIRONMENTAL SETTINGS AND THEIR POSSIBLE RELATIONSHIP TO THEIR PROTECTIVE FUNCTION

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Plants have evolved strategies to survive stressful environments. One of these responses is the accumulation of late embryogenesis abundant (LEA) proteins. LEA proteins lack a well-defined three-dimensional structure but can change this disordered structural conformation upon environmental perturbations. Changes in the structural conformation of LEA proteins also result in changes in the protective function they exert on other proteins. This suggests that the ability of LEA proteins to change their disordered structural conformation when their environmental surroundings change is related to their protective function. In this work, we chose four LEA proteins from different groups of *Arabidopsis thaliana* and we characterized their structural conformation by changing their environmental settings. In vitro, using the solution space scanning methodology, we characterized the expansion and compaction of the proteins in different osmolytes (denaturing agents, salts, sugars, polymers of different lengths, among others). In addition, we expressed the constructs of our selected group in yeast cells and subjected them to hyperosmotic shocks with NaCl, and we measured the changes in their structural conformation using FRET. Our results in vitro showed a diverse behavior for the different LEA proteins tested. In addition, this behavior is reproduced under conditions of hyperosmotic shock in vivo. This project will allow us to better understand how LEA protein's function and eventually we may be able to enhance their protective function to benefit plants and other organisms under stressful environmental conditions.

ABSCISIC ACID INCREASES THE TOLERANCE OF *P. REPLICATUM* PROTONEMATA TO ULTRA-FREEZING STRESS

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The moss *P. replicatum* is known to have a high tolerance to abiotic stress, including salinity, drought, and osmotic stress. This study aims to evaluate the tolerance limits of this moss (monosporic line) to ultra-freezing at a protonema stage. The sample groups used in the study were the following: Control (1), 24 h acclimatization at 4 °C without ABA pretreatment (2), 24 h acclimatization at 4 °C with ABA pretreatment (3), and 24 h ABA pretreatment (4). All samples were subjected for 10 days at -78ºC and then transferred to standard growth conditions to monitor subsequent growth. Immediately after the freezing stress, the efficiency of Photosystem II (QY) decayed in the first two groups. In contrast, in groups 3 and 4, surprisingly, the PSII was still active, showing values of QY of 0.5 and 0.49, respectively. After the freezing stress, all samples were transferred to standard growth conditions (23ºC, 16 h light/ 8 h darkness) to monitor the recovery. After 5 d and 10 d, samples from the first two groups appeared bleached entirely; however, after 15 d of recovery, small areas of green protonema were grown, and the PSII values started to recover. On the other hand, samples from the ABA-treated groups always remained green during the recovery period and showed an improved PSII. Moreover, Evans's blue staining revealed that ABA helps to preserve cell integrity. These findings suggest that *P. replicatum* possesses protection mechanisms against ultra-freezing stress, which are ABA-dependent and probably also ABA-independent. This was reflected in the QY values, indicating that *P. replicatum* remained dormant during ultra-freezing. Understanding this can provide valuable insights for improving the tolerance of sensitive crops affected by low temperatures.

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Comparative analysis of Cornichon gene homologues of *Pseudocrossidium replicatum* and *Physcomitrium patens* in response to extreme abiotic stress

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Cornichon proteins serve as cargo receptors and play a crucial role in the selection, transport, and targeting processes during anterograde vesicular trafficking. Their function has been well-documented in yeast and other organisms such as vascular plants, where they are responsible for mobilizing ion transporters, growth factors, and other proteins involved in extracellular signals detection. However, the specific role of Cornichon proteins in non-vascular plants (bryophytes) remains uncertain. Bryophytes constitute a clade comprising liverworts, hornworts, and mosses, representing some of the earliest plant species to colonize terrestrial environments. These plants have developed mechanisms to allow them to colonize harsh environments, from deserts to arctic regions. In *Physcomitrium patens* model moss it has been identified two Cornichon protein homologs, whose function has been associated with protonemal cell development. *Pseudocrossidium replicatum*, a highly desiccation-tolerant moss is an excellent candidate for studying extreme stress tolerance responses. We have recently identified a Cornichon gene homologue (*PrCNIH)* in this plant, and its characterization could be important to understand its role in extreme abiotic conditions. In this work, we performed a phenotypic and *CNIH* gene expression analysis of *P. replicatum* gametophores response to extreme temperatures (45°C heat and -80°C freezing) as well as desiccation, compared to P. *patens*.

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Controlled elicitation effect in swiss chard (*Beta vulgaris* L. var. Cicla) in open field with hydrogen peroxide

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Swiss chard is one of the most popular leafy vegetables. Its low cost and easy production makes this crop a feasible commercial option. It grow in open fields and stressed by factors. These factors can modify morphology, primary and secondary metabolism triggering the immune system, and transcriptional factors resulting in a cocktail of antioxidant compounds (AOx) to deal with the damage caused by them. The main objective of this study was analyzed the effect of controlled elicitation with hydrogen peroxide (H_2O_2), through total polyphenol content, and 1,1-diphenyl-2-picrylhydrazyl (DPPH•), 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+), $DPPH^{\bullet}$ and $ABTS^{\bullet+}$radicals scavenging activity. In addition, it was measured the catalase (CAT), superoxide dismutase (SOD) and phenylalanine ammonia-lyase (PAL) activities. Furthermore, the salicylic acid method for nitrate determination. Ours results show that the 75 mM H_2O_2 treatment was a bio-stimulant dose increasing the antioxidant activity, PAL enzymatic activity, polyphenol content, and the dosage did not affect the nitrates concentration on chards. Therefore, the elicitation with H_2O_2 can be a strategy to increase the functional and nutraceutical properties of chard, which can encourage its consumption in both humans and animals.

Keywords**:** hydrogen peroxide, eustress factor, elicitation, chard

Titanium signalling pathway study in *Arabidopsis thaliana*

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Titanium is the second most abundant transition metal on Earth, widely used in industries such as dyes, chocolate softness, and makeup. In agriculture, this element has also been studied for its potential in crop improvement. It has been found to enhance chlorophyll content and photosynthesis, improve fruit quality and biomass, strengthen tolerance to biotic and abiotic stresses, and promote nutrient uptake. Although there is numerous evidence supporting the beneficial effects of titanium fertilization on plants, little information is available on the genetic signaling pathways activated by titanium application in plant tissues. Recently, our research group performed a transcriptome analysis to uncover the molecular signals that are triggered in Arabidopsis plants when treated with titanium. This transcriptome analysis revealed that titanium activates the abscisic acid and salicylic acid signaling pathways. Consequently, this activation leads to enhanced resistance against drought, high salinity, and infection with *Botrytis cinerea* in Arabidopsis. To validate these findings, we performed a genetic analysis using mutant lines associated with the abscisic acid and salicylic acid signaling pathways. The results confirmed that both signaling pathways are involved in titanium responses, promoting improved growth and development in *Arabidopsis thaliana*.

Study of changes in carbohydrate synthesis in heat-tolerant bread wheat genotypes

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Wheat *(Triticum aestivum L.)* is one of the world's most important staple food crops. Wheat grows in both tropical and subtropical regions around the world, these regions tend to experience different climates, leading to various abiotic stresses. However, this crop is sensitive to temperature changes. Heat stress affects wheat´s growth and development, leading to morphological, physiological, and biochemical alterations. The aim of this work was to analyze the heat stress effect in sugars synthesis in four wheat genotypes (2, 13, 18 and 24). Herein, two field experiments at two different temperatures were done, temperature control and HS during the vegetative stage. Chlorophyll content was measured by Arnon method; RuBisCO activity was measured according with Li; soluble sugars were quantified according with Romero-Reyes et al.; and total starch by Zeeman & Smith method. Genotypes 02 and 18 maintained their total chlorophyll content while genotypes 13 and 18 had a decrease in these photosynthetic pigment under HS. The RuBisCO activity increased in genotype 13 under HS, in the other three genotypes the activity did not change. Starch content decreases in genotypes 2 and 13, meanwhile it increases in genotypes 18 and 24 in heat-stressed plants. The glucose and fructose content, increased in all genotypes under HS. However, the sucrose content decreased due to heat stress. Decrease in the chlorophyll content suggest that the light energy transfer diminish and Calvin cycle could be less efficient; but our results show that RuBisCo activity is maintained in three of the wheat genotypes reason why the glucose and fructose content did not decrease.

Evaluation of *Rhodotorula sp* (Rh4) effect on germination and root growth of *Zea mays* L. and *Arabidopsis thaliana*

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Methyl parathion is one of the most widely used insecticides in Mexico even though its use has been restricted by the authorities. This compound inhibits acetylcholinesterase, so it is not only toxic to pest insects but also to pollinating insects, predators, and any other animal with a nervous system: fish, birds, and mammals, including humans. Being a hydrophobic compound, methyl parathion can also cause osmotic imbalances in the soil and particularly in the rhizosphere. The use of microorganisms as bioremediators are capable of degrading xenobiotic compounds, and some of these are plant growth promoters, therefore are ideal for application in crops of commercial importance. An example of these microorganisms is the yeast *Rhodotorula*. The yeast *Rhodotorula sp.* (Rh4) isolated by Tapia et al. (2020*) from the *Arenaria* genus plant that inhabits the crater of the Xinantécatl volcano has been well characterized in terms of its ability to degrade aromatic compounds, like methyl parathion and as a promoter of plant growth in plants. The objective of this project is to evaluate if the Rh4 strain is capable of degrading parathion and see if it acts as a growth promoter for *Zea mays* L. seedlings. To achieve this, the hydrotropic response of the root will be measured for evaluating whether the presence of Rh4 modifies root growth influence by a moisture gradient.

*Tapia-Vázquez I, Sánchez-Cruz R, Arroyo-Domínguez M, Lira-Ruan V, Sánchez-Reyes A, Sánchez-Carbente MR, Padilla-Chacón D, Batista-García RA, Folch-Mallol JL. (2020). Isolation and characterization of psychrophilic and psychrotolerant plant growth promoting microorganisms from a high-altitude volcano crater in Mexico. Microbial Research 232 (126394). https://doi.org/10.1016/j.micres.2019.126394.

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Accumulation of amino acids and photosynthetic pigments in Mexican soybean genotypes subjected to water deficit

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Drought is the main abiotic threat to soybean production. To cope with drought, the accumulation of some metabolites can be altered to maintain homeostasis in plants. This work aimed to determine free amino acids and photosynthetic pigment content in leaves of soybean genotypes with different levels of drought tolerance. One early (H02-2309) and two intermediate (H98-1240 and Huasteca 700) genotypes were evaluated in a randomized block design with at least two replicates under well-watered (WW) and water-deficit (WD) conditions. The WD was applied at the R2 stage of plants by reducing soil irrigation gradually (from 11% to 3% gravimetric humidity) for 17 days. Plants under WW were irrigated normally. After that, accumulation profiles of amino acids, chlorophyll, and carotenoids were measured in the third leaf. The number of leaves plant^{-1}, and the wilting rate of plants were also recorded. Under WD, proline increased drastically in H02-2309 and Huasteca 700 genotypes (almost 60-fold). Histidine, cysteine, tyrosine, and glycine were raised (1.37-3.16-fold) in both genotypes. Meanwhile, Huasteca 700 excelled in asparagine and threonine (3.04 and 7.61-fold, respectively). Early genotype highlighted in aspartic acid, valine, and isoleucine (1.39, 10.28, and 3.34-fold, respectively). On the other hand, Huasteca 700 showed the highest accumulation in total chlorophyll (1.75-fold), chlorophyll A (1.74-fold), chlorophyll B (1.66-fold), and carotenoids (1.54-fold) content. Interestingly, Huasteca 700 recorded the highest number of leaves plant^{-1} and the lowest wilting rate under WD. Therefore, Huasteca 700 showed better adaptative characteristics to WD, since it accumulated important amino acids for osmotic adjustment and pigments related to photosynthetic efficiency, contributing to producing more leaves and a lower wilting rate. Proline accumulation increased in drought-tolerant soybean genotypes.

Participation of ABA and ROS in the strawberry fruit response to UV-C postharvest application

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The strawberry (*Fragaria x ananassa*) is a fruit with a high content of anthocyanins, compounds that give the red color and confer high antioxidant activity, a characteristic that links its consumption with the prevention of cardiovascular diseases and some types of cancer. Plants increase the production of phenolic compounds, such as anthocyanins as a defense mechanism, in response to different types of stress like short-wavelength ultraviolet light (UV-C). We know that the application of UV-C to strawberry fruits is an efficient method to increase their anthocyanin content; however, the molecular mechanism by which UV-C can increase the production of these compounds is not clear. We have hypothesized that abscisic acid (ABA) and reactive oxygen species (ROS) could be the first molecular signals in the response of strawberry fruit to UV-C light. ABA is a hormone involved in the response to stress and in strawberry fruit it's also linked to maturation and coloration. Furthermore, as it is harmful to the plant, UV-C could cause the production of ROS, a signal that would induce the activation of defense mechanisms, such as the production of anthocyanins. To investigate the possible mechanism, we measured the ABA content by High-Performance Liquid Chromatography (HPLC), in ripe postharvest strawberry fruits treated with UV-C (2 kJ/m^2) and in non-irradiated fruits. Also, the enzymatic activity of superoxide dismutase (SOD), an enzyme that mediates the production of superoxide anion, was measured spectrophotometrically at different post-irradiation times (0, 12, 24 and 36 h). We found that ABA content and SOD activity were significantly increased by UV-C application compared to controls. This confirms the involvement of ABA and ROS in the UV-C signaling mechanism in postharvest treated strawberry fruits.

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Translational regulation analysis of the phosphate deficiency response in *Arabidopsis thaliana*

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Phosphorus (P) is an essential macronutrient for plant development. Although the content of this element in the soil is high, plants growth and productivity are limited due to P low availability. The P low availability is caused by different factors as plants only take and metabolize P in the form of phosphate (Pi). Under Pi deficient conditions, plants activate an array of responses to cope with the lack of this nutrient. These responses have been extensively studied at the genetic level, focusing on transcriptional regulation, however, it is known that the mRNA and protein levels are not directly correlated, and little research has been done on posttranscriptional regulation, such as the regulation of translation, which is vital for determining the final expression at the level of proteins. Using polysome profiling, we determinate the global changes in the translational regulation of phosphate starvation-responsive genes in *Arabidopsis thaliana* plants subjected to Pi deficiency. We obtained 18 traductomes of three different treatments, 12 hours and seven days in Pi deficiency, and two hours in Pi resupply after seven days in Pi deficiency, that were compared with transcriptomes in the same conditions. Our results suggest that there are regulation mechanisms at the translational level in phosphate deficiency, more specifically, the positive regulation of translation of mRNAs related to processes involved in increasing the root system exploration area, such as lateral roots formation and the increase of root hairs length and density.

Phylogenetic analysis reveals conserved residues from the MYB-CC gene family potentially involved in the regulation of the Phosphate Starvation Response in monocot and dicot species

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The phosphate starvation response (PSR) is an essential adaptive mechanism in plants that allows the coordination of several responses to alleviate low phosphate (Pi) availability. At the center of this coordination is PHR1, a master transcription factor involved in the expression of hundreds of genes. PHR1 consists of different domains, including an MYB domain responsible for DNA binding and a coiled-coil domain implicated in dimer formation and protein–protein interactions. Posttranslational modification has been suggested as the mechanism for PHR1 regulation. Recent research from our group revealed that PHR1 undergoes phosphorylation at S11 by kinases of the SnRK1 family, which negatively impacts its transcriptional activity. This was demonstrated through the transient expression of a reporter gene controlled by a Pi deficiency-inducible promoter. To determine whether S11 and other putative phosphorylation sites are conserved in orthologs of PHR1, we compared and analyzed the sequences of MYB-CC gene family members from various monocot and dicot species. A multiple sequence alignment was performed to reconstruct the molecular phylogeny of this transcription factor family. The alignment of the putative orthologs of PHR1 showed a high level of conservation in several residues that have been identified as phosphorylated residues in PHR1 from Arabidopsis, such as S11, S19, T287 and S297. These residues are located at the N-terminal site and between the MYB and CC domains, suggesting a potential regulatory effect. We will discuss the relevance of these findings.

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Determination of *Capsicum annuum* L. capsiate producer as growth promoting on C57BL/6J mice

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Capsiate is a non-pungent analogue of capsaicin in pepper (*Capsicum* spp). The absence of pungency, in addition to the multiple capsaicin-like biological activities such as anti-obesity, anti-inflammatory, antimicrobial, and antioxidant properties, makes capsiate an excellent alternative to expand its use in health and nutrition. Moreover, few sources of chili producing capsinoids have been reported and the natural production of secondary metabolites in plants is minimal. *Capsicum annuum* L. (accession 509–45-1) is known for producing capsiate, previously we found that through elicitation by foliar application of hydrogen peroxide (H_2O_2 200 mM) before harvest it is possible to increase the capsinoid content in the fruit to more than double, compared to non-elicited plants. In this work, nutritional composition and antioxidant capacity of elicited capsiate-producing chili and habanero chili were compared, as well as the effects of their addition in the diet of C57BL/6 male mice. Results showed major content of fiber, flavonoids and tannins in capsiate-producing chili, while habanero chili has a higher content of protein, phenols and antioxidant activity. *In vivo* tests showed that the addition of dried chili accession 509-45-1 (50 ppm of capsiate) in mice diet acts as a growth promoter by stimulating weight gain and femur growth, furthermore, supplementation also modifies the gene expression of toll-like receptors (TLR), upregulating TLR2 expression in jejunum and downregulating TLR4 in jejunum and colon compared to mice that did not receive supplementation. TLR´s are related to immunity, metabolism and intestinal composition of the microbiota. The addition of habanero powder (capsaicin 30 ppm) to mice diet only stimulated femur growth. Therefore, as a strategy to face the growing demand for protein of animal origin and the antimicrobial resistance phenomenon caused by the use of antibiotics as growth promoters, capsiate producer chili seems to be an attractive alternative in animal nutrition.

MADS-Box XAANTAL1 transcription factor plays a crucial role under osmotic stress in Arabidopsis

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Plants are sessile organisms and must overcome several environmental threats such as abiotic stress. Particularly, osmotic stress has acquired significant importance given the increase in temperatures, and frequent and longer drought periods due to climate change. This has led to the need to better understand how plants respond to low-water stress and what genes and hormones are mainly involved in this response. Development and stress responses are tightly linked to each other, as the defense against stress could suppress growth or the plant´s correct functioning which is, in fact, due to defects in developmental processes.

In our laboratory, we are interested in unraveling the role of genes relevant for development in osmotic stress. For instance, the MADS-Box gene family codifies transcription factors whose functions are pivotal for plant development. *XAANTAL1* (*XAL1*/*AGL12*), whose name derives from the mayan ¨going slower", since the lost-of-function mutant has shorter primary roots and its cells have a slower cell cycle compared to the WT; hence, XAL1 is a root development promotor. Furthermore, *XAL1* orthologous in rice, *OsMADS26*, acts as a negative regulator of drought as its lost-of-function mutant is more tolerant to drought than the WT in laboratory and field conditions, whereas the over expression lines are more sensitive to this stress.

In this work, we pursue the integration of the developmental and osmotic stress response roles of XAL1 in Arabidopsis through the analysis of high-throughput sequencing data and showed that XAL1 may be functioning as a modulator of the interplay between plant stress and plant development.

Salicylic acid regulates the *CRK33* expression during leaf and fruit development in *Arabidopsis thaliana*.

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Over time, abiotic stress has played a fundamental role in the development of physiological and metabolic processes in plants. The intensity and duration of stress influence the effects and the ability of plants to resist it. One of the main effects of stress is found in the growth, height, stem, and roots development, as well as morphological and structural changes in leaves and fruits. Stress response mechanisms can be measured at various levels, from the whole plant to the molecular level since the responses are controlled by the genome. Due to the seriousness of this phenomenon, several researches have been carried out to deal with it, that is why it has been proposed to use salicylic acid as a factor that helps to reduce the effects of stress since this is a plant hormone, which is present in all plant organs and plays a fundamental role in the regulation of growth, development and interaction of plants with pathogenic organisms, as well as in the induction of plant defense against different types of environmental stresses such as drought, salinity, flooding, temperature changes, among others. Among the signaling factors that respond to biotic and abiotic stress conditions are also CRKs (cysteine-rich receptor-like proteins) family, *CRK33* was identified in previous reports and related to tolerance to water stress. However, it is still unknown how CRKs are regulated. For the present project, the regulation of salicylic acid on *CRK33* expression was analyzed. For this purpose, salicylic acid was applied at different times and concentrations on leaves and fruits. The results showed that there is a regulation of stomata density by *CRK33* and salicylic acid.

Key words**:** Fruit, leaves, CRK33, salicylic acid.

Analysis of water stress tolerance of amaranth varieties.

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Amaranth belongs to the Amaranthaceae family, which has about 70 genres and within these genres we find Amaranthus with its different species (*hypochondriacus, cruentus, caudatus, edulis*). Amaranth is a plant with agronomic and nutritional qualities, since it contains a high protein and vitamin value, which can compete with cereals such as rice, wheat, and barley. Amaranth seed has taken high potential for food and production in Mexico. Amaranth has been reported as a plant resistant to different types of stress, either biotic or abiotic. Within the abiotic stresses we can find drought and high temperatures, which in recent years have been a very important variable for agronomy, since the combination of water scarcity and high temperatures that we are experiencing due to climate change, and which leads to the concern of achieving sufficient food production. Therefore amaranth has become an important plant for the study of these factors, as it has been shown that it contains a high resistance to these factors. The present project consists of studying the behavior of 5 varieties of amaranth: nutrisol, revancha, tlahuicole, (*A. hypochondriacus*) amaranteca and Benito *(A. cruentus*), subjected to a drought stress. The results reveal that the amaranteca variety is one of the most resistant to drought, since it has a higher percentage of water content compared to the other varieties. Additional studies are still being carried out to evaluate the yield of the seeds and agronomics traits|.

Key words: drought, resistance, yield

Antioxidant and Metabolic Responses of Diverse Bread Wheat Plants Under Heat Stress

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Wheat (Triticum aestivum L.) is one of the main crops worldwide, providing more calories and proteins than any other cereal. Wheat crops across many areas of the world experience high-temperature episodes, significantly reducing grain yield. Heat stress induces the accumulation of ROS in the cells, which leads to oxidative stress. Therefore, the study of the antioxidant and metabolic responses is crucial for developing HS-tolerant wheat genotypes able to maintain grain yield and quality, which is vital to food security and economical profits. Previously, we performed two field experiments (optimal and HS during reproductive stage) and selected 10 out of 26 wheat genotypes based on yield reduction (6 heat-sensitive and 4 heat-tolerant) for further analysis. Herein, we aimed to evaluate the antioxidant and metabolic response of the selected genotypes to heat stress by performing catalase and ascorbate peroxidase enzymatic activity assays as well as the antioxidant activity using Trolox equivalents antioxidant capacity (TEAC) assay, and by determining the concentration of osmolytes glycine betaine and proline. In most of the genotypes, catalase activity was reduced in heat-stressed plants, while ascorbate peroxidase activity was increased, thus indicating that the latest is preferred in these plants for the H_2O_2 scavenging. The scavenging of 1,1-diphenyl-2picrylhydrazyl (DPPH) radical determined by TEAC, showed a genotypic-dependent behavior as it was decreased in most tolerant, and increased in most sensitive wheat genotypes under HS, representing an interesting mechanism for the heat-sensitive genotypes to cope with the oxidative stress caused by HS. Furthermore, the osmolyte glycine betaine (GB) concentration was higher in all stressed plants, while proline was decreased. Both GB and proline are important osmoprotectants that accumulate in response to abiotic stress; however, proline increases more under salinity stress than HS, which may explain the preference for wheat plants to accumulate GB under the HS.

Possible role of the protein Vacuolar Protein Sorting 29 from *Mesembryanthemum crystallinum* on salt stress tolerance in *Arabidopsis thaliana.*

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High salt concentrations in soils affect up to 20% of the total global cultivated land causing agricultural productivity losses and economic losses of up to US $27.3 billion/year [1]. Halophyte plants are a good model for understanding the salt tolerance mechanisms, such as *Mesembryanthemum crystallinum*, which can tolerate high concentrations of NaCl by transporting the ions from the root to the leaves for their accumulation in the vacuole [2]. Previous research in the workgroup showed that the protein Vacuolar Protein Sorting from *M. crystallinum* (Mcvps29) increased its abundance in plants treated with 200 mM of NaCl [3]. VPS29 protein is part of the retromer complex, which sorts cargoes from early endosomes to the plasma membrane, the vacuole, or the late endosomes [4]. To gain more information about the role of McVPS29 in salt conditions, we proposed to study its overexpression in a TDNA mutant line of vps29 obtained from ABRC (accession number, SAIL_158_B03). We detected one independent homozygous line of vps29 mutant (*vps29-5*) without the transcript of the vps29 gene. The mutant vps29-5 exhibited a smaller morphology in the root and cotyledons on 100 mM of NaCl compared with wt at eight DAG. This result could indicate that Atvps29 plays a role in salinity conditions. On the other hand, *vps29-5* showed a delay in the emerging time of the stem, and the rosette was smaller than the wt. The *vps29-5* mutant will be complemented with the Mcvps29 gene to analyze its effect on salinity tolerance in Arabidopsis.

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References

1. Qadir, M., et al. *Nat. Resour. Forum*. 2014. 282-295.
2. Barkla, B. J. et al. *Prot*. 2012. 12, 2862–2865.
3. Lastiri-Pancardo, G. M. 2016. Tesis de maestría, Universidad Nacional Autónoma de México
4. Gershlick, D. C., and Lucas, M. *Curr. Biol*. 2017, R1233-R1236.

Molecular analysis of *Arabidopsis* ECT8 protein in mRNA recognition and processing

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Post-transcriptional gene regulation plays a critical role in controlling the fate of transcripts. There are different pathways that regulate how long a transcript lasts in space and time. A unique regulatory mechanism is the selective addition of N6-methyladenosine (m^6A) to mRNAs and its recognition via RNA-binding proteins (RBP) containing a YTH domain. In *Arabidopsis thaliana,* an 11-member RBP family termed Evolutionarily conserved C-terminal region proteins (ECT) has been identified. Although a few proteins in this YTH family have been studied, how do they contribute to spatio-temporal control of mRNAs remains to be elucidated. To address this question, we first determined the subcellular location of the m^6A reader protein ECT8 using a translational fusion to GFP. Whether it forms condensates or not and its colocalization with P-bodies and stress granules was also analyzed. Confocal microscopy analysis of *Nicotiana benthamiana* leaf discs transiently overexpressing ECT8:GFP revealed that it can be localized in both nucleus and cytoplasm forming protein aggregates in response to ABA, PEG, and NaCl. Which are the ECT8 target transcripts? Are they methylated? To know this, an ECT8-based pull down assays are under way to demonstrate ECT8-mRNA interactions *in vitro*. We are also using as heterologous system the moss *Physcomitrium patens* to express a Cas13b-guided ECT8 protein along with a gRNA targeting GFP mRNA which will serve as a reporter. We expect these strategies will allow us to understand the function of this reader protein as an activator of translation or inducer of transcript degradation.

Far-red light PHYTOCHROME A receptor controls cell proliferation/survival in the root meristem

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Shoot and root meristematic cells are vulnerable to environmental factors that damage DNA and cause programmed destruction of dividing cells, which lead to growth restriction. The molecular mechanisms underlying this process in plants remain largely unexplored. Here, we tested the possible role for red-light photoreceptors in the protection of *Arabidopsis* root against genotoxic stress. Genetic and molecular analysis show that the PHYA photoreceptor was critical for the protection of root meristems from cell death caused by genotoxic agents. *PHYA* expression was located in the tip of primary roots, where it influences the expression of genes related to DNA repair and cell regeneration such as *ERF115* and *RAD51*. Interestingly, *phyA-211* mutants treated with zeocin did not express the repressor of cell cycle progression MYB3R3, which leads mitotic cyclin CycB1, suggesting that PHYA is required for safeguarding the DNA integrity during cell division. Moreover, light/darkness experiments and the growth of the primary roots of PHYA downstream component HY5 indicates that cell viability and DNA damage responses within root meristems takes place via an independent mechanism from the canonical light and photomorphogenesis signaling pathway. Together, our data revealed a new role of PHYA as a key player for cell division, stem cell niche maintenance and DNA damage responses, which are critical for the function of meristems and proper root growth.

Correlation of the root hydrotropic and gravitropic response with cell wall lignification of teosinte (*Zea mays mexicana* L.)

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The hydrotropic response of maize is highly diverse since some accessions developed root curvatures from 120º to 20º. The lines with root curvatures higher than 40º were classified as robust, and those with curvatures <39º as weak. Those lines with robust hydrotropic response (RHR) are highly resistant to drought. Teocinte is the direct ancestor of maize; however, their hydrotropic response has not been tested yet. We examine the hydrotropic response of teosinte using teosinte lines from different geographical areas of Mexico. We found that teosinte readily responded to hydrostimulation Teocinte roots also showed a diverse hydrotropic response with curvatures from 100º to 25º. Since Teocinte roots showed lower elongation growth than maize in the hydrotropic assay, we analyzed under the microscope root sections and stained them with safranin 'O' to discern whether lignin is synthesized in response to water deficit. We selected roots with RHR and roots in control conditions (water) that showed gravitropic response. Stained root sections were analyzed with the RootScan program that allows quantitative morphological analysis. Our results showed that roots with a RHR increased the xylem area and decreased both the number and size of cortical cells compared to those with a gravitropic response, which reduced energy expenditure in water deficit conditions. Analysis of the images with the ImageJ software revealed that there is a positive correlation between the distribution and abundance of lignin in the vascular bundles in hydrostimulated roots since the relative amount of lignin increases under water deficit conditions compared to those in control conditions. Hence, Teocinte responds to hydrostimulation and increases lignin synthesis in xylem cells, suggesting that these mechanisms provide less water loss and greater tolerance to drought.

Participation of HXK on the tolerance of *Arabidopsis thaliana* to cold acclimation and freezing temperatures.

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Plants are exposed to many types of stress such as cold temperatures. Chilling (0 and 15 ºC) and freezing temperatures (<0 ºC) affect metabolic activities causing osmotic stress in plants, freezing temperatures, led the formation of ice crystals which can permanently damage the cells. Many plant species have developed a set of mechanisms to content the negative effects of cold stress. Cold acclimation is a process that involves numerous physiological and biochemical changes and increases the plant freezing tolerance. On the other hand, Hexokinases (HXKs) are well known as glucose phosphorylating enzymes but also in eukaryotes they are moonlighting proteins, with a wide range of other functions such as glucose sensing proteins, part of a repressor complex, protein kinase and others. The moonlighting function of the HXKs have an impact in different processes such as regulating the photosynthetic activity, stomate closure, programmed cell death, increase in the resistance to salt and biotic stress. MdHXK1 protein kinase stabilizes MdbHLH3 to regulate the expression of anthocyanin biosynthesis genes. In Arabidopsis seedlings have been shown significant anthocyanin accumulation induced by low-temperature treatments. In this work, we explore the role of the HXK during cold acclimation and the freezing tolerance on different Arabidopsis plants, wild-type ecotype Ler, gin2-1 mutant unable to synthesize the glucose sensor HXK1, and in the gin 2-1 complemented ZmHXK4 plants (glucose sensor). Anthocyanin synthesis is induced after 5 to 7 days of acclimation on WT plants and in the gin 2-1 complemented with ZmHXK4. However, gin2-1 plants did not accumulate anthocyanin.

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Genome-wide *in silico* identification and classification of RLK genes in *Capsicum annuum* and expression analysis in response to cold an UV-B combined stress

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The receptor-like kinases (RLKs) family is one of the largest gene families in plants, with its members playing an important role in plant growth, development and response to stresses. Members of this family have been identified in many plants but in *Capsicum annuum*, an economic important crop, only a few RLKs have been identified. Thus, in this study we identified and classified 804 RLKs in *Capsicum* genome by using a hidden Markov models approach. These sequences were aligned with RLKs from other species for the construction of a maximum likelihood phylogenetic tree. Chromosome distribution of the genes was determined as well. Following, RLKs gene expression during cold and UV-B combined stress was analyzed from RNA-seq data, revealing a diverse regulation pattern and suggesting a potential role for the many RLKs during the response to these conditions.

Arabidopsis primary meristem activity modulated by Iron and SOMBRERO during Phosphate deficiency

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Plant development and yield are depending to the perception and their adaptation to adverse environmental traits, including nutrient scarcity. Phosphorus (P) is an essential macronutrient for plant growth as it is necessary to build lipids and nucleic acids, but also participates in different metabolic and signalling processes. P is mainly absorbed by roots as inorganic phosphate (Pi), but in soils its availability is low due limited mobility and the interaction whit different compounds including those containing iron.
Has been highly documented that Pi scarcity modify plant root system, inducing branching to increase Pi absorption from upper soil layers where is deposited, but also the primary root growth is inhibited stopping the exploration and uptake of water and other nutrients in deep soil regions (1). Primary root inhibition by meristem exhaustion under Pi deficiency coincides with iron (Fe) increases and oxidative burst in the stem cell niche (2).
Recently, we found that under Pi scarcity, the stem cell niche proliferates producing a thicker root tip as an early adaptative response before the meristem exhaustion of *Arabidopsis*, a process modulated by the transcription factor SOMBRERO (3). However, the modulation of Fe dynamics by SOMBRERO during the stem cell proliferation under Pi deficiency is unknown. Here we will discuss the meristematic responses depending on Fe increases modulated by SOMBRERO in *Arabidopsis* plants under Pi deprivation.

1. Sánchez-Calderón *et al*., Phosphate starvation induces a determinate developmental program in the roots of Arabidopsis thaliana. *Plant & cell physiology* 46, 174-184 (2005).
2. Müller *et al*., Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. *Developmental cell* 33, 216-230 (2015).
3. Ravelo-Ortega *et al*., Early sensing of phosphate deprivation triggers the formation of extra root cap cell layers via SOMBRERO through a process antagonized by auxin signaling. *Plant molecular biology* 108, 77-91 (2022).

Species-specific phyllosphere responses to external pH change

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The leaf surface (the phylloplane) is the first point of contact in the interaction between the plant and the aboveground environment. These plant-environment interactions can involve pH changes, such as when the phylloplane is in contact with pesticide application, acid rain, microbes, and pests. It has been shown that a plant can modify its pH on the phylloplane, and this buffering ability is species-specific. Genes and/or pathways involved in responses to external pH changes, and how they can be related to other abiotic and biotic stress signaling pathways haven't yet been described. The pH response to external pH changes was characterized in different species to include a broad range of phylloplane pH (*Gossypium,* pH ~8.8, *Beta,* pH ~7.8, *Nepenthes,* pH ~4.8). Leaf surface pH was measured for each species on a dry control and in response to each pH treatment (pH 6.5, 4, and 2) to examine pH regulation ability. A comparative transcriptomic analysis was performed with the same multifactorial experiment design. Orthologues annotation across species was done with OrthoFinder. We found specific buffering ability and differentially expressed genes linked to each genus. *Gossypium* were the only species that showed a strong buffering ability. At the pH 6.5 and pH 4 treatments, both *Gossypium* species alkalinized phylloplane pH slightly higher than the dry control pH, and even increased allowing the pH 2 treatment to be around pH 6 in 5 minutes. At the transcriptional level, each species showed specific differentially expressed isoforms, which suggests that unique mechanisms are used to sense and buffer external pH changes. ATPase-H^+ pumps, K^+ potassium transporters, auxin responsive protein and ABC transporters were differentially expressed in response to pH changes mainly in both *Gossypium*. This, altogether with gene ontology enrichment analysis suggest a link between pH stress and other abiotic stresses such as drought, salt, and chemical stresses.

Role of the m^{6}A-reader protein ECT8 in the response to stress conditions in *Arabidopsis thaliana*

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Methylation of adenosine at the N6 position (m^{6}A) is the most common internal modification found in eukaryotic mRNA that affects the fate and function of the modified mRNA, thus regulating fundamental biological processes. Most "reader" proteins recognize m^{6}A through a highly conserved domain known as YTH. In Arabidopsis, the transcript encoding for a protein with this domain, AtYTH06/ECT8 showed increased accumulation under different conditions associated with low water availability or upon abscisic acid (ABA) addition.

Furthermore, in germination tests, we observed a reduced sensitivity to ABA when we used three different T-DNA insertional mutant lines for this gene. In addition, during germination we observed that the *ECT8* transcript accumulates after 9 hours of treatment with ABA, and this accumulation is not observed in the later hours of treatment. *ECT8* expression is affected during germination in the *abi5-4* mutant and on the other hand, the expression of *ABI5* is affected in the *ect8* mutants, indicating that they regulate each other. To determine the role of ECT8, we are currently performing the identification of differentially expressed genes in response to ABA during germination in the *ect8* mutant. We obtained pECT8:ECT8:GFP/*ect8* complemented lines to perform co-immunoprecipitation assays to determine the proteins and transcripts with which ECT8 interacts, and to determine the subcellular localization of ECT8 using confocal microscopy. Finally, we identified LARP1a as a potential ECT8 interactor. During germination in the presence of ABA, the *larp1a* mutant has a phenotype similar to that of *ect8* mutants and the *ect8*/*larp1a* double mutant is even less sensitive to the hormone. These results suggest that ECT8 and LARP1a are involved in the response to ABA treatment during germination, prompting us to evaluate this possibility further. This work will contribute to characterize the molecular role and biological function of ECT8 during germination in the presence of ABA.

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ABSTRACT

Submergence stress diurnally activates subsets of transcripts of the different glycolytic pathways in *Brachypodium distachyon.*

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Glycolytic pathways are core to primary metabolism. In plants, there are three catabolic pathways for glucose: the Embden-Meyerhof-Parnas (EMP); the Entner-Doudoroff (ED); and the Oxidative Pentose Phosphate (OPP). During submergence stress, oxygen diffusion is limited and the EMP pathway is activated, leading to the overreduction of the cell. The fermentative pathways oxidize NADH to NAD+, regenerating the capacity to carry on EMP. However, the roles of ED and OPP under submergence stress are less clear. In this work, we aimed to quantify the transcriptomic response of all three glucose-catabolic pathways. For that purpose, we studied the response of the transcriptome of the model monocotyledonous plant, *Brachypodium distachyon*, during one day of submergence (S) in two ecotypes with differential tolerance, Bd-21 (sensitive) and Bd21-3 (tolerant). As controls, we used normal growth (NG) and low light (LL). Using the genome annotations of Phytozome and TAIR, we were able to digitally isolate 119 transcripts coding for all enzymes in these pathways. We employed Counts Per Million and the clustering tree of the transcriptomic values at five points in the day. Most enzymatic steps were coded by several family members. Interestingly, they are radically divided into two main clusters of responses: up- and down-regulated under stress. In both groups, we could quantify transcripts coding for all enzymes in the three different glucose-catabolic pathways. We also observed the transcriptional regulation of these transcripts in a diurnal manner because there were zenith peaks detected during the different times of day under submergence. We observed that in tolerant Bd21-3, during submergence, there is a subset of transcripts with an early response at ZT0 and ZT8. We concluded that the three glucose-catabolic pathways are activated in *Brachypodium distachyon* during stress in a time-dependent manner and identified possible enzymatic bottlenecks in the three different glucose-catabolic pathways.

The ATL-C Family: Functional Diversity and Neofunctionalization orchestrate Response to Water Stress in Arabidopsis.

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The ATL-C group is a family composed of 15 members with common features. They possess a highly conserved transmembrane region as well as a highly conserved RING-H2 domain. Among them, nine genes show functional diversity in response to water stress. A neofunctionalization event has been described in ATL78 which arose from ATL77. This event strengthened its expression through a short repetition containing a TATA box that occurred about 22 million years ago and improved the tolerance of *A. thaliana* to drought stress. ATL72 and ATL73 play a role in anther dehiscence. About 75% of the genes involved in anther dehiscence respond to water deficit, indicating a relationship between these processes. We suggest that the dehydration mechanisms associated with anther dehiscence and drought tolerance in vegetative tissue share several similar traits. On the other hand, ATL80 regulates early responses to water deficit, with its expression rapidly activated within 30 minutes and then declining. ATL80 regulates 90% or more of the genes involved in these responses, and we suggest that ATL80 is involved in the reprogramming of early gene expression in response to water stress deprivation that progress in transcriptional waves.

Influence of organic fertilizers at different environmental conditions on the quality of corn seed.

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The application of organic fertilizers has been used to improve the growth and development of crops, especially with the purpose of increasing the quality of grains of economic importance crops, in this ca se, it has been a great field of study. The objective of this project is to analyze the quality of corn seeds obtained under four different environmental conditions and the use of organic fertilizers, as well as its relationship with the stress response in plants obtained from these seeds. For this work, a bromatological analysis of seeds was carried out. The seeds were obtained from crops grown in four different growing areas that have different environmental conditions and doses of organic fertilizer. The analysis of stress response genes in plants, providing relevant data in relation to the influence of the use of organic fertilizers on seed quality in a comparative manner between the study areas. So far, preliminary results provide significant differences between the analyzed areas and the use of organic fertilizers.

Anatomical changes and sucrose metabolism in common bean (*Phaseolus vulgaris* L.) pod subjected to water restriction

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The common bean (*Phaseolus vulgaris* L.) is one of the most important legumes for direct human consumption in the world. However, qualitative studies on temporal and spatial aspects of flowering have shown that under water restriction the flower number and pods are severely reduced. By contrast, pod walls retained the green color for several days longer than leaves and the reserves are used for rapid seed growth during pod filling. The objective of the present study was to determine whether the pathway of sucrose distribution in pod wall structures (pedicel, funiculus and seed) are implicated in the mechanism of resistance to water restriction. Plants of common bean cultivar OTI were exposed to growth under well-watered and terminal drought condition. Our results have shown evidence that under water restriction stress, the breakdown of sucrose was promoted in the pod wall; enzymatic activity of SUS and INV enzymes were correlated with fluctuations in sucrose and fructose levels. In addition, we tested the uptake and apoplastic distribution of fluorescent sucrose analog (esculin) which is recognized and transported similarly by sucrose transporters (SUTs). We have found that the water restriction modified the area of the pedicel, therefore parenchyma size was significantly increased. Interestingly, the esculin was concentrated in the vessel of phloem while the funiculus and seed have not showed treatment-dependent alterations. These findings suggest that other structures associated with pod wall are implicated in water restriction responses as a key process in common bean.

Identification of Transcriptional Factors involved in the signaling of the responses mediated by ABA or abiotic stress in the desiccation-tolerant moss *Pseudocrossidium replicatum*

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Plants are subjected to different stresses, particularly abiotic ones, which cause large crop losses worldwide. Therefore, studying the transcriptional factors (TFs) involved in the stress response is of the utmost importance to develop strategies for future biotechnological applications in plants of agricultural interest. To study the molecular response of *P. replicatum* to abiotic stress, 21 transcriptomes were generated from 7-d protonema subjected to different conditions for 3 h: control, 10 µM ABA, 200 mM NaCl, 300 mM glucose, 400 mM sorbitol, dehydration 63 % HR, and rehydration with water, all conditions by triplicate. The sequences obtained were analyzed with the Trinity v2.14.0 package, and gene expression was quantified and evaluated using the Kallisto program. To generate a collection of reference genes for RT-qPCR studies, we selected genes with a low level of variation in their expression among all the abiotic stimuli analysed (validated using Bestkeeper and GeNorm), traditional reference genes, and classic abiotic stress response genes that were validated with endpoint PCR and RT-qPCR tests. In addition, the 21 transcriptomes described above were analyzed to identify TFs that were repressed or induced under the different abiotic stress conditions analyzed. We have identified several TFs closely related to abiotic stress in other species in various stimuli and some specific to one type of stress. Here are some examples: DREB2A, ERF, Homeobox-leucine zipper, ABI5, WRKY, GATA, AREB1/ABF2, VP1/ABI3, ABI5, HAT5, MYB106, bHLH87, and BepR-HTH. Using the reference genes obtained in this work, we are currently characterising the expression of these FT-codifying genes by RT-qPCR, and our advances will be presented. MAVL thanks to CONACYT grant A1-S-35357, SRM, AAB and MAVL thanks to SIP and COFFA IPN funds, RMNN thanks to CONAHCYT and BEIFI IPN fellows.

The role of intrinsically disorder regions in the activity of transcription factors of the MYC family

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A high proportion of eukaryotic proteins (30%) are intrinsically disordered proteins (IDPs) or contain intrinsically disordered regions (IDRs) (70%)[1]. In *Saccharomyces cerevisiae* and in *Arabidopsis thaliana* a substantial proportion of transcription factors (TFs) and signaling proteins are part of this set of proteins[1, 2]. Of note is the high representation of these type of regulatory proteins in stress response signal transduction pathways. In this work, we address the hypothesis that the physicochemical properties that generate intrinsic structural flexibility are associated with TFs ability to detect changes in the intracellular environment through modifications in its conformation. We focused on RTG1, an intrinsically disordered TF (IDTF) of the MYC family involved in the yeast nutrient and osmotic stress response signaling pathways[2]. To evaluate RTG1 possible function as a sensor of hyperosmotic stress, we fused two fluorescent proteins at its N- and C-ends to detect conformational changes by determining the Förster resonance energy transfer (FRET) upon different yeast growth conditions. RTG1 sensor construct produces FRET in the presence of high concentrations of NaCl, KCl, and PEG-4000. Yeast cells grown under 0.8 M NaCl induced the migration of RTG1 to the nucleus and the formation of biomolecular RTG1 condensates. The search of RTG1 orthologous within the *A. thaliana* genome revealed the presence of MYC-type TFs containing IDRs with high similarity to the RTG1 N- and C-terminal IDRs. Examples of these are SPEECHLESS and MYC2 TFs. The first one acts as an integrator of the stomata and brassinosteroid signaling pathways to control stomatal development, while the second one mediates the activation of jasmonate signaling to regulate the plant growth and stress response. The role of the structural disorder in the function of IDTFs involved in the control of cell stress responses will be discussed.

[1] French-Pacheco, et al. PLOS.ONE. 2022,17(3):e0265422.
[2] Salladini, et al. Int.J.Mol.Sci. 2020,21(24):9755.

Proteomic analysis of tepary bean seed germination reveals changes in storage protein with low water potential

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Tepary bean (*Phaseolus acutifolius* L.) is one of the five species domesticated of the genus *Phaseolus* with genetic resistance to biotic and abiotic stress. To understand the mechanisms underlying drought-stress responses, here, we used a comparative morpho-physiological and proteomics analysis approach to monitor the changes in germinating tepary bean seeds exposed to water (control) and polyethylene-glycol-induced low water potential stress (PEG-6000) at ψ_W of -0.49 MPa for 24, 48 and 72 hours. Our physiological analysis showed that seed germination in water reached maximum after 48 h; in contrast, at -0.49 MPa seeds spend 72 h to get 85 % germination. In each case, the differences was not statistically significant at 48 and 72 h; however, root growth at -0.49 MPa was 40-50 % less than the control. Based on the protein banding patterns by SDS-PAGE abundant seed storage proteins showed changes in Phaseolin (50 KDa) and lectins fractions (20-30 KDa). Two-dimensional gel electrophoresis (2-DE) analysis revealed the mayor proteins with pI value in the 4–7 range. A second group of proteins was observed in the alkaline pI range with more than 50 proteins with a pI of 8.5–9. Furthermore, storage proteins were altered only with -0.49 MPa and clearly spots were diminished with molecular weight between 23-30 kDa. To identify the proteins, we used mass *spectrometry* (MS)-based *shotgun* proteomics and Uniprot's legume database with 99 % probability score (PLGS score) and "OK" value equals to 2. The Gene Ontology (GO) analysis show that GO terms proteins in PEG-6000 condition were involved in binding process of carbohydrate. Using Protein interactomes String database, we found that lectin phytohemagglutinin interact with serine/threonine-protein phosphatase. These findings suggest that tepary bean seed proteins provide valuable information with potential of being used in genetic improvement and could be part of the drought stress response.

Functional characterization of the Arabidopsis chloroplast ribosomal protein CL15, which interacts with Glycine-Rich Domain Protein 2 (AtGRDP2)

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AtGRDP2 protein is constituted by a DUFF1399 located at the N-terminal, a potential RNA recognition motif in the central region, and a Glycine-rich domain at the C-terminal. This protein plays an important role in response to abiotic stress and development in Arabidopsis. *AtGRDP2* gene overexpression lines display enhanced growth and development, early flowering, and increased tolerance to abiotic stress, whereas *atgrdp2* mutants exhibit the opposite phenotype. In a previous study, we identified CL15 protein as an interactor of AtGRDP2 by yeast two-hybrid and plant BiFC assays. The CL15 protein is part of the large subunit of the chloroplast ribosome and is essential for embryogenesis in Arabidopsis. In this study, we generated Arabidopsis *CL15* overexpression lines, which were subjected to 125 mM and 150 mM NaCl, 4% mannitol, and 1 μM ABA. Our results showed that the *CL15* overexpression lines did not exhibit statistically significant differences in the germination and development of Arabidopsis seedlings under 1 mM ABA and 4% mannitol. However, under 125 mM and 150 mM NaCl, they showed a decrease in fresh weight compared to the WT seedlings. Interestingly, some of the *CL15* overexpression lines presented a chlorotic phenotype, which did not produce seeds. We are currently overexpressing *CL15* in *E. coli* for further functional characterization of this gene.

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ABSTRACT

A rapid, non-invasive method to predict drought survival in Arabidopsis using quantum yield under light conditions

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Survival rate is frequently used to compare drought tolerance level among different plant genotypes. However, determining the critical point for recovery irrigation has represented an issue that relies directly on a qualitative inspection by the researcher or on the employment of non-straightforward and invasive techniques that invalidate the subsequent use of the tested individuals. Here, we present a simple, instantaneous and non-invasive method to estimate the survival probability of Arabidopsis plants after severe drought treatments. Quantum yield or efficiency of photosystem II was monitored in the last stage of the drought treatment, before recovery irrigation, in darkness (Fv/Fm) and light (Fv'/Fm') conditions. We found a high correlation between a plant's Fv'/Fm' value before recovery irrigation and its survival phenotype 7 days after. This correlation was maintained in the Arabidopsis ecotypes Col-0, Ler-0, C24, and Kondara under the same conditions. To test the applicability of Fv'/Fm' as a survival predictor, it was applied to control the survival rate of Col-0 and compare its drought tolerance with transgenic lines overexpressing key transcription factors involved in the molecular networks that regulate Arabidopsis seed desiccation tolerance, founding significant differences in survival rate respect to the control line. The results obtained in this work demonstrate that the chlorophyll *a* fluorescence parameter Fv'/Fm' can be used as a survival predictor that gives a numerical estimate of the Arabidopsis drought survival rate before a recovery irrigation. The procedure employed to get the Fv'/Fm' measurements is rapid, non-destructive and requires inexpensive and easy-to-handle equipment. In conclusion, Fv'/Fm' as a survival predictor offers an overview of the photosynthetic state of the tested plants and determine the best timing for rewatering to assess the survival rate of tested lines more accurately, especially when the symptoms of severe dehydration between genotypes are not contrasting enough to identify a difference visually.

Pseudocrossidium replicatum owns tolerance mechanisms to salinity at different developmental stages

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Salinity is the second abiotic stress that affects crop productivity worldwide. Thus, studying plant mechanisms to face this injury is pivotal to secure food production in the future. Recently, moss *P. replicatum* was reported as highly tolerant to desiccation (Ríos-Meléndez et al., 2022). The present work evaluated the tolerance of *P. replicatum* under salinity at different developmental stages. First, the germination kinetics of *P. replicatum* spores on $PpNH_4$ media supplemented with various NaCl concentrations were addressed. The germination of Control was 91%, whereas, under NaCl 0.05 M, 0.1 M, 0.2 M, and 0.3 M, the germination decreased to 89%, 90%, 87.5%, and 45%, respectively. After germination, *P. replicatum* could develop green protonema even at 0.2 M NaCl. However, at higher concentrations of NaCl, the protonema growth was abolished after germination. To explore a possible role of ABA in this response, an exogenous ABA pretreatment was addressed in 10 d old *P. replicatum* protonema 24 h before the exposure to 0.2, 0.4, 0.6, and 0.8 M NaCl for 10 d. After the stress period, we found that PSII was detectable in all ABA pre-treated samples. These findings suggest that ABA could induce a significant cellular protection response to protect the *P. replicatum* PSII. Additionally, *P. replicatum* protonema can recover growth and greening after 0.8 M NaCl, even without ABA pretreatment. Moreover, at the gametophore stage, the accumulation of soluble sugars under salinity and ABA reaches similar levels suggesting that the NaCl tolerance exhibited by *P. replicatum* could be ABA-mediated. Finally, a transcriptome analysis of *P. replicatum* under 200 mM of NaCl revealed a set of DEGs that will be presented. MAVL thanks to CONACYT grant A1-S-35357, SRM, AAB and MAVL thanks to SIP and COFFA IPN funds, GAGP thanks to CONAHCYT, BEIFI IPN, and IPN-QMUL fellows.

Biochemical response of four bread wheat genotypes subjected to heat stress.

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Wheat (*Triticum aestivum L.)* is one of the most consumed food crops in the world, being a vital component in the human diet since it provides more calories and protein than any other cereal. The elevated temperatures can cause heat stress (HS) which inhibits wheat growth, metabolism and adversely affects its productivity worldwide. As a response, these plants induce physiological, biochemical, and metabolic changes to tolerate HS. The main consequence caused by HS is the overproduction of reactive oxygen species (ROS), which leads to oxidative stress. Therefore, it is of major importance to investigate the biochemical response of heat-stressed wheat genotypes to generate new crop strategies and maintain food security. Previously, two field experiments were conducted: one under optimal temperature conditions and other under HS during the reproductive stage, using diverse wheat genotypes donated by CIMMYT and INIFAP. These genotypes were classified as tolerant or sensitive to HS based on reduced grain yield. Herein, we selected four genotypes (two tolerant, one intermediate, and one sensitive to HS) to evaluate the biochemical response to HS by determining the concentration of chlorophyll and carotenoids, performing ascorbate peroxidase and catalase enzymatic assays, and accumulation of glycine betaine and proline. All genotypes maintained the concentration of photosynthetic pigments, however tolerant genotypes showed a tendency to increase, while sensitive to decrease, which may lead to impaired photosynthetic machinery. Furthermore, in most genotypes, catalase (CAT) activity decreased under HS, while ascorbate peroxidase (APX) activity increased, showing that the H_2O_2 scavenging under HS is mainly by APX. GB content was increased in tolerant genotypes, while proline was decreased in most genotypes under HS. Both proline and GB are important osmoprotectants that accumulate under abiotic stress; however, proline increases more under salinity stress than HS. This could explain why wheat plants preferentially accumulated GB under HS.

CHARACTERIZATION OF MACROMOLECULAR CROWDING OF *Arabidopsis thaliana* ROOT CELLS USING GENETICALLY ENCODED MULTIMERIC NANOPARTICLES

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The cell cytoplasm is densely packed with macromolecules such as proteins, nucleic acids, and polysaccharides. The resulting macromolecular crowding is crucial for the efficient function of the cell, as it influences different events such as molecular transport, protein-protein interactions, and the kinetics of biochemical reactions. Changes in crowding occur in response to environmental perturbations, for example, in response to hyperosmotic stress. Thus, monitoring crowding changes inside cells is required in order to understand how this property impacts cell functions. One method to study crowding *in vivo* is passive microrheology, a technique that tracks the movement of tracer particles within the cell. The genetically encoded multimeric (GEM) nanoparticles are homomultimeric scaffolds fused to a fluorescent protein that self-assemble in the cell into bright tracer particles of defined shape and size. Through confocal microscopy, we can estimate the diffusion coefficient of these nanoparticles, which is a rheological parameter indicative of particle mobility inside the cell. Using this tool, we measured macromolecular crowding in different cell types and developmental zones of *Arabidopsis thaliana* roots in standard conditions and under hyperosmotic stress conditions induced with increasing concentrations of NaCl or sorbitol. In this way, we aim to elucidate how the rheological dynamics of the cytoplasm of different cell types of the root are modulated, from the perception of stress to long-term acclimation. Quantifying how crowding dynamics are modulated in plant cells will have several implications for the field of plant biology, and will contribute to understanding how plant cells sense, respond, and acclimate to environmental stress conditions.

GENETICALLY ENCODED FLUORESCENT BIOSENSORS TO STUDY THE RELOCATION OF PROTEINS IN RESPONSE TO HYPEROSMOTIC STRESS

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Cell homeostasis is perturbed when the environment is altered by changes on different physical-chemical properties. One of them is the osmolarity inside the cell. During stress conditions, the osmolarity and macromolecular crowding levels change dramatically. Genetically encoded fluorescent biosensors are biomolecular tools that can sense and report events that are occurring in living cells dynamically and in a non-destructive manner. A series of biosensors were designed to study osmotic stress in vivo in the budding yeast *S. cerevisiae* using intrinsically disordered regions (IDRs) and a pair of fluorophores (mCerulean3 as the donor and Citrine as the acceptor). IDRs can be useful to follow how proteins are re-located in response to changes in the intracellular environment caused by osmotic stress. Using confocal microscopy, the localization of different IDRs to changes in osmolarity (hyperosmotic treatment with 0.5 M NaCl) was followed before and after the treatment. Imaging was done immediately after the treatment with NaCl. The liquid properties of condensates formed by two IDRs, IDRBS144 and IDRBS163, were evaluated with the treatment of 1,6-hexanediol at different concentrations. In this work, we showed that biosensors are capable to re-localize to different compartments, including liquid-liquid phase separated condensates upon stress and these condensates were dissolved by 1,6-hexanediol *in vivo*. These results will help to understand how hyper-osmotic stress induces the re-location of intracellular proteins.

AFP1 transcriptional activation through a lncRNA under abiotic stress conditions

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Plants, as sessile organisms, are often exposed to different environmental conditions leading to the possible evolution of sophisticated developmental mechanisms that ensure plastic responses to conditions experienced as they grow. For instance, abscisic acid (ABA) is the major stress phytohormone and takes part in plant adaptation through regulation of physiological processes. ABI5 is a transcription factor that promotes ABA-responsive genes expression and it is activated by drought and salt stress during seed germination within a short developmental window and its activity causes the inhibition of germination or early seedling growth. In addition, ABI5 activity is regulated at the protein level via protein interaction and posttranslational modification. It's been reported that ABI FIVE BINDING PROTEIN 1 (AFP1) mediates the proteasomal degradation of ABI5, nonetheless, the mechanism of this regulation is still unknown. Moreover, long non-coding RNAs (lncRNAs) have emerged as major products of the eukaryotic transcriptome with regulatory importance and an intergenic lncRNA was identified within *AFP1 locus*, hence we named it as *lincAFP*. In this study, we characterized the function of *lincAFP*, and we showed that acts positively in the regulation of *AFP1* expression by recruiting the chromatin remodeling complex COMPASS-like and, therefore, generates a positive effect upon germination in presence of ABA. In conclusion, the evidence presented shows the role of *lincAFP* as a novel regulatory factor within ABA signaling pathway.

Identification of *Pseudocrossidium replicatum* transcriptional factors involved in response to salt stress.

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One of the main problems in agriculture is soil salinization, which can be caused naturally or by the wrong design of irrigation systems, poor quality drainage, and excessive use of chemical products. In crops, it affects the yield and lowers the quality of the products. Plants, being sessile organisms, are constantly exposed to abiotic stress. Abiotic stress is those environmental changes that cause development and growth deficits in plants and even death. However, through evolution, some plants have managed to obtain adaptive mechanisms. *P. replicatum* is a moss classified as totally tolerant to desiccation, which has physiological, biochemical, and molecular adaptive mechanisms to reactivate its photosynthetic system and recover quickly (Ríos-Meléndez et al., 2022). However, it can also tolerate different types of abiotic stress, including cold stress at -80°C and salinity stress at concentrations up to 800 mM with an ABA pretreatment. Due to these characteristics, *P. replicatum* is of biotechnological interest. Here, through a transcriptomic analysis (RNA-Seq by Illumina), differentially expressed genes were analysed in a NaCl treatment at 300 mM for 3hr. We only used the 250 more induced genes and the 150 more repressed genes for this analysis. Based on BLAST, we predicted four induced transcriptional factors commonly associated with abiotic stress: AREB1/ABF2, DREB2A, VP1/ABI3, and HAT5, and only one repressed (Zinc finger AN1 and C2H2 domain). Advances in the characterization and temporal expression in response to the NaCl of these genes will be presented. MAVL thanks CONACYT grant A1-S-35357, SRM, AAB and MAVL thanks to SIP and COFFA IPN funds, TRL thanks to CONAHCYT and BEIFI IPN fellows.

Genome-wide transcriptome and translatome analysis of nodulated soybean roots under water deficit

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Soybean can establish a mutualistic interaction with nitrogen-fixing soil rhizobacteria. Via this interaction, the plant can obtain most of its nitrogen requirements through symbiotic nitrogen fixation, reducing (or even eliminating) its nitrogen fertilizer needs. This crop is susceptible to water deficit (WD); evidence suggests that its nodulation status—whether it is nodulated or not—can influence how it responds to water restriction, although it is uncertain which molecular mechanisms are responsible for the differential response. The translational control step of gene expression has proven relevant in plants subjected to WD since it allows them to respond rapidly. Here, we analyzed the differential responses of nodulated soybean roots to WD. Also, these responses were classified depending on their transcriptional, translational, or mixed (transcriptional + translational) regulation level. Thus, the transcriptome (total RNA fraction) and translatome (polysome-associated RNA fraction) of four combined-treated soybean roots—including the nodulation and WD conditions—were analyzed. We identified gene modules associated with the nodulation and WD conditions of soybean plant roots through a weighted gene co-expression network analysis (WGCNA) followed by differential expression analysis (DEGs). Protein-protein interaction network analysis was performed for some subsets of mixed DEGs of the modules more associated with the plant responses to nodulation, WD or the combination of nodulation + WD. Our research reveals that the stand-out processes and pathways in the before-mentioned plant responses partially differ; terms related to glutathione metabolism and hormone signal transduction (2C protein phosphatases) were associated with the response to WD, terms related to transmembrane transport, response to ABA, pigment metabolic process were associated with the plant responses to nodulation + WD. Still, two processes were common: galactose metabolism and branched-chain amino acid catabolism. A comprehensive analysis of these processes could lead to the identification of new sources of tolerance to drought in soybean.

Photosynthetic, anatomical, and transcriptomic characteristics of leaves from two drought resistant *Phaseolus* species

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Drought is a deleterious condition mostly affecting rainfed crops. It impairs the plant photosynthetic functions and consequently their growth, mainly due to the decrease in CO_2 entrance and low water availability in cells. To compensate for these limitations, plants have developed mechanisms at the molecular, physiological, and anatomical level. Common bean (*Phaseolus vulgaris)* is one of the most affected crops by water shortage. By studying two drought resistant *Phaseolus* species, we found that they present different transpiration strategies to optimize their water use efficiency[1]. To get insight into these differences, in this work we examined the relation between their physiological adjustments and anatomical modifications in response to drought. We compared different photosynthetic related characteristics, water status and leaf anatomical parameters between *P. vulgaris* var. Pinto Saltillo and *P. acutifolius* T32. The alterations in leaf anatomy were evaluated in cross-sections using stereoscopic, optical, and electron microscopy. To identify possible molecular mechanisms involved in these responses, we performed a comparative transcriptomic analysis of leaves from both species, grown under irrigation or water deficit. The anatomical analysis showed that, upon drought treatments, leaves in both species kept the same proportion of air space and thickness of palisade parenchyma as under well irrigated conditions, indicating that in these tolerant cultivars water shortage does not impact the efficiency of CO_2 diffusion and the abundance of photosynthetic cells, even though they have contrasting stomatal dynamics. We also found that *P. acutifolius* did not show changes in the major vein density under drought, whereas for *P. vulgaris* a reduction was observed. This finding suggests that this decrease in veins density might represent a common bean's adaptation to keep soil water, in consonance to its early stomatal closing[1]; hence, exhibiting a water saving strategy. The enrichment of transcripts related to these functions will be discussed.

[1] Polania JA, Salazar-Chavarría V, González-Lemes I, Acosta-Maspons A, Chatter CCC, Covarrubias AA. (2022) Front Plant Sci*13:894657. doi: 10.3389/fpls.2022.894657*
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Unveiling the role of iron in dehydration adaptation dependent of ubiquitin ligase ATL78 in *Arabidopsis thaliana*

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Water deficit has a significant impact in almost all biological activities. Consequently, plants have developed a phenotypic plasticity that enables them to respond to stressful events threatening their survival. Iron, being an essential micronutrient for plants, plays a critical role in multiple biological processes. Previous studies have revealed that the activation of ubiquitin ligase ATL78 expression, during the evolution of Brassicaceae, is fundamental for water deficit adaptation in plants. A transcriptomic analysis of the *Arabidopsis thaliana* atl78 mutant under hydric stress has revealed substantial changes in gene expression patterns related to iron metabolism, specifically in iron acquisition, translocation, and storage. The identified proteins include IMA1, IMA2, FER1, as well as transcription factors like MYB29, bHLH38, and lncRNA-FER. Our work aims to establish the involvement of iron in plant adaptive mechanisms under water deficit conditions, as well as to determine the potential role of ATL78 ubiquitin ligase in regulating iron-related proteins.

The *Fabaceae* miR2199 induced by water deficit downregulates a transcription factor involved in root development.

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Post-transcriptional silencing is a mechanism that prevents the translation of mRNAs by inducing cleavage of targeted transcripts guided by a family of small RNAs (21-23 nt. length) known as microRNAs (miRNAs). The stimulus that induces the expression and activity of miRNAs can be a consequence of a programmed change, such as a development step, for example, or as a response to changes in the environment. For plants, the lack of water availability affects cell volume, compromises gas exchange through stomata and photosynthesis activity, in addition to affecting enzymatic reactions and other metabolic processes, which combined can cause an arrest in the growth of the organism. Our group has been studying the functions of miRNAs present in legumes such as *Phaseolus vulgaris* (common bean), a species of agronomic importance, and in the model species *Medicago truncatula*. Among of them, miR2199 is induced by water deficit in *P. vulgaris*[1], and in *M. truncatula*[2]. Transcripts encoding for members of the bHLH family (basic-helix-loop-helix) of transcription factors were bioinformatically predicted as targets of miR2199, and in *M. truncatula* the bHLH factor TSAR1 was experimentally confirmed as the target for this miRNA[2]. This transcription factor is required to initiate the pathway for saponin biosynthesis in *M. truncatula*[3]. In assays employing compound transgenic roots in *M. truncatula* plants we have observed that increasing the abundance of TSAR1 results in a phenotype of short root architecture. The next step is to identify the relationship between water deficit, the induction of miR2199 and its role on the abundance of TSAR1 and the effect of this regulation module on the development of the root system of *M. truncatula* plants.

[1]Arenas-Huertero, C. et al. (2009) *Plant Molecular Biology*.
[2]Romero-Pérez (2015) Tesis de maestría, UNAM.
[3]Devers, E. A. et al. (2011) *Plant Physiology.*
[4]Mertens, J. et al. (2016) *Plant Physiology.*

Biolistics cisgenic vector transformation of *Capsicum annuum* L. tissue cultures (cv. 'serrano') with pyrophosphatases fused to the iLOV fluorescent reporter.

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The plant adaptive response to phosphate (Pi) deficiency shows a sharp change in gene expression. Proteins changing their expression include haloacid dehalogenase superfamily (HADsf) members known as phosphate-response 2 (PS2), as well as soluble and membrane bound inorganic pyrophosphatases (PPa & PPv, respectively). Unlike Arabidopsis, the economically important crop *Capsicum annuum* L. is highly sensitive to Pi deficiency; yet its genome encodes seven PS2-like, more than seven PPa and three PPv encoded sequences, though none have been studied. Here, *C. annuum* proteins were expressed using a pCAMBIA 1300 vector with a cisgenic cassette composed of the *C. annuum* promotor for fructose-1,6-bis-phosphate aldolase, one *C. annuum* ORF in frame with the fluorescent reporter iLOV (differing only by three mutations from the photosensitive domain of *C. annuum* phototropin receptor) and one of four putative terminators from the *C. annuum* genome. The constructs were verified by sequencing, by transient expression in onion epidermis, and by stable expression in C. annuum tissue cultures, using biolistics and visualized by confocal microscopy. The *C. annuum* tested ORFs were one PS2-like protein, one membrane bound PPv and one classic PPa. The results revealed a fluorescent signal of enough intensity and stability for localization and expression studies under confocal microscopy. The photo physical properties of the iLOV reporter make it an excellent alternative to GFP, with its advantageous smaller size and quick photobleaching recovery. Observations of iLOV fluorescence fused to different *C. annuum* ORFs resulted in patterns consistent with the expression of cytosolic proteins (PS2-like ORF) and with membrane vesicles for the membrane bound (PPv ORF). The results support the use of iLOV as an efficient cisgenic reporter in plants. Cisgenic constructs such as the one reported here pose a reduced risk of ecological perturbation to natural environments, since they lack foreign sequences.

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Long noncoding RNA involved in the response to heat stress in *Arabidopsis thaliana*.

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Long non-coding RNAs (lncRNAs) are RNAs with a size greater than 200 nt. and it is participate in the regulation of gene expression. In plants, various lncRNAs have been identified, but only some of them have been characterized. The lncRNAs are involved in biological processes such as flowering, morphogenesis, and response to biotic and abiotic stresses. In the case of abiotic stress response, they have been found to be involved in responding to stressors like drought, salinity, cold, heavy metals, and heat. Regarding heat stress, several lncRNAs have been identified to be over-expressed under this condition. One of these lncRNAs is known as *lincEIN2,* which is located downstream of the *EIN2* gene. The *EIN2* gene encodes the main protein in the ethylene perception pathway. EIN2 is known to be involved not only in ethylene perception but also in the response to various stresses, including heat stress. It participates in the expression of heat-responsive genes such as heat shock proteins. Furthermore, *in silico* studies suggest that *lincEIN2* interacts with the EIN2 protein through its C-terminal-EIN2 domain. This interaction implies that *lincEIN2* plays a regulating role of the EIN2 protein. Under heat stress conditions, *lincEIN2* is found to be overexpressed, indicating that this lncRNA might play a possible role in the heat stress response mediated by EIN2. Therefore, characterizing this lncRNA under heat stress conditions could be crucial in understanding the heat stress response. pathway and finding a strategy to enhance resistance to this stress.

The role of eIF4E and eIF(iso)4E proteins in root development during cold acclimatation

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Eukaryotic initiation factor 4E (eIF4E) binds to 7-methyl-guanosine cap structure on mRNA 5´ end for translation initiation. During cold response (4ºC) *eIF4E* and *eIF(iso)4E* isoforms are up-regulated concomitantly with *TCF1* and *COR15A* cold responsive genes. Both isoforms participate in cold-stress response since rosette leaves of *Arabidopsis thaliana eif4e* and *eif(iso)4e* null mutants are more vulnerable to freezing damage than Col-0 WT plants (Salazar-Díaz et al., 2021). Nonetheless, *eif4e* and *eif(iso)4e* mutant root development might also be altered (Martinez-Silva et al, 2012; Liu et al, 2022). It was previously shown that phosphate transporter 1 (*PHO1*), sucrose transporter 3 (*SUC3*) and cold- *TCF1* mRNA translation is impaired in the absence of eIF(iso)4E (Martinez-Silva et al., 2012), while eIF4E is involved in root development via auxin signaling pathway (Liu et al, 2022). Here we present a work focused on understanding the role of eIF4E and eIF(iso)4E in root development during cold stress acclimatation. Under control conditions at 22ºC and cold acclimatation at 4ºC, both, *eif4e* and *eif(iso)4e* mutants, had reduced root growth compared to Col-0 WT. When both mutants were treated with abscisic acid (ABA), a cold-related hormone, the root growth was further inhibited to a greater extent than Col-0 WT. The synergistic effect between cold and ABA on root growth reduction was maintained in all lines, but *eif4e* displayed a more sensitive phenotype than *eif(iso)4e*. To understand the possible effect of either eIF4E isoforms on root growth under stress conditions, each line was crossed with fluorescent PLT1 and CycB1 reporters to analyze root meristems. In addition, the distribution of root development- and cold response-related mRNAs along polyrribosomal profiles from mutants and Col-0 WT was screened to decipher the molecular relevance of eIF4E isoforms.

Reference.
1. Salazar-Díaz K., et al. (2021). *Frontiers in Plant Science* **12**:69858.
2. Martínez-Silva A. V., et al. (2012). *PLOS ONE* **7**:e31606.
3. Liu T., et al. (2022). *Front. Plant Sci.* **13**:938476.

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Humidity restriction, high temperature, and elevated CO_2 on phenotype and physiology of *Agave salmiana*

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The concentration of CO_2 in the atmosphere has risen rapidly. This condition envisages the combined effects of high temperatures and droughts, which are effects of climate change. Crassulacean acid metabolism (CAM) plants show considerable plasticity, varying in response to environmental conditions and with developmental state. The objective of the study was to evaluate simple and combined effects of elevated CO_2 (eCO_2), high temperatures and humidity restriction on the physiology and phenotype of plants of *A. salmiana*. Plants were grown in pots at maximum field capacity (100% FC). Afterwards, four sets of plants were set aside each kept during 30 days at: 1) 100% FC and ambient temperature (AT), 2) 25% FC and AT, 3) 100% FC and high temperature (45°C, HT), and 4) 25% FC and HT (combined stress). Then, all plants were watered (100% FC) and subsequently separated into two subsets maintained for 30 days at: 1) ambient CO_2 (400 ppm) and 2) nocturnal eCO_2 (800 ppm). Evaluation of phenotype using RGB images showed that 25% FC and 25% FC and HT sets recovered at 800 ppm showed a 15-20% increase in area represented as leaf angle compared to the other treatments. This suggests that the eCO_2 effect depends on environmental conditions. Also, the effects of eCO_2 on plant growth and acidity were evaluated in mature, medium, and young leaves. The fresh weight did not show clear differences among leaf ages. In addition, eCO_2 + 25% FC had a lowered percentage of acidity, while in combined stress, no changes were observed with respect to the control. The results of this study enhance our understanding of the positive effects of CO_2 on the growth of *A. salmiana* and will help researchers devise adaptation strategies for crops in agricultural systems.

The function of the ABI4 transcription factor during the evolution the land plants and its roles in *Marchantia polymorpha*

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The colonization of the terrestrial environment by the basal plants (liver, moss and bryophytes) had different morphological and physiological adaptations occurred that allowed them terrestrialization. Fossil studies suggests that the first plants that colonized terrestrial habitats were ancestors of liverworts, such as *Marchantia polymorpha*, making this plant a good model for studying ancestral signaling pathways important for colonization and adaptation including the recruitment and functional specialization of key transcription factors (TF). In this project, we are interested in the analysis of the function of the ABSCISIC ACID INSENSITIVE 4 (ABI4) of *Marchantia polymorpha*, which participates in the perception and signaling of different biotic and abiotic factors such as sugars, ABA hormone and desiccation in vascular plants, but whose molecular function still remains not fully understood. ABI4 is conserved in plants, and the characterization of the possible ortholog from *M. polymorpha* could generate relevant information about its function and evolution. We generate mutants and overexpress plants of this TF where we have demonstrate that ABI4 participates in developmental processes including division, thallus morphogenesis and reproductive structures. Also, we analyzed its participation in stress responses including sugar and ABA signaling and desiccation responses. Our data so far support that ABI4 has relevant function in signaling responses to environmental signals. Finally its expression pattern show that this TF has is present in the meristematic regions of the plant.

Determination of genomic diversity and identification of favorable alleles for breeding of traditional and hybrid corn

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Corn breeding strategies in Mexico, are mostly focused on monocropping along with high throughput practices including an intensive usage of chemical fertilizers. This has led to the lost of alleles that would help interacting with microorganisms u other plants that would facilitate nutrient availability in roots. The implementation of corn breeding programs that consider including favorable alleles related with a higher efficiency of fertilizer usage without decreasing yields, is highly desirable. Based on a list of candidate genes related to the assimilation of N, Fe and P, with expression in roots, obtained using a comparative genomics strategy among elite varieties, landraces and teosinte germplasm (Hufford et al., 2012), identified 3 transporters PHT, 3 NRT and 5 nodulin-like (MtN3) and a gene related with the synthesis of phyto-siderophores (DMAS), with loss of allele diversity due to breeding and domestication. DNA Sequencing of landrace populations would allow the discovery of alleles that increase the interaction with microorganisms that would decrease the usage of chemical fertilizers. These alleles would be incorporated to commercial maize lines or for local farms usage. In this sense, but with the goal of increasing the nutritional quality of corn seed, crossing schemes using favorable alleles of *DGAT1-2*, *FAD2* y *WRI1* were stablished. These alleles were discovered in recurrent selection breeding populations by using DArTseq genotyping. Experimental hybrids were obtained with oil contents with 8% or more. Crosses that considered high genetic distances allowed to keep yields above 11 Ton / Ha. Favorable allele mining in maize genomes allows to develop varieties and lines that improve nutritional quality and fertilizer use efficiency.

Characterization and evolution of the large RIP family in *Agave tequilana* var. *azul.*

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Ribosome inactivating proteins (RIPs) are rRNA N-glycosylases enzymes that cleave an adenine (A4324) from 28S rRNA and arrest protein synthesis. RIPs are found in fungi and bacteria, but they are more abundant in plants [1]. Plant RIPs are commonly classified into three types: type A, formed by a single catalytic chain of approximately 30 kDa; type AB, are heterodimers of ~ 60 kDa formed by catalytic chain A plus a B chain with lectin properties: type AC, are inactive precursors that require proteolytic processing to obtain a functional RIP [1]. RIPs are involved in the plant response to abiotic stress, pathogens, and herbivory, but their biological roles are still sketchy.
Few RIPs from monocots are known so far, and only one report describing RIPs from Agavoideae subfamily has been published [2]. Using both genomic and transcriptomic data we identified 44 type A RIPs from *A. tequilana* var. *azul.* Transcript and protein expression profiles for several members of the family were confirmed by RT-PCR, and by mass-spectrometry, respectively. Phylogenetic reconstruction showed that RIPs from *A. tequilana* var. *azul* form two clades differentiated by the presence/absence of a signal peptide. We traced the two lineages to the Agavoideae and other subfamilies within the Asparagaceae indicating that they diverged during the origins of Asparagaceae were already differentiated in early stages of the divergence of Asparagaceae. Modeled 3D structures also showed structural differences in the C-terminus among the two lineages.
Our data contribute to the understanding of RIP family evolution. Some RIPs from *A. tequilana* might be used for biotechnological applications and as molecular markers to derive phylogenies within Agavoideae.

1. de Virgilio, M. et al. (2010). Toxins 2: 2699-2737.
2. Lledías, F. et al. (2020). Frontiers in Plant Science 11: 753.

Multifactorial regulation of gene expression mediated by ATAXIN-2 orthologs in *Arabidopsis thaliana*

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Ataxin-2 (ATXN2) is an RNA-binding protein (RBP) that interacts with poly(A) binding protein (PABP), RNA helicase (DDX6), and poly(A) polymerase D4 (PAPD4) as part of a ribonucleoprotein complex. It plays a role in the formation of stress granules (SG) and processing bodies (P-bodies). Originally identified in human neurodegenerative diseases, ATXN2 has also been found to be involved in RNA metabolism in flies, worms, and mice. Transcriptome profiling studies have revealed that *Arabidopsis thaliana* orthologs of ATXN2, known as CID3 and CID4, act redundantly to promote floral transition and are also involved in leaf growth dynamics. These genes exhibit similar expression patterns. In plants, a subclass of ATXN2, represented by CID16 and CID17, may associate with PABP assemblies, leading to interference with paralogs. Additional modes of regulation include ubiquitination, as E3 ligases that interact with CID4 have been identified, and alternative splicing events, as CID4 generates splicing variants. To understand the role of ATXN2 in plants, we are using the florigen component FLOWERING LOCUS T (FT) as a model. FT transcript levels are downregulated in the *cid3cid4* mutant, and these mutant lines display a late flowering phenotype. Intriguingly, the 3' untranslated region (3'UTR) of FT contains potential ATXN2 binding sequences.

The role of the lncRNA gene located downstream of EIN2 in the regulation of crosstalk between ABA and ethylene in *Arabidopsis thaliana*.

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The long non-coding RNAs (lncRNAs) are a diverse group of RNA molecules that are greater than 200 nucleotides in length and have limited protein-coding potential. These molecules play important regulatory roles within the genome by interacting with other RNAs and proteins. They are classified based on their orientation with respect to other genes, such as antisense, intronic, promoter, or intergenic. While it was previously believed that these lncRNAs primarily function as non-coding RNAs, recent studies have revealed that some of them can also be translated into small peptides with specific physiological functions (sORFS). In this study, we characterize by analysis *in silico* the conservation of a putative lncRNA-encoded peptide downstream of the *Ethylene Insensitive 2* (*EIN2*) gene, which is a central component of ethylene signaling in plants. We analyzed the genomic context of the *EIN2* gene and identified a conserved region downstream of it where lncRNA-coding genes were present in several angiosperm plant species. These lncRNAs had variable lengths and showed conservation of secondary structure and sequence similarity in a small conserved region that corresponded to an sORF. We proposed that these lncRNA-coding genes downstream of *EIN2* might have originated from an ancestral protein-coding gene through a neofunctionalization process. We hypothesized that the retained sORF within the lncRNA might represent a remnant of the protein-coding region of the ancestral gene. The conservation of this lncRNAs, its proximity to the *EIN2* gene and the altered phenotype in the triple response in *knockdown* lines of the lncRNA, suggest that it could have functional relevance, either as a translated peptide or as a functional RNA molecule with a secondary structure. However, further investigations are needed to elucidate the precise roles and putative of these lncRNAs and their encoded peptides in ethylene signaling and plant development.

Leveraging the evolutionary information in genomic and epigenomic databases to reconstruct DNA methylation systems

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The basal unit of biological information is the DNA sequence. Furthermore, DNA nucleotides can be methylated, impacting genome structure and readout. DNA methylation can be epigenetically inherited over many generations, is crucial for plant and animal development, and its mis-regulation underlies several human diseases. Despite its importance, our understanding of DNA methylation is far from complete, partly because it is absent from the most common genetic model organisms. To overcome this challenge, we are leveraging the available genomic and epigenomic data to uncover the evolution of DNA methylation. We systematically sampled multiple databases to recover proteins bearing a functional catalytic DNA methyltransferase (DNMT) domain, particularly focusing on poorly studied branches of the tree of life. This data, combined with bisulfite sequencing of representative species, has allowed to shed light on multiple peculiarities of the evolutionary history of the eukaryotic methylation systems. In particular, we are exploring the role of DNA methylation in Zygnematophyceae, the green algae lineage leading to plant terrestrialization.

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Skotomorphogenic growth patterns and genes associated to deep-sowing tolerance in maize landraces.

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Maize deep-sowing is an ancient and effective agricultural strategy used in arid and semiarid regions, such as in the Southwest of the USA and in many States of Mexico: Oaxaca, Puebla, Tlaxcala, Ciudad de Mexico, Estado de Mexico, Morelos, Nayarit, and Chihuahua. Some deep-seeding landraces can tolerate planting up to 40 cm depth to ensure adequate soil moisture for germination, emergence, and post-emergence vegetative growth during several weeks or months before the start of the rainy season. Deep-sowing tolerance depends primarily on the vigorous elongation of the mesocotyl, a phenotypic trait regulated by several QTLs with small phenotypic effects. To identify genes associated to deep-seeding tolerance in maize landraces, we collected, from traditional farmers, seven landraces managed under traditional deep-sowing practices and five landraces managed under traditional shallow-sowing practices. The skotomorphogenic growth of 30 seedlings from each one of the 12 accessions was assessed for seven days in the laboratory on a deep-planting assay system in the dark. Each seedling was individually genotyped by DArTseq to obtain SNPs prior to Genome-wide association analysis. The deep-sowing tolerant landraces showed a canonical skotomorphogenic growth pattern: long mesocotyls, short primary leaves, unbroken coleoptiles, and undeveloped adventitious roots from the coleoptilar node. In contrast, shallow-sowing landraces displayed, in the dark, patterns more related to a constitutive photomorphogenic growth: short mesocotyls, long primary leaves that ruptured the coleoptilar tip, and adventitious roots arising from the coleoptilar node. We found that genes associated to deep sowing tolerance are involved in cell wall synthesis, phytohormone signal transduction and transport, ion transport, transcriptional regulation, cell division, and response to light.

The plant growth promoting rhizobacterium *Achromobacter* sp. 5B1 helps *Arabidopsis* seedlings to grow under phosphate scarcity

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Phosphate scarcity is one of the major problems in agriculture because around 70% of the plantations lack sufficient amounts of this nutrient to grow properly due to acidity or alkalinity conditions. Under these conditions, phosphate combines with cations forming sparingly soluble precipitates, unavailable for root absorption. Plants manifest highly efficient adaptive responses to increase phosphate solubilization, uptake and transport from roots to shoots through modifying the root system architecture, secretion of protons, organic acids and enzymes, as well as reinforcing symbiosis events with soil bacteria and fungi. A single bacterial isolate, *Achromobacter* sp. 5B1, was previously characterized as a beneficial microorganism helping *Arabidopsis* plants to resist salt stress and instructing root movements through modulating auxin transport and response within the root tip (1). Here, we investigated the influence that this bacterium could exert in plants grown under varied amount of applied phosphate. Noteworthy, increasing concentrations of phosphate into the medium augmented root biomass production by the bacterium, which correlated with massive formation of lateral roots, which extended the root surface area. In addition, *Achromobacter* sp. 5B1 provided advantages to *Arabidopsis* seedlings to grow under phosphate deficiency, opening the possibility to use this bacterium to improve the nutritional status of plants under different growth conditions.

(1) Jiménez-Vázquez KR, *et al*. (2020). The plant beneficial rhizobacterium *Achromobacter* sp. 5B1 influences root development through auxin signaling and redistribution. *The Plant Journal*, 103(5), 1639-1654.

Peroxisomal polyamine oxidation in the *Arabidopsis-Botrytis* interaction

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Regulation of polyamine levels during plant infection through their catabolism has a particular impact depending on the pathogen's lifestyle. Polyamines such as spermidine and spermine are catabolized by FAD-dependent polyamine oxidases. This study aims to understand the role of peroxisomal spermidine and spermine oxidation during the *Arabidopsis thaliana - Botrytis cinerea* interaction. In *A. thaliana* plants of the wild-type Col-0 ecotype, an increase in PAO activity for the substrate's spermidine and spermine was observed 24 hours post-inoculation with the fungus. Furthermore, the lesion size caused by *B. cinerea* was determined in *A. thaliana* leaves treated with the PAO inhibitor 1,8- diaminooctane and with polyamines (spermidine and spermine), or with combined treatments. Expression levels of the Arabidopsis *AtPAO* gene family were determined, showing that the *AtPAO3* gene expression increased 72 hpi. Little is known about the participation of the *AtPAO3* gene encoding a peroxisomal enzyme in plant defense, for this purpose, a T-DNA insertional mutant line and two *35S::AtPAO3* overexpression lines were used. Both mutant and *35S::AtPAO3* overexpression lines showed smaller lesion sizes compared to the parental Col-0 ecotype. The role of reactive oxygen species and polyamine oxidation mediated by the *AtPAO3* gene in plant defense is discussed.

Deciphering the mechanism by which Trichoderma elicitors EPL1 and SM1 act in plants

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The beneficial interaction between plants and diverse microorganisms has been demonstrated, one of these ways is through the presence of elicitors in different fungi. These elicitors activate plant defenses, providing resistance against pathogenic microorganisms. Among the fungi that release elicitors to plants are Trichoderma, which produces ceratoplatanin proteins such as EPL1 and SM1 in *Trichoderma atroviride* and *T. virens*, respectively. Herein, we generated *Arabidopsis thaliana* lines that express the EPL1 gene from *T. atroviride*. It was observed that 35S::TaEPL1 expression lines exhibited increased resistance to pathogen attacks and improved growth compared to wild-type plants (WT). Additionally, an increase in the expression of genes responsible for the response to salicylic acid (SA) and jasmonic acid (JA) was detected, suggesting a possible association with pathogen resistance. We detected significant increases in hydrogen peroxide accumulation in the TaEPL1-expressing lines compared to the WT plants. We are currently in the process of generating Arabidopsis lines that express *T. virens* SM1 to assess its efficacy compared to its EPL1 ortholog. Furthermore, we are determining the subcellular localization of both elicitors within the plant cell. These findings clearly demonstrate the significant beneficial impact of Trichoderma-secreted elicitors on plants, indicating their potential application in enhancing crop resistance against pathogens and promoting growth for agricultural improvement.

Defensive host responses of *Prosopis laevigata* against mistletoe *Psittacanthus calyculatus*

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Mesquite (*Prosopis laevigata*) is an endemic and the most abundant tree in Guanajuato state in central Mexico. Mesquite is currently threatened by the invasion and heavy parasitism of *Psittacanthus calyculatus* mistletoe. Mistletoe parasitizes approximately 65% of the mesquites in urban zones. This parasitism is characterized by continuous stress that eventually results in the host´s death. The *P. calyculatus* infective cycle starts when frugivorous birds disperse the mistletoe's mature seeds onto the host tree branches and finish when the haustorium penetrates the branch and connects with the xylem. Successful establishment of infection relies on cell wall-degrading enzymes secretion such as cellulases, β-1,4-glucosidases, and endo-glucanases, as well as molecular and genetic factors that inhibit the host immune system. However, chemical signals derived from the host tree are involved in the mistletoe parasitism perception, are remain unknown. The aim of this work is to study the activation of initial mesquite's defense responses by the infestation with mistletoe. Here we show that *P. calyculatus* trigger early and late resistance responses in the host tree *P. laevigata*, from the activation of signaling pathways through phenotypic resistance traits during the initial infection stage. For this purpose, we inoculated mistletoe seeds onto young mesquite branches, and quantified H_2O_2 production, superoxide dismutase, catalase, and peroxidase enzymes activities, as well as phenylalanine ammonia-lyase enzyme activity and phenolic compounds production, as early and late responses. We found that the host increases resin production, suggesting that it is an additional important trait in mesquite resistance against the mistletoe infection. Our results will improve our understanding about the initial host defense mechanisms against mistletoe infection and they will contribute to designing novel strategies to reduce or eliminate mistletoe infections from mesquite and other trees.

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Transcriptional characterization of plant innate immunity using the mutant *eca2* of *Arabidopsis thaliana* as a model

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The transcriptional activation of early response genes is part of the responses activated by plants in the presence of pathogens. However, the molecular elements involved in their regulation are not complete known. Five mutants, called *eca*, have been isolated because they constitutively express the elicitor-induced early response gene *ATL2*. Recently, it has been determined that the *eca2* mutant is resistant to the fungus *Botrytis cinerea* and the bacterium *Pseudomonas syringae*, in addition to that, the permeability of the cuticle is increased in *eca2* due to the decrease in the content of waxes and cutin monomers. Considering the importance of *ATL2* in the defense responses and in order to identify molecular elements that intervene in the regulation of *ATL2* mediated by *ECA2,* we performed genetic screening to identify *reca* mutants, which are revertants of the constitutive expression of *ATL2* in the *eca2* background. We isolate the mutant *reca1* and it presented a reduction of the rosette size, as well as an increase in the number of leaves. The *reca1* mutant is susceptible to *B. cinerea*, but is resistant to *P. syringae*, and has a more permeable cuticle compared to the wild plant. It was determined that the phenotype of *reca1* is mediated by an independent recessive gene of *eca2* mutation. Therefore, *RECA1* could be acting as a new molecular element, which positively regulates the expression of *ATL2*. According to the phenotypes evaluated in *reca1*, we determined that are not completely reverted to those observed in the wild plant, but in many of the cases, *reca1* presented intermediate phenotypes between the wild plant and the *eca2* mutant. In this work, we characterize the transcriptomic response of *reca1* that might allow us to elucidate the molecular mechanisms that intervene in the regulation of the early response genes part of the plant immune response.

Common bean (*Phaseolus vulgaris*) STYLISH (STY) transcriptional factors (TF) as key regulators of nitrogen fixation symbiosis with *Rhizobium etli*.

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The symbiotic nitrogen fixation (SNF) occurring in the legume-rhizobia association is responsible for most of the nitrogen incorporation into biological systems and has the potential to reduce the chemical fertilization used in agricultural management. This is a complex and finely regulated process, in both symbionts, that includes transcription TF and microRNAs, among other regulators.
In the model legume *Lotus japonicus*, NIN, the master TF regulator of symbiosis, was shown to regulate NF-Y (A, B, C) that in turn, regulate STY TF. This signal transduction pathway is required for auxins biosynthesis and local accumulation in root cortical cells, inducing their division as an initial step in nodule organogenesis (1)
The goal of this work is to analyze the role of STY TF in SNF of common bean, the most important legume for human consumption. The common bean genome encodes 10 STY TF genes that are highly expressed at different stages of nodules / roots during SNF (2). Recent qRT-PCR analyses confirmed the increased expression pattern of the *STY* genes in inoculated roots and nodules of the BAT93 common bean genotype in symbiosis with *R. etli*. In addition, the expression of NFY-A1/B7/C1 and NIN TFs were also increased in symbiotic tissues. *In silico* bioinformatic analysis of the promoter of the *STY10* gene -that reaches its maximum expression value in mature nodules- identified *cis*- elements related to nodulation or root development, as well as CArG and CCAAT boxes, consensus sequences that are recognized by MADS/AGL TF and NFY TF, respectively. Experiments in progress show the transcription regulation of common bean *STY* genes not only by NF-Y TF (1) but also by AGL TF and indicate their relevant role in SNF.

1. Hossain et al. *MPMI* 2016, 29 (12) 950-964
2. O'Rourke, et al. *BMC Genomics* 2014, 15:866

Double-stranded RNAs (dsRNAs) as biofungicides against *Penicillium digitatum* for the protection of orange fruits (*Citrus sinensis*) in the postharvest stage

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Orange fruits (*Citrus sinensis* L.) are affected by *Penicillium digitatum,* which is the main cause of losses in orange production at the postharvest stage. A new technology known as SIGS (Spray-Induced Gene Silencing) is based on the application of exogenous, double-stranded RNAs (dsRNAs) on the surface of plants and fruits that can be taken and processed by the plant or the pathogen and, once inside the cells, initiate a process to silence key genes in the pathogen. Previous in vitro assays showed that dsRNAs reduced the germination of *P. digitatum* spores by up to 80% with respect to water-treated spores. With the aim of evaluating the potential of SIGS on orange fruits, three genes involved in the virulence of *P. digitatum,* called *FET5* (iron transporter), *Mic-33* (ethylene precursor protein), and *NIP* (necrosis inducer protein), were selected as potential targets for SIGS. Selected regions of these genes were amplified, flanked by the T7 promoter, and used as a template for the *in vitro* synthesis of double-stranded RNAs (dsRNAs). The dsRNAs were mixed in equimolar concentration and applied over previously injured orange fruits, which were then inoculated with *P. digitatum* spores. The analysis of the lesions developed five days after the infection revealed a slight reduction in fungal growth in the oranges treated with dsRNAs. Ongoing assays will reveal the effects of applying additional doses of dsRNAs and the use of MgAl-LDH nanoparticles as carriers to improve the delivery and stability of dsRNAs.

Endoglucanase gene family in *Solanum lycopersicum*: Genome-wide identification and characterization, and expression profiling analysis during arbuscular mycorrhizal symbiosis.

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The main load-bearing polymer of the cell wall (CW) is cellulose, which is comprised of chains of β (1,4) D-glucopyranosyl units that form crystalline structures. Enzymatic degradation of cellulose in CW is achieved through the synergistic action of enzymes called cellulases, including endoglucanases (EC) that cleave glycosidic bonds in amorphous regions of cellulose. In plants, EC enzymes are encoded by the glycosyl hydrolase family 9 (GH9) gene family and may have a variety of roles in cell wall biosynthesis and remodeling. *Solanum lycopersicum* is one of the most important crops worldwide and has become a model for physiological and genetic studies. Several biotic and abiotic stresses could challenge the sustainable production of this crop. However, many beneficial plant microbes can modulate plant defense responses. Arbuscular mycorrhizal (AM) symbiosis induces systemic responses. CW modification genes expression profiles as ECs may have an important role in triggering a priming mechanism that may lead to defense and resistance against pathogens. This study, identified and characterized 22 EC genes encoded in the tomato genome, classified into three subfamilies. Subcellular localization prediction showed that 16 SlEC proteins are secreted and localized in the extracellular region, while six are in the plasma membrane. An analysis of regulatory elements indicated functions of SlEC genes in gibberellins, SA, auxins, and ABA signaling, cell development, and defense and stress responses. Finally, to provide information for further functional studies, the expression patterns of these genes in several tomato tissues and in response to the AM symbiosis, were characterized by qRT-PCR analysis. Differential expressions of *SlEC1*, *SlEC2,* and *SlEC8* were observed in roots and leaves in response to the symbiosis, which are now targets for future studies.

The *XTH2*: a potential gene involved in cell wall biogenesis in *Solanum lycopersicum* during arbuscular mycorrhizal symbiosis.

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Xyloglucan (XyG) is the most important component of hemicellulose, composing up to 25% of the cell wall (CW). XyG is a polysaccharide formed by β1–4-linked D- glucan backbone branched with various glycosyl residues, playing essential roles in growth regulation and biotic and abiotic stress responses. Xyloglucan endotransglucosylase/hydrolase (*XTHs*) are a family of XyG modifying-enzymes mainly responsible for cleavage and rearrangement of XyG backbones in plants. Arbuscular mycorrhizal (AM) symbiosis induces an immune response, in which CW modification genes, including *XTHs*, are essential to trigger a priming mechanism that improves defense against pathogens. *Solanum lycopersicum* is one of the most important crops worldwide due to its increasing commercial production, and its use as a model for genetic and physiological studies. In the present study, we identified all potential *XTHs* genes encoded in the *S. lycopersicum* genome, and we analyze the possible role of *XTH2* gene in the CW biogenesis in leaves of mycorrhizal plants. We identified 37 *SlXTH* genes through genome-wide screening using bioinformatics approaches. The relative expression by qRT-PCR revealed that *SlXTH* genes had a differential expression in colonized plants, in which, *SlXTH2* was the only overexpressed gene in *S. lycopersicum* leaves. Protein-protein interactions revealed that SlXTH2 regulates proteins involved in CW biogenesis such as: SlEXPA2, SlEXPA5, SlEC7, SlCslE2, SlXTH14, SlXTH16, and SlXTH33, that at transcriptional levels are differentially induced in response to AM symbiosis. 3D predicted protein showed that SlXTH2 uses D-xylopyranose, D-glucopyranose, and D-galactopyranose as donor substrates, which could be responsible for the chemical modifications of XyG molecules, which was consistent with the sugar analysis by HPLC in colonized leaves. This supports the idea that CW modification genes play an essential role during AM symbiosis.

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The maize defense compound, benzoxazolinone, induces oxidative stress and fumonisin B1 production in the fungal pathogen *Fusarium verticillioides*

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Fusarium verticillioides is a fungal pathogen that causes several diseases in maize (*Zea mays L.*) that include seedling blight, stem rot, and ear rot. *F. verticillioides* ability to colonize maize plants depends on the production of various glycohydrolases and toxic metabolites. Among the latter, fumonisin B1 (FB1) stands out because it is synthesized early during the plant – pathogen interaction and has several targets in the plant cell. Maize plants produce several metabolites that contribute to its defense against pests and pathogens. 2- Benzoxazolinone (2-BOA) is an hydroxamic acid derivative that accumulates in maize tissues and has insecticidal and antifungal activities. However, *F. verticillioides* has evolved to tolerate this compound as it is able to break it down into a non-toxic molecule. Herein, we set to study the diversity among four *F. verticillioides* strains isolated from maize on their capacity to produce FB1, to metabolize 2-BOA and to cause seedling blight. We found that the four strains differ in FB1 production in maize seedlings (range 2.0-13.7 nmol FB1/g tissue), which were associated with growth inhibition of the main root. For the high-FB1 producing strain (MY3), 2-BOA stimulated FB1 synthesis *in vitro*. Although this strain was quite competent in transforming 2-BOA into non-toxic metabolites, we found that 2-BOA induced oxidative stress, detected through (Nitroblue tetrazolium chloride and 3,3'-diaminobenzidine) staining of the mycelia. Because the cell redox status might influence mycotoxin production, our results suggest that *F. verticillioides* response to plant defense molecules is through boosting FB1 synthesis. (Acknowledgements: DGAPA-PAPIIT IN217720 and PAEP-2023).

PDLP5 protein participation on root development of *Arabidopsis* during its interaction with *Azospirillum*

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Azospirillum brasilense Sp245, when interacting with *Arabidopsi*s, arrests primary root (PR) growth and increases lateral roots number (LRN), phenotype attributed to bacterial auxins. The natural auxin, Indole-3-acetic acid (IAA), is synthesized in the aerial part of the plant from where is distributed a to the root through: i) a rapid transport throught phloem and ii) a cell-cell transpor, where regulates root system development. Carrillo-Flores *et al*., 2022 observed that *Azospirillum* caused during the first days of interaction with *Arabidopsis* an auxins increase in differentiation zone of lateral roots (LR) of the PR and later the auxins were mobilized towards the LR meristems. On the other hand, it has been reported that IAA can difunded through plasmodesmata (PD) (intercellular channels small that connect the cytoplasm of neighboring cells) by a symplastic transport, which depends of PD permeability. This permeability is regulated by callose accumulation (polymer og glucoses linked by β-1,3 bond), throught the synthases (CALS/GSL) and clases two of proteins that help polymer deposition in PD neck: PLASMODESMATA CALLOSE-BINDING PROTEIN (PDCB) and PLASMODESMATA-LOCATED PROTEIN (PDLP), while callose degradation is carried out by β-1,3-glucanases. Regarding PDLP, it has been shown that PDLP5 regulates a transient symplastic isolation of auxins during LR development to ensure the emergence of these organs. The objective of this study is to analyze whether PDLP5 are involved in LR development, for which we analyzed the auxins level and the callose accumulation in the *pdlp5-1;DR5:GUS* mutant and the lines *PDLP5OE;DR5:GUS* overexpression of *Arabidopsi*s. The results showed that LR decrease in *pdlp5-1* seedlings inoculated with the rhizobacteria, a phenotype that could be related to a transient decrease of auxins caused by the PD opening in said mutant. These results suggest that PDLP5 participates on LR development of *Arabidopsis* when it is exposed to *Azospirillum*.

Bibliography

Carrillo-Flores E., Arreola-Rivera J., Pazos-Solís D.M., Bocanegra-Mondragón M., Fierros-Romero G., Mellado-Rojas M.E. and Beltrán-Peña E. 2022. Participation of Auxin Transport in the Early Response of the Arabidopsis Roor System to Inoculation with *Azospirillum brasilense*. Phyton 91: 2383-2401.

Sequencing of the microbiome in the rhizosphere and endosphere of a MAGIC population for Mexican landraces of maize

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A microbiome is a community of commensal, symbiotic, and pathogenic microorganisms that share the same environment and are related to each other; the microbiome is the sum of microorganisms and their genomic elements and is commonly composed of bacteria, archaea, fungi, algae, and small protists. The soil microbiome has an impact on the biogeochemical cycle of macronutrients, micronutrients, and other important elements essential to plant growth. In this work, we will identify and compare microbial taxa in the soil, rhizosphere, and endosphere of a multi-parent mapping population (MEXI-MAGIC population). The MEXI-MAGIC population was generated with 8 Mexican varieties of maize coming from a broad range of environments. To obtain the sequences of the microorganisms associated with each compartment, we have obtained soil, rhizosphere, and endosphere samples for 180 families of the MEXI-MAGIC population grown under optimal field conditions. The study of the role of the microbiome in the rhizosphere and the endosphere of Mexican maize varieties will allow us to better manage biological processes such as plant nutrition, pest control, and biotic interactions.

Molecular characterization of small peptides involved in plant-microorganism interactions

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Peptides have been described to participate as key components in the regulation of plant growth, development and stress responses. During biotic interactions, peptides have been identified as modulators of signaling and response pathways. Recently, peptides from 2 to 100 amino acids (small peptides) have gained great relevance as signaling molecules and/or as antimicrobial compounds. In our laboratory, we characterized at the molecular level the defensive responses induced by the exogenous application of the rare-earths element Gadolinium during the interaction between *Arabidopsis thaliana* and *Botrytis cinerea*. Among the genes with the higher expression, we identified the *A. thaliana AT3G04184* and *AT5G23411* genes, which encode for small peptides, the function of these genes remains unknown. To determine the participation of the innate immunity of these genes during the *A. thaliana-B. cinerea* interaction, we performed a functional analysis in homozygous knockout mutant plants. The preliminary results show a significant increase in the lesion size mediated by *B. cinerea* in leaves of the *at3g04184* and *at5g23411* mutants compared to their control, wildtype plants (Col-0). Currently, the characterization of these mutants at the molecular level is being carried out to know the defensive responses modified during the plant-pathogen interaction. Future experimental work on the small peptides will be contribute to expand our understanding of plant-microorganism relationships.

The role of phytosulfokine in the modulation of root immune responses to beneficial rhizobacteria Pseudomonas simiae WCS417 in Arabidopsis and Camelina.

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It is widely known that rhizobacteria play an important role in helping the development of several plant species, however, before colonization by mutualistic microorganisms may provide any benefits, such microorganisms must first avoid antagonizing the immune system of their host plants. In this context, our project sought to investigate the role of the plant peptide phytosulfokine (PSK) in the modulation of host immune responses to the plant-growth promoting rhizobacteria Pseudomonas simiae WCS417, specifically pertaining to Arabidopsis thaliana and Camelina sativa. The role of PSK in attenuating microbially induced immune responses and conversely increasing the susceptibility of plants to bacterial colonization has been long characterized. Furthermore, it has been shown that live WCS417 bacteria possess, in interaction with Arabidopsis, a capacity to attenuate host immune responses otherwise triggered by its flg22 flagellin proteins. We thus hypothesized that an inhibition of PSK-related pathways – as seen in mutants lacking functional tyrosylprotein sulfotransferase (tpst) or receptor PSKR1/PSKR2 (r1r2) genes – could affect such modulation of root immune responses to colonization by live WCS417, thus interfering with the establishment of a growth-promoting relationship. Protocols for evaluating microbially-induced differential growth promotion and gene expression[1] were adapted and applied to the loss-of-function Arabidopsis mutants tpst and r1r2 as well as to Camelina sativa, whose interaction with WCS417 was tested in the presence of exogenous PSK. Our findings help illustrate PSK's importance in the establishment of such beneficial plant-microbe interactions, for while the addition of exogenous PSK was not found to induce significant changes in the effects of bacterial colonization in Camelina, the loss of PSK-related genes severely inhibited or even reversed the growth-promoting effects of WCS417 in Arabidopsis.

[1] Banday ZZ, Cecchini NM, Speed DJ, et al. Friend or foe: Hybrid proline-rich proteins determine how plants respond to beneficial and pathogenic microbes. Plant Physiol. 2022;190(1):860-881. doi:10.1093/plphys/kiac263

Functional characterization of the NPR1-NPR3 interaction in the *Pseudomonas syringae-Arabidopsis thaliana* pathosystem

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Salicylic acid (SA) is a phytohormone that plays a key role in the activation and regulation of multiple responses to biotic and abiotic stresses, particularly to induce systemic acquired resistance (SAR), a mechanism used by plants to resist pathogen attack.
In *Arabidopsis thaliana*, SA is perceived by a nonexpresser of pathogenesis-related genes 1 (NPR1), NPR3, and NPR4 receptors, which are involved in the plant immune response. The co-activator NPR1 is the master regulator in SA perception whereas NPR3 and NPR4 were suggested to function as adaptors of the Cullin ubiquitin E3 ligase to promote NPR1 degradation in an SA-regulated manner.
The regulatory mechanism between these proteins is complex, therefore understanding how their interaction takes place and how it can be modified by different stimuli allows us to better understand their role in plant immunity. Although the interaction between these proteins has already been analyzed, the interaction domains have not been characterized.
In this work, the domains involved in the NPR1-NPR3 interaction were characterized *in planta* by the Bimolecular Fluorescence Complementation (BiFC) approach. The sequences encoding residues 1-200, 201-400, and 401-587 of the NPR3 protein were amplified and cloned to accomplish this. These sequences include the BTB/POZ and ankyrin repeats domains, and the SA binding site of NPR3, respectively. We also generated deletions of only one of the NPR3 domains were done. In addition, translational fusions of the full length of NPR3, as well as of the obtained fragments of NPR3, were generated with a green fluorescent protein.
The translational fusions were transiently expressed in leaves of plants col-0 and the *npr3-2* mutant of *A. thaliana*, making it possible to evaluate the susceptibility to infection of these plants as well as changes in the subcellular localization of NPR1 and NPR3 during infection with *pseudomonas syringae.*

Participation of the *AtPUT2* gene, encoding a polyamine transporter, in the plant defense response against *Pseudomonas syringae*.

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The interaction between plants and microorganisms is recurrent in nature. Therefore, plants have developed a complex defense system that is activated just after pathogen recognition. The regulation of the plant defense system depends on several mediators, among which are polyamines. These amines are present in all organisms, where they are essential for cell viability. In plants, among other processes, polyamines participate in the establishment and regulation of the defense response, through modulating their metabolism, specifically their biosynthesis, conjugation, and catabolism. However, it is unknown whether polyamine transport is involved in the plant defense response. In our study, the importance of Polyamine Uptake Transport 2 (*At*PUT2) in the interaction between *Arabidopsis thaliana* and *Pseudomonas syringae* pv. *tomato DC3000* was analyzed. We found that *AtPUT2* expression is induced in response to *P. syringae* in the early stages of interaction. In turn, the absence of the transporter (in the *Atput2-1* mutant line) induces *Arabidopsis* resistance to the bacterium. Under normal conditions and during interaction with the pathogen, the mutant line shows changes in the expression levels of genes involved in the salicylic acid pathway, suggesting that the resistance phenotype appears to be associated with the dysregulation of SA-mediated hormone signaling. Interestingly, using a reporter promoter line (*promAtPUT2::GUS*) it was found that *AtPUT2* gene expression decreased in response to salicylic acid treatment. On the other hand, we found that the *Atput2-1* mutant line does not adequately develop the hypersensitive response, an important process in the plant defense response. However, the mechanism underlying this response remains to be determined. These results provide evidence for the involvement of the *AtPUT2* transporter in the defense response of *Arabidopsis* against *Pseudomonas syringae*.

Characterization of bacteria from the skin of axolotls (*Ambystoma* spp.) as promoters of plant growth and defense

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Chemical fertilizers and fungicides have been used to improve the development and growth of plants and to protect the plants against pathogens. However, the excessive use of these chemicals entails negative effects on the environment and human health. Therefore, new ecological methods have been looked at for replacing these agents, such as the use of beneficial microorganisms. Previously, our team identified five bacterial strains from the skin of the endemic Axolotl (*Ambystoma* sp.) with an inhibitory role against the phytopathogenic fungus *Botrytis cinerea*. However, we do not know if these strains could be also used as phytostimulant agents. To characterize the morphological effects on plants, *in vitro*- and greenhouse- interactions of each bacteria with the *Arabidopsis thaliana* model plant were carried out. *In vitro* interactions revealed a phytostimulant effect in the primary root length and root hairs number. While under greenhouse-conditions, tests revealed that bacteria-inoculated plants are resistant to *Botrytis cinerea*. The results prove the role of bacteria strains isolated from the skin of axolotl as an ecological alternative to the use of chemical fertilizers and fungicides.

Induced systemic response study in the interaction *Arabidopsis thaliana - Streptomyces ambofaciens*.

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Induced Systemic Response (ISR) is a defense response in plants that generates immunity against a broad spectrum of pathogens with diverse lifestyles (hemibiotrophic, biotrophic and necrotrophic). This response is triggered by non-pathogenic bacteria, mainly plant growth promoting bacteria (PGPRs), which can be recognized by the plant and through different inducing molecules; for example, lipopolysaccharides, antibiotics, and flagellin. The ISR is regulated by the jasmonic acid (JA) and ethylene (ET) pathways, and different genes are known to participate in triggering this response; for example, *MYB72* and *MYC2* that participate in the initial processes of ISR in the root, as well as genes related to the JA and ET pathways, such as EIN2, EIN3, COI1, JAR1. Although there is knowledge about the pathways through which the ISR is activated and despite the fact that there are studies that show changes in gene expression in the interaction of plants with PGPRs, there is still a long way to go to understand how the ISR is established at the molecular after beneficial bacterium recognition. In a previous study analyzing the interaction *A. thaliana-S. ambofaciens*, it was determined that this actinobacteria can promote plant growth. The evaluation of different parameters such as lateral root density, rosette area, root biomass and aerial part of the plant showed that *S. ambofaciens* is a PGPR. In the present work, evidence is shown that it is also capable of inducing a systemic response against foliar pathogens such as *Pseudomonas syringae* and *Botrytis cinerea*. Likewise, the expression profiles of various molecular markers of the jasmonic acid and ethylene signaling pathways were determined, as well as ISR markers, finding the most important changes 2 days post-inoculation. This work constitutes an important precedent to broaden the study of Arabidopsis-Actinobacteria interactions.

The Amaranth cystatin inhibits the growth of *Meloidogyne incognita* in tomato plants

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Plant cystatins are proteins with the ability to inhibit the activity of cysteine proteases from different microorganisms. Based on this property, cystatins are considered a plant defense mechanism against phytopathogenic microorganisms. The root-knot nematode *Meloidogyne incognita* is one of the most damaging plant parasitic nematodes in the world. In this study, the effect of a cystatin from *Amaranthus hypochondriacus* (AhCPI) as a potential control agent for *M. incognita* was evaluated in greenhouse conditions. Recombinant AhCPI obtained in *E. coli* was applied to the soil around the stems of thirty-day tomato plants. Five days after treatment, plants were inoculated with about 10,000 *M. incognita* eggs per pot, and three subsequent AhCPI applications to tomato plants were made every month for 3 months. Non-treated plants, only AhCPI-treated plants, and only *M. incognita*-infected plants were included as controls. The experimental design was completely randomized with four treatments with five plants per treatment. Results showed that the three applications of 10 mL of AhCPI (1.4 mg /mL) to *M. incognita*-infected tomato plants, reduced the number of galls by 93 ± 8%, as compared to the control *M. incognita*-infected plants. In addition, the application of AhCPI to the infected plants increased the yield (10.7%) of harvested tomato fruits, as compared to infected plants. These results show the potential of AhCPI for the control of *M. incognita* in tomato plants.

Transcriptional profiling of Arabidopsis *polyamine oxidase 1* mutant and overexpression line in interaction with *Pseudomonas syringae*

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Polyamine catabolism by polyamine oxidase enzymes mediates signaling and stress responses through hydrogen peroxide production. Five genes encode polyamine oxidases in the *Arabidopsis thaliana* genome, with cytoplasmic and peroxisomal localization. Recently, we analyzed the phenotype of *Atpao* single mutants in response to *Pseudomonas syringae* pv. *tomato* DC3000 and identified that the *Atpao1-1* single mutant was resistant to bacterial infection. This mutant line has lower spermine oxidation activity, accumulates reactive oxygen species (hydrogen peroxide and superoxide anion radical), and shows increased RBOH activity and alterations in the activity of different antioxidant enzymes. In addition, several markers of the salicylic acid pathway were found to be deregulated in the mutant line in comparison to the WT (*A. thaliana* ecotype Columbia). All these data suggested that cytoplasmic polyamine oxidation through *AtPAO1* impacts hydrogen peroxide production and hormonal signaling. To further understand the participation of the *AtPAO1* gene in plant defense the *Atpao1-1* mutant and a *35S::AtPAO1* overexpression line were sequenced by RNAseq. Herein, we present an analysis of the *Atpao1-1* mutant and *35S::AtPAO1* transcriptomes under control and stress conditions (24 hpi with *P. syringae*). In the WT, biological processes such as the jasmonic acid mediated signaling pathway, plant-type hypersensitive response, glutathione metabolic process, and response to hydrogen peroxide, were up-regulated in response to the pathogen while in the *Atpao1-1* mutant, these processes were down-regulated 24 hpi. In accordance with previous observations, the ethylene synthesis and signaling pathway was repressed in the *Atpao1-1* mutant line in control and stress conditions. Furthermore, the auxin and cytokinin signaling pathways were deregulated in the mutant *Atpao1-1* mutant and a *35S::AtPAO1*. The participation of polyamine oxidation in plant defense and in the modulation of hormonal response in discussed.

EFFECT OF LIPOPOLYSACCHARIDES FROM *Azospirillum baldaniorum* Sp245 ON THE FUNCTION OF PHOSPHOLIPASE D AND TOR PROTEIN IN *Arabidopsis thaliana*

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Lipopolysaccharides (LPS) are amphiphilic molecules. They present in their structure anhydrophilic región with polysaccharide chains and a lipid region that anchors to the outer membrane of Gram-negative bacteria. It has been reported that LPS from the rhizobacteria Azospirillum baldaniorum Sp245 participate in the stimulation of plant growth and development. In Arabidopsis thaliana this increase is related to an increase in the expression of the TOR (Target of Rapamycin) protein, which regulates cell growth and development through the integration of various environmental signals. On the other hand, phospholipase D (PLD) metabolizes phospholipids in the cell membrane to produce phosphatidic acid, a second messenger that activates signaling pathways related to developmental processes. In the present investigation, the effect of A. baldaniorum LPS on the activity of PLD and its relationship with the TOR protein during the growth promotion of A. thaliana is studied. Enzymatic assays showed an increase in PLD activity after 5 min of interaction with LPS. Furthermore, an increase in the production of phosphatidic acid was detected by TLC. Finally, through histochemical assays with the TOR::GUS reporter, an increase in TOR expression was observed in plants treated with LPS and with different molecular types of phosphatidic acid.

Bioinformatic characterization of the cellulose synthase gene family in tomato (*Solanum lycopersicum*) reveals the involvement of *SlCslD2* during arbuscular mycorrhizal symbiosis.

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The cellulose synthase (*CesA*) and the cellulose synthase-like (*Csl*) genes belong to glycosyltransferases 2 family, which are involved in cellulose and hemicellulose biosynthesis, respectively. *CesA* and *Csl* are essential for the structural integrity of plant cell wall (CW). Symbiosis with Glomeromycota fungi induces a systemic response in plants that regulates the expression of many genes, in which CW modification genes, including *CesA* and *Csl,* are essential to trigger a priming mechanism that improves defense against pathogens. However, the complete characterization of all *the CesA* family members in *Solanum lycopersicum* and their expression under symbiotic conditions are limited. In this work, we identified 16 *CesA* and 20 *Csl* genes in the tomato genome bioinformatically based on the presence of the PF03552.16 domain. These genes were grouped in one CesA subfamily and four Csl subfamilies (SlCslB, D, E, G). The majority of SlCesA and SlCsl proteins were located in the Golgi membrane. Promoter analysis showed that some members of *SlCslD* subfamily have MeJA and stress-responsive elements. *In silico* analysis revealed that for some genes, expression is ubiquitous, whereas, for others, it is tissue specific. During pathogenic interactions with *Sclerotinia sclerotiorum* and *Botrytis cinerea* the expression levels were differential for *CesA* and *Csl* genes; *SlCslD2* was the only gene that showed a higher expression level in response to AM symbiosis by RT-qPCR. The 3D predicted protein showed that *SlCslD2* uses D-mannose as donor substrates, which could be responsible for the chemical modifications of CW, which was consistent with the sugar analysis by HPLC in colonized leaves. We performed a comprehensive analysis of the *SlCesA* and *SlCsl* gene families, which lays the foundation for future studies at a functional level and their role in cellulose and hemicellulose biosynthesis in tomatoes. This work was funded by CONACYT (CB A1_S_31400) and SIP-IPN (20232056, 20230746) (RGB (14, 16, 26)).

The *Xyloglucan xylosyltransferase* gene family: bioinformatic characterization reveals its involvement during arbuscular mycorrhizal symbiosis in *Solanum lycopersicum.*

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Plant cell wall consist of cellulose, hemicellulose, pectin, lignin, and proteins, which confer mechanical properties to plant structures. Hemicellulose is a principal constituted by xyloglucan (XyG), a molecule composed of glucan backbone decorated with α-(1,6)-xylose, α-(1,2)-galactose or α-(1,2)-fucose. XyG biosynthesis involves a diversity of glycosyltransferases (GTs). GT family 34 (GT34) belongs to xyloglucan xylosyltransferases (*XXTs*), which transfer UDP-xylose for the XyG backbone. Arbuscular mycorrhizal (AM) symbiosis is an important interaction between most plants and the Glomeromycota fungi. In AM plants, many genes are differentially regulated, both in roots and leaves, and it has been reported that AM plants respond more effectively and faster against pathogens, a phenomenon known as "priming". Glycosylation is important in enhancing plant resistance to abiotic and biotic stress, by modifying the cell wall composition. However, the characterization of the GT34 family and its relation to the priming during AM symbiosis is still unclear in *Solanum lycopersicum.* We identified five GT34 genes (*SlXXT1-5*) based on the presence of the GT34 domain (PF05637). Gene structure analysis and the identification of the conserved catalytic domain in XXT proteins supported this idea. Synteny and phylogenetic relationship analysis of SlXXTs and other model plants were also evaluated. *In silico* expression profiles showed that *SlXXT* genes have differential expression patterns in several tissues and on pathogenic interactions. The relative expression by qRT-PCR revealed that *SlXXT1* and *SlXXT2* genes display differential expression levels in *S. lycopersicum* leaves and roots in response to AM symbiosis. Finally, 3D predicted protein analysis corroborated that XXT members uses UDP-xylose as donor substrate, in which xylose concentration significantly differed under symbiotic conditions in *S. lycopersicum* leaves. All these results provide a comprehensive understanding of *XXT* genes as priming-related genes induced by AM symbiosis. This work was funded by CONACYT (CB A1_S_31400) and SIP-IPN (20232056, 20230746).

N-acetyl transferases are involved in Arabidopsis response to *Pseudomonas syringae* infection.

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The study of polyamine metabolism under biotic stress has recently gained significant relevance. It has been demonstrated that putrescine (Put) biosynthesis is induced during infection, using arginine as a primary metabolic precursor in *Arabidopsis thaliana*. Put is a central molecule in polyamine (PA) metabolism because it is the primary precursor for synthesizing higher PAs, such as spermidine and spermine. Put can also be acetylated to form N-acetyl putrescine (AcPut) by the activity of N-acetyl transferases. In mammals, polyamine acetylation is crucial for their catabolism and transport outside the cell. However, PA acetylation's role in plants, especially related to plant-pathogen interaction, remains unknown.

In *Arabidopsis thaliana*, two genes have been identified that encode for N-acetyl transferases: *AtNATA1* and *AtNATA2*. It has been reported that *At*NATA1 catalyzes the acetylation of Put to AcPut and the acetylation of ornithine and 1,3-diaminopropane, a terminal product of PA oxidation. Regarding biotic stress, only one study suggested that the activity of *At*NATA1 negatively affects Arabidopsis resistance to *Pseudomonas syringae* (*Pst*) by suppressing antimicrobial defenses. However, the role of *At*NATA2 regulating *Arabidopsis* response to *Pst* remains unknown.

Our study aimed to characterize single mutant lines of *Atnata1-1* and *Atnata2-1* during *Pst* infection, with and without Put supplementation. We employed a non-invasive high-throughput screening (HTS) method based on RGB and chlorophyll fluorescence images from which the Plant Biostimulant Characterization (PBC) index was calculated as a quantitative measure of phenotypic traits. As the most remarkable result, both mutants presented bigger rosettes than wild-type plants. Additionally, *Atnata1-1* exhibits resistance to *Pst*, and the Put supplementation further improved it. Moreover, the *Atnata2-1* mutant line exhibited the most favorable response with or without *Pst*, and Put supplementation. Altogether, the results pointed to the regulation of Put levels via N-acetyl transferases as an essential step in defining *Arabidopsis* resistance to *Pst.*

Unraveling the polyamine back-conversion catabolism enigma: the role of AtPAO2 in Arabidopsis resistance to *Pseudomonas syringae* infection

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Polyamine catabolism, orchestrated by polyamine oxidase enzymes, is crucial in plant defense against pathogen infection. In *Arabidopsis*, the cellular localization of PAO enzymes (cytoplasm or peroxisomes), the type of catabolism (terminal or back-conversion), and the relation to other signaling pathways through the production of reactive oxygen species make polyamine oxidation relevant to understand plant-pathogen interactions. However, the specific mechanisms and role of polyamine oxidation remain elusive. In this study, we characterized the role of the *AtPAO1* and *AtPAO2* genes in the Arabidopsis-*Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) interaction employing PAO single and double mutant lines (*Atpao1-1*, *Atpao2-1,* and *Atpao1-1 x Atpao2-1*) and overexpression lines (*35S::AtPAO1*) by combining high-throughput phenotyping screening, targeted metabolomics, and gene expression analysis. Our results demonstrated that *At*PAO1 regulates *Arabidopsis* resistance by modulating the RBOH (respiratory burst oxidase homolog) activity in the presence or absence of *Pst*, and that response is influenced by spermine supplementation.
Furthermore, we reveal that resistance to *Pst* relies on the fine tune balance between *At*PAO back-conversion and terminal catabolism, which significantly affects the overall *At*RBOH activity and the content of various polyamines. Interestingly, we found that peroxisomal polyamine catabolism, mainly through *At*PAO2, is the primary regulator of defense against *Pst*. Specifically, *At*PAO back-conversion facilitates putrescine accumulation, reduction of spermine and conjugated/bound polyamines levels, and increase of specific amino acids, including glutamate, acetylornithine, γ-aminobutyric acid, β-alanine, proline, and glycine. These metabolic changes ultimately improve plant resistance. Our study indicates that *Pst* resistance depends on the plant strategy. Whereas a strong upregulation of terminal catabolism (mainly by cytoplasmic *At*PAO1) to produce 1,3-diaminopropane can condition plant resistance, Spm catabolism via back-conversion (by peroxisomal *At*PAO2), which conduct to putrescine production, confer resistance.

Role of the root cap in the interaction between *Achromobacter* sp. 5B1 and *Arabidopsis* that modulates root growth direction

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The root cap is a multilayered tissue that assists the root in exploring the soil. Its location at the distal end of roots protects the meristem and directs root growth toward gravity and nutrients. Although the root cap maintains a close interaction with the rhizosphere, its role in signaling with soil microorganisms is unknown. Here, through direct root cocultivation with *Achromobacter* sp. 5B1, we show that specific elements in the development of the root cap control the direction of growth. In *Arabidopsis* seedlings with mutations in the transcription factor FEZ, the rhizobacterium caused susceptibility of roots to form supercoils, whereas in seedlings harboring mutations in *SOMBRERO* the change in root growth direction did not occur. These effects coincided with expression analysis of *FEZ::FEZ:GFP* and *SMB::SMB:GFP* in the root cap. Measurements of the root cap and amyloplast content revealed that interaction with *Achromobacter* sp. 5B1 modifies the structure of the root cap; increases the volume of the tissue and alters the accumulation of amyloplasts in columella cells. In addition, wild-type seedlings, and *fez-2* and *smb-3* mutants inoculated with other plant growth-promoting bacteria did not modify the direction of root growth, indicating specificity in the response. Our data indicate that the root cap senses the rhizobacterium *Achromobacter* sp. 5B1 and contributes to the deviation of root growth forming turns, coils and branches that help plants to more efficiently explore the soil.

Phenotype assay of miR161 and its target in *Arabidopsis thaliana* during the interaction with the mutualistic symbiotic fungi *Serendipita indica*

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Serindiptica indica (syn. *Piriformospora indica*) is a filamentous root endophyte fungus that belongs to the order *Sebacinales* (Basidiomycota). *S. indica* colonizes the root epidermal and cortex cells without penetrating the central cylinder and displays a biphasic colonization strategy. During the initial phase of biotrophic colonization, the fungus invades the root cells inter- and intra-cellularly. Subsequently, *S. indica* switches to a host cell death-associated phase, although a defined switch to necrotrophy with massive cell death does not occur. *S. indica* colonization exhibits various effects on host plants including enhanced growth, improved assimilation of nitrate and phosphate, increased tolerance to abiotic stresses, and resistance against pathogens. Has been reported that *S. indica* establishes symbiotic interactions with a wide range of plant hosts, including monocots plants like orchid, rice; and also the dicot model plant *Arabidopsis thaliana*, nevertheless there are a few information about the posttranscriptional regulation, specifically from microRNA in the colonization of *S. indica* in the root of *A. thaliana.*
Previously, our team performed an analysis to elucidate the microRNA and their targets differential expressed in the early stages of the colonization of *S. indica* in *A. thaliana.* In this study, we found that the mir161 and its target, the gene AT5G55840 belonging to the pentatricopeptide repeat (PPR) proteins, were differentially expressed, and degraded, respectively 3 days after inoculation with the fungus. To further characterize the role of miR161 and its target, we are performing a phenotyping analysis in mutants seedlings from miR161 and AT5G55840 during the interaction of *S. indica,* using a spore solution of the fungus to inoculate the root with a constant spore concentration and documenting the reported early stages of colonization for *S. indica* in *A. thaliana* roots.

The cilantro (*Coriandrum sativum L.*) from Puebla and analysis of the presence of *Cyclospora cayetanensis*

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The fresh produce trade between Mexico and the United States has increased due to the demand for nutritional and safe foods. However, since 2014 the Food and Drugs Administration of the United States (FDA) implemented Import Alert 24-23, which prohibits the entry of cilantro (*Coriandrum sativum* L.) from Puebla, Mexico. This is due to the previous association of fresh cilantro consumption imported from Puebla with outbreaks of cyclosporiasis (diarrheic illness caused by the protozoa parasite *Cyclospora cayetanensis*) within the USA from 2012 to 2015. Yet, it is proposed that humans are the only host for this parasite. It is well known that contaminated food and water are vectors of transmission of oocysts. The knowledge about *C. cayetanensis* biology, reproduction, ecology, and molecular mechanism regulating its life cycle needs to be improved and better understood. The present research aims to find the principal source of contamination for the cilantro with *C. cayetanensis* oocysts using Real-Time PCR following the protocols described in the Bacteriological Analytical Manual (BAM) of FDA. To identify the origin of the contamination, we plan to analyze potential sources of oocyst, such as soil, water, seeds, and wild fauna commonly found in the production zone of cilantro. Furthermore, we intend to contribute to the knowledge of cilantro ecology by performing a metagenomic study of the cilantro rhizosphere in plants grown under several conditions. MAVL thanks CONACYT grant A1-S-35357, AAB and MAVL thanks to SIP and COFFA IPN funds, LLA thanks to CONAHCYT, COFAA and BEIFI IPN fellows.

Pseudomonas aeruginosa LasI-dependent plant growth promotion requires the host nitrate transceptor AtNRT1.1/CHL1 and the nitrate reductases NIA1 and NIA2

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Cross-kingdom communication with bacteria is crucial for plant growth and productivity. Here, we show a strong induction of genes for nitrate uptake and assimilation in *Arabidopsis* seedlings co-cultivated with *P. aeruginosa* WT (PAO1) or *ΔlasI* mutants defective on the synthesis of the quorum-sensing signaling molecule *N*-(3-oxododecanoyl)-*L*-homoserine lactone. Along with differential induction of defense-related genes, the change from plant growth repression to growth promotion upon bacterial QS disruption, correlated with upregulation of the dual-affinity nitrate transceptor CHL1/AtNRT1/ NPF6.3 and the nitrate reductases NIA1 and NIA2. *CHL1-GUS* was induced in *Arabidopsis* primary root tips after transfer onto *P. aeruginosa ΔlasI* streaks at low and high N availability, whereas this bacterium required high concentrations of nitrogen to potentiate root and shoot biomass production and to improve root branching. *Arabidopsis chl1-5* and *chl1-12* mutants and double mutants in NIA1 and NIA2 nitrate reductases showed compromised growth under low nitrogen availability, and failed to mount an effective growth promotion and root branching response even at high NH_4NO_3. WT *P. aeruginosa* PAO1 and *P. aeruginosa ΔlasI* mutant promoted the accumulation of nitric oxide (NO) in roots of both the WT and *nia1nia2* double mutants, whereas NO donors SNP or SNAP did not improve growth or root branching in *nia1nia2* double mutants with or without bacterial cocultivation. Thus, inoculation of *Arabidopsis* roots with *P. aeruginosa* drives gene expression for improved nitrogen acquisition and this macronutrient is critical for the plant growth promoting effects upon disruption of the *LasI* quorum-sensing system.

Contransting *Arabidopsis* develomental effects of *P. brassicae* and *P. chlororaphis* under Phosphate deficiency

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Bacteria have interacted for millions of years with plants by modulating positive aspects such as the host's hormonal status, nutrient solubilization, and root growth or by producing negative virulence factors that affect plant health. In this work we compare the effects of the inoculation of 2 bacteria belonging to the genus *Pseudomonas* that share the property of solubilizing phosphate. Despite the fact that nutrient solubilization can be considered a desirable trait for the promotion of plant growth, the effects that each of the bacteria have in *Arabidopsis thaliana* plants are highly contrasting, while *P. brassicae* increases the biomass accumulation of both shoot and root systems, *P.chlororaphis* does not. To find out if *Pseudomonas* isolates could modify root architecture, *Arabidopsis* seedlings were inoculated with pure cultures of the bacteria, where we obtained that both species inhibit the growth of the primary root; however, inoculation with *P. brassicae* increases the formation of lateral roots while inoculation with *P. chlororaphis* does not. As it is believed that a greater formation of lateral roots is associated with an auxin response, we decided to evaluate the expression of the auxin inducible gene *DR5:GUS* in roots, where interestingly *P. brassicae* did not present an increase in the expression of this gene compared to the control plants, instead plants inoculated with *P. chlororaphis* cause a sharp increase in auxin levels within the plant. Lateral roots are involved in plant adaptation to challenging environments, for this reason, we compared the expression of the cell cycle reporter line *CYCA3:GUS* in lateral and primary roots of *Arabidopsis* plants inoculated.
Inoculation of *P. brassicae,* but not *P. chlororaphis,* improved shoot and root growth in medium supplemented with calcium phosphate as the sole Pi source. Collectively, our data indicate the high potential of *P. brassicae* to manage agriculture in a more eco -friendly manner.

Gene expression related to biotic stress by application of peroxyacetic acid in *Capsicum annuum* L and induction of geminivirus resistance.

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In this work, a strategy for the induction of resistance to geminivirus in *Capsicum annuum* was proposed and evaluated through the application of peroxyacetic acid as an inducer. Peroxyacetic acid was applied in a foliar way, directly to the substrate and a combined application to previously inoculated chili plants by bioinjection with a mixture of geminivirus, PEPGMV and PHYVV. The applied applications of peroxyacetic acid were divided into 0 ppm, 80 ppm, 100 ppm, 120 ppm and 140 ppm, after 17 days the plants treated for the symptoms of the disease and at that time the sample was taken. Each treatment and the response to the oxidative stress generated by the plant was processed to the measurements of the analysis solution, PCR was detected to follow the viral mobility within the plant, likewise the differential expression of the PAL genes was evaluated by PCR, CAT, NPR1, PR1, MnSOD and β-ACT. In addition to the above, phenological characteristics such as stem diameter, plant height and leaf area by treatment were evaluated.

Exploring Secondary Metabolite Profiling in Opuntia Cultivars: Insights into Resistance Mechanisms Against Dactylopius coccus Costa

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Cactus pear (Opuntia spp.) is a vital source of livelihood in arid and semi-arid regions globally. However, it faces challenges from both abiotic and biotic stressors, with cochineal (*Dactylopius spp.*) being recent a significant threat. Mismanagement of domestic cochineal (*Dactylopius coccus*) in some areas has worsened the problem, impacting cactus pear productivity. Using resistant varieties shows promise for controlling cochineal infestation, but resistance traits vary across varieties and environmental conditions. Understanding the basis of resistance traits is crucial for effective breeding strategies.

To study metabolic profiles in response to cochineal infestation, we analyzed three resistant and three susceptible cultivars using untargeted DLI-ESI-mass spectrometry, and analyzed the obtain metabolic features by unsupervised and supervised learning clustering methods PCA and PLS-DA. Out of the 376 mz features detected by DLI-ESI, we identified 81 relevant features distinguishing infested and non-infested cladodes, with 28 induced by *D. coccus* infestation, potentially related to defense responses. We define 49 features involved in a basal response to domestic cochineal for all cultivars, highlighting conserved responses among the different cultivars, and 22 features specifically relevant for defense responses on resistant cultivars, which ultimately determine the effectiveness of their response in countering the herbivore's attack. Furthermore, by comparing mature and young cladodes and their relationship to induced responses, we identified 11 features which may explain why infestation by *D. coccus* does not occur typically on these tissues.

Overall, this study provides insights into the metabolic plasticity of Opuntia cultivars in response to herbivores, shedding light on its defense mechanisms. Understanding these mechanisms provides valuable information for the development of sustainable strategies to protect cactus pear crops from the menace of *D. coccus* infestation and to enhance food security in arid regions.

Keywords**:** *Opuntia spp.***,** Cactus pear, (DLI-ESI)-mass spectrometry, metabolic profile, plant resistance, Arid crop plants, domesticated cochineal, *Dactylopius coccus*

Identification and functional analysis of genes involved in symbiotic interactions through comprehensive approaches in the model legume *Lotus japonicus*

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The legume family is composed by numerous species of agricultural relevance such as chickpea, soybean, peanuts, beans, pea, among others. Additionally, legumes play a relevant role in their ecosystems, by establishing mutualistic associations with soil microorganisms such as arbuscular mycorrhizal fungi and rhizobia. These microorganisms provide phosphorous and nitrogen resources to the legume host in specialized organs called arbuscules and nodules, respectively. These interactions are regulated by complex genetic networks and through chemical dialogues between the legumes and their partners. To fully understand these processes is crucial to identify and characterize the genes involved in these interactions. The model legume *Lotus japonicus* (*Lotus*) has been extensively used to study the legume-microbe associations, since valuable biological resources have been developed for this organism, for instance the *Lotus*-retrotransposon (LORE1) mutant population and the abundant transcriptomic data of roots colonized by rhizobia. In our group we have combined several approaches to exploit this useful data and biological material. We performed a large-scale mutant screening in 200,000 LORE1 mutants to assess their nodulation capacity with rhizobia. After additional screenings, the symbiotic phenotype was confirmed for 100 different mutant lines. A flanking sequence tag pooling and identification (*FST poolit*) protocol was followed to track the location of the novel transposon insertions in the genomes of 40 mutant lines, revealing candidate genes affected by these transposable elements. In addition, by analyzing the transcriptome response of *Lotus* roots inoculated with different rhizobial species, we found that several cell wall- and metabolic-related genes were upregulated. For several candidate genes we have obtained homozygous mutants and constructs to monitor their promoter activity and subcellular localization during *Lotus*-microbe associations by confocal microscopy. These approaches unveil novel players required for mutualistic interactions in legumes.

Expression analysis of the *PR-1* gene in CRISPRa-edited tomato plants in combination with the use of acibenzolar-S-methyl and in response to pathogens

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Tomato (*Solanum lycopersicum*) is an important vegetable in Mexico, being the first exporter worldwide. However, this crop has been greatly affected by bacteria such as *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), a pathogen against which there are still no commercial resistant varieties. Currently, alternatives to the use of pesticides include, for example: (a) the use of agrochemicals, e.g. synthetic analogs to the salycilic acid (SA), which promote "defense priming", an increased readiness of defense induction against pathogens; (b) epigenetic edition via CRISPR-activation (CRISPRa). CRISPRa allows the upregulation of target endogenous gene expression levels (like Pathogenesis-related genes, *PRs*), thanks to the use of transcriptional activators, such as the synthetic activator domain VP64 (M12) or the histone lysine methyl-transferase SET-domain (ST4). We hypothesized that there is a synergism amongst the application of acibenzolar-S-methyl (ASM, a priming activator compound) and the use of epigenetically edited tomato plants for the *PR-1* gene, against pathogens like Cmm. Thus, our main goal was to analyze *PR-1* gene expression and plant resistance against Cmm, in CRISPRa-edited plants and in combination with the use of ASM. Our results show that 6 h after pathogen infection there is an increase in *PR-1* transcript levels in edited and ASM-treated tomato plants, in contrast to the control and WT plants. Furthermore, we detected reduced leaf damage and disease severity (M12=80%, ST4=90%. WT=60%) in edited plants, when compared to our controls. We conclude that CRISPRa-edited tomato plants treated with ASM show a synergism and developed greater resistance against Cmm.

Down-regulation of a *Phaseolus vulgaris* aquaporin Pvpip2-4 impairs the nodulation

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Plant aquaporins are a large family of proteins that function as transporters of solutes (water, sugar, NH4, etc.) and that play important roles in several physiological processes in living organisms. On the other hand, hydrogen peroxide (H_2O_2) levels and transport have been related with plant growth, development, and biotic and abiotic stress responses. It has been proposed that aquaporins can also transport H_2O_2, regulating its subcellular distribution, and thus, the strength of de associated signaling. However, little is known about this process. It´s well known that reactive oxygen species (ROS) are highly involved in polar growth, as well as during mutualistic interactions such as mycorrhizal or rhizobia-legume associations. ROS generated in the apoplast by NADPH oxidases and SOD activity, such as H_2O_2, need to be transported from the extracellular side to the cytoplasm. However, we know little about this process.
The functional role of aquaporins in *P. vulgaris* and their potential role to transport H_2O_2 in root hairs during the polar growth and during rhizobia-legume interaction, has been poorly studied. In this study we determined the role of *PvPIP2-4*, a gene encoding for an aquaporin that could be involved in the H_2O_2 transport. By silencing and overexpression of the gene in *Phaseolus vulgaris*, we have also evaluated the effect on the nodulation process. We have found that *PvPIP2-4* depict an early increased transcript accumulation in roots inoculated with *Rhizobium tropici* CIAT899; however, at later stages, the level of transcript decreased considerably. Results describing the subcellular localization, nodulation, and nitrogen fixation phenotype, will be presented and discussed.

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EFFECT OF MULTIVARIATE WALL CARBON NANOTUBES AND LIPOPOLYSACCHARIDES FROM *Azospirillum baldaniorum* Sp245 IN *A. thaliana* PLANTS´ GROWTH.

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Azospirillum baldaniorum Sp245 is a Gram-negative bacteria that belongs to a group of plant growth-promoting bacteria (PGPR). One of the components of its outer membrane is lipopolysaccharides (LPS). It has been reported that LPS from *A. baldaniorum* Sp245 modulates different aspects on the growth and development of *A. thaliana*, such as an increase in primary root length and number of lateral roots. On the other hand, multivariate wall carbon nanotubes (MWCNT) are a type of nanomaterial with a variable effect on plant growth that depends on the dose, the type of nanomaterial and the plant in question. For *A. thaliana*, MWCNTs are known to increase reactive oxygen species and the activity of some antioxidant enzymes. However, in other plant species, they stimulate germination and promote fruit growth. In the present investigation, we analyze the effect of MWCNT alone and conjugated with LPS isolated from *A. baldaniorum* Sp245 on the growth and development modulation in plants of *A. thaliana*. This is because it has been described that in animal systems MWNTC can bind to certain biomolecules, including LPS, and function in this way, among others, as biosensors. However, there is no literature with reports of said union for plant systems.

Common bean patatin-related phospholipases A (*PvpPLA-IIγ*) negatively regulate nodule development

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Patatin-related phospholipases A (pPLA) are plant enzymes that hydrolyze both phospholipids and galactolipids. These enzymes are involved in several biological processes such as the response to pathogens, vegetative growth, and cellular elongation. In particular, in legume symbiosis with arbuscular mycorrhizal fungi, a signal molecule released by the hydrolytic activity of pPLA (lysophosphatidylcholine), was reported to induce the expression of phosphate transporters. However, the participation of pPLA in rhizobial symbiosis has not been explored to date. This symbiotic interaction occurs between legumes and nitrogen-fixing bacteria called rhizobia, generating a new organ in the roots of legumes, the symbiotic nodule. In this work, we identified the pPLA family in common bean (*Phaseolus vulgaris* L.) and other legume models through in silico analysis. By using qPCR-based approaches, and reverse genetics we functionally characterized the common bean pPLA gene, *PvpPLA-IIγ*, in bean-rhizobia interaction. In silico analysis showed that the genomes of common bean and *Medicago truncatula* Gaertn. encode 21 and 16 *pPLA* genes, respectively, which are grouped into five clades. This differs from that reported in non-leguminous species such as rice and Arabidopsis. It is important to highlight that we observed gene duplication in both legume species, *PvpPLA-IIγ* being one of the duplicated genes; this evolutionary characteristic has not been reported to date in another plant model. Transient expression in *Nicotiana benthamiana* leaves revealed a cytosolic localization of PvpPLA-IIγ. Quantification of *PvpPLA-IIγ* transcript levels by qPCR indicated high expression in nodules at 14 days after rhizobia inoculation. Interestingly, overexpression of this gene significantly reduced the number of nodule primordia and nodules at 7 and 14 days after inoculation, respectively. Overall, our results show that legume pPLA families exhibit typical phylogenetic features not found in other plant species. In particular, *PvpPLA-IIγ* seems to function as a negative regulator in rhizobial symbiosis.

IDENTIFICATION AND CHARACTERIZATION OF COMMON BEAN (*Phaseolus vulgaris*) NON-NODULATING MUTANTS ALTERED IN RHIZOBIAL INFECTION (1)

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The symbiotic N_2-fixation process in the legume–rhizobia interaction is relevant for sustainable agriculture. The characterization of symbiotic mutants, mainly in model legumes, has been instrumental for the discovery of *ca.* 200 relevant symbiotic genes, but similar studies in crop legumes are scant (2). For common bean, only one symbiotic mutant has been molecularly characterized (3). To isolate and characterize common bean symbiotic mutants an ethylmethanesulphonate-induced mutant population from the BAT 93 (wt) genotype was analyzed. Our initial screening of *Rhizobium etli* CE3-inoculated mutant lines revealed different alterations in nodulation. We proceeded with the characterization of three non-nodulating (*nnod*), apparently monogenic/recessive mutants: *nnod*(1895), *nnod*(2353) and *nnod*(2114). Their reduced growth in a symbiotic condition was restored when the nitrate was added. A similar *nnod* phenotype was observed upon inoculation with other efficient rhizobia species. A microscopic analysis revealed a different impairment for each mutant in an early symbiotic step. *nnod*(1895) formed decreased root hair curling but had increased non-effective root hair deformation and no rhizobia infection. *nnod*(2353) produced normal root hair curling and rhizobia entrapment to form infection chambers, but the development of the latter was blocked. *nnod*(2114) formed infection threads that did not elongate and thus did not reach the root cortex level; it occasionally formed non-infected pseudo-nodules. The current research is aimed at mapping the responsible mutated gene for a better understanding of SNF in this critical food crop. Comparative whole genome sequence analysis led to identify six candidate mutated early symbiotic genes for each of *nnod* mutants. Mutants from other legumes, defective in our proposed candidate genes, showed similar phenotypic alterations as the ones we have observed (1, 2).

1. Reyero-Saavedra R *et al*. (2023). *Plants*. 12, 1310-1327.
2. Roy *et al.* (2020) *Plant Cell*. 32, 15-41.
3. Ferguson *et al.* (2014). *Plant Biotechnol. J.* 12, 1085-1097.

Symbiotic and non-symbiotic phenotype of mutants disrupted in *EXPANSINS* in the model legume *Lotus japonicus*

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Legumes can establish symbiotic relationships with nitrogen-fixing bacteria (rhizobia); these bacteria reside in specialized root organs called nodules. This symbiosis requires an organogenesis and infection program, and the latter can be either intra- or intercellularly. These mechanisms of symbiotic colonization induce the expression of genes related to the biomechanics of the cell wall, for example: genes encoding expansins.

In our group we found that mutants affected in genes encoding expansins of *Lotus japonicus* (*exp1*-1 and *exp2*-1) showed reduced nodule formation. Also, we detected strong promoter activity of *EXPANSIN1* at early events of the rhizobial infection process. Similarly, a EXPANSIN1-YFP protein was located in root-hair infected cells and nodule organogenesis, confirming its participation in the symbiotic process.

In this work we are evaluating the symbiotic phenotype of a second mutant allele for *EXPANSIN1* (*exp1*-2) and the non-symbiotic phenotype of the mutants *exp1*-1, *exp1*-2 and *exp2*-1.

We found that the *exp1*-2 mutant showed a significant reduction in the nodule number compared to the wild type, at 1-6 weeks post-inoculation with rhizobia, reflecting a comparable symbiotic performance to the mutant allele *exp1*-1. The non-symbiotic phenotype tested in plants grown in nitrogen-replete medium revealed that in the *exp1*-1 mutant, the aerial part was 25% shorter compared to the wild type, but the *exp2*-1 was not significantly affected in this parameter. However, these mutant lines didn't show any significant difference in the length of the main root respect to the wild type plants.

These results demonstrate that *EXPANSIN1* and *EXPANSIN2* are relevant players in the establishment of the legume-rhizobia symbiosis. In order to understand the interplay of these proteins in the symbiotic process, we are generating constructs to monitor the promoter activity and subcellular localization of EXPANSIN2 as well.

Reproductive capacity *in plant* of endophytic strains of *Bacillus thuringiensis*

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Bacillus thuringiensis (Bt) is a Gram-positive bacterium used in agriculture to control insect pests due to its ability to produce parasporal inclusions of proteinaceous nature that are toxic against insects. However, few is known about its ecology. Bt strains have been isolated from various habitats such as water, soil, air, dead insects, and plants, including the phylloplane, the rhizosphere, and very recently, from internal plant tissues as entophytic bacteria. In this work we demonstrated that Bt is a natural endophytic bacterium capable of reproducing within the plant. The vertical transmission of this strain was monitored through several generations and the toxicity presented in each of the generations was evaluated by performing semiquantitative bioassays with *Trichoplusia ni* neonate larvae. The HD-73 strain labeled with the *gfp* gene and the *Arabidopsis thaliana* model plant were used. Plants were inoculated in the rhizosphere with a suspension of the spore-crystal complex, which later was translocated to the upper internal tissues, verified by detecting the labeled strain from superficially sterilized leaves. This detection was observed through a series of generations, were CFU counts increased throughout each generation, indicating that the bacterium is vertically transmitted through the seed. To assess whether the toxicity was maintained, toxicity on *T. ni* neonate larvae were tested on plants from each generation, as well as in control plants (without inoculation). The number of living larvae showed no significant difference between treatments; however, the size of larvae varied significantly in the treated plants, while larger, uniform larvae were observed on the control plants, indicating that the vertically transmitted Bt was affecting their development. This work demonstrates that Bt can translocate and reproduce *in planta* and affect susceptible larvae throughout generations.

Lipid-producing green microalgae *Neochloris oleoabundans* responds to extracellular self-DNA as a damage-associated molecular pattern (DAMP)

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Damage-associated molecular patterns (DAMPs) are endogenous molecules coming from damaged cells, indicating an injury, and inducing stress responses on the neighbor intact cells. One of most reported DAMPs is extracellular self-DNA (self-DNA), which is extracellular DNA from the same species. Microalgae, as any other organism, are permanently exposed to factors that can potentially damage them. Our study model, *Neochloris oleoabundans* is a lipid-producing green microalga with high potential in industry. *N. oleoabundans* can respond to extracellular self-DNA application by increasing peroxidase enzymatic activity, phenolic compounds content and lipids content. However, mechanisms involved in lipids biosynthesis by extracellular self-DNA application remains unknown. The main objective of the present work is to characterize the *N. oleoabundans* responses to extracellular self-DNA regarding lipids production; at phenotypic, biochemical, and proteomic levels using microscopy analyses to determine oxidative stress, lipid and protein quantification, as well as proteomic approach through mass spectrometry analysis. Here we show that *N. oleoabundans* respond to extracellular self-DNA application by increasing the production of reactive oxygen species (ROS), as well as duplicating the lipid content at 24 h and 48 h after extracellular self-DNA application. Furthermore, we'll look for differential protein profiles between treated and control cells. Our results demonstrate that self-DNA application activates metabolic machinery in *N. oleoabundans,* shedding light on the biochemical mechanisms involved in this phenomenon on microalgae. This knowledge will contribute to understand mechanisms involved in this interaction, to improve the lipids production for biofuels and other important commercial products.

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Bacteria from amphibians' skin help plants to grow and protect themselves against the *Botrytis cinerea* pathogen.

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Various chemical agents have been used for years as plant growth stimulants. However, their frequent use has been questioned to their negative impact on the environment and human health. This has led to the search for new ecological alternative solutions such as biological control agents (BCA) or biostimulants, which can help in the promotion of growth plants and increase their immune system. Recently, bacterial communities have been discovered on the skin of frogs, which protect them from the pathogenic chytrid fungus *Batrachochytrium dendrobatidis* that has caused amphibian declines worldwide. However, it is unclear whether these bacteria can help to growth plants, and cure diseases caused by pathogenic fungi. To investigate this, a study was conducted to determine whether neotropical amphibian skin bacteria possess the ability to control the development of the pathogen *B. cinerea*. Through dual experiments, we identified three potential candidates for biocontrol activity. The compounds released by the bacteria were found to inhibit the germination process, and the inhibition was dose-dependent. The bacteria and filtrates also conferred a protection system in the model plant *Arabidopsis thaliana* against *B. cinerea* infection. Additionally, we found that bacteria can modify the root structure of *A. thaliana* and increase the growth of tomato plants. The results suggest that bacteria from amphibian skin may have excellent potential to control diseases caused by phytopathogenic fungi affecting plants, and act as natural biostimulants, providing an ecologically-friendly alternative to chemical agents.

Search of maize seed endophytes with antagonistic activity against *Fusarium verticillioides*

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Mexico produces over 22 million tons of maize (*Zea mays L.*) annually as this crop is the main caloric source, consumed in several cooked forms. Maize production is hindered by, both abiotic and biotic factors, and among the latter, *Fusarium verticillioides* is the main fungal pathogen in this crop. It is an ascomycete that can survive as an endophyte in maize tissue or as saprophyte in stubble. This pathogen causes seedling blight, stalk rot and ear rot, and synthesizes several mycotoxins that have deleterious effects on humans and animal. Fumonisin B1 is the main toxin produced by this fungal pathogen, acting as a virulence factor that promotes fungal colonization and as a contaminant in moldy corn. Because chemical control of this plant pathogen is not economically feasible, biological control agents offer an alternative. Here, we isolated several fungal and bacterial endophytes from maize seeds and identified them through microbiological and molecular techniques. Monosporic or single-colony cultures were obtained, and genomic DNA was purified for PCR amplification. Fungal isolates were analyzed by DNA sequencing of the ribosomal RNAs Internal Transcribed Sequence (ITS) and the following genus were identified: *Fusarium* sp., *Acremonium* sp., *Phialemoniopsis* sp., *Talaromyces* sp. For bacterial isolates, the 16S ribosomal RNA gene was amplified and sequenced. An isolate (CEL1) identified as *Bacillus subtilis* inhibited the growth of several *F. verticilliodes* strains in a plate assay. We are currently analyzing the metabolites excreted by this bacterium and investigating whether it inhibits fumonisin B1 production by *F. verticillioides.*

Analysis of resistance to *Clavibacter michiganensis* subsp. *michiganensis* in tomato plants epigenetically edited via CRISPRa/dCas for *WRKY29* gene activation

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To ensure global food security and adequate nutrition, increasing crop production and addressing agronomic constraints are critical. Tomato (*Solanum lycopersicum*) is the second most important horticultural crop globally. However, abiotic and biotic stresses limit its production. Accordingly, the most significant production losses in tomato are caused by the bacterium *Clavibacter michiganensis* subs. *michiganensis* (Cmm). Inadequate control measures of this pathogen can generate negative impacts on both the environment and human health.

Thus, we hypothesized that in epigenetically editing tomato plants, activation of gene expression of plant defense genes, via CRISPR-activation, promotes plant defense against pathogens.

Consequently, we fused the synthetic activator domain VP64 and the histone lysine methyl-transferase SET-domain, from ATX1, to the dCas9 (dCas9-VP64:SET, or TS3H construct), and the VP64 domain to dCas12 (dCas12-VP64, or LF9H construct), and used such vectors for biolistic transformation of tomato cotyledonary explants. Guide RNAs were designed to the promoter region of the *WRKY29* gene, which codes for a transcription factor related to plant development and defense processes. Plants were regenerated *in vitro*, characterized for the insertion and gene expression, and propagated.

Next, independent lines were inoculated with Cmm and the effect of pathogen infection on epigenetically edited tomato plants was determined. Our results indicate that in TS3H, expression of *WRKY29* was 9 times higher, when compared to WT (whereas in LF9H it was 7.9 times higher). Colony forming units per 100 mg of tissue were reduced in the edited plants (when compared to WT). Also, TS3H (62.5%) and LF9H (81.25%) had lower leaf damage and disease severity, than WT. Interestingly, the root area of TS3H (15.7 cm^2) and LF9H (12.7 cm^2) was larger than in WT (6.9 cm^2). Also, the edited plants had higher fruit production compared to the controls. In conclusion, CRISPRa is a promising biotechnological approach for developing Cmm-resistant tomato plants.

Bioinformatic characterization of the β-galactosidase gene family reveal its involvement during fungal interactions in *Solanum tuberosum*

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β-galactosidases (β-gal) are a family of plant proteins that belong to the glycosyl hydrolase 35 (GH35), that catalyze the removal of terminal galactose residues from carbohydrates, glycoproteins, and galactolipids in the cell wall of living organisms. In plants, β-galactosidases are involved in defense processes as a response to biotic or abiotic stress in several phenological stages, fruit ripening, as well as in the metabolic recycling of galactolipids and glycoproteins, and the renewal of signaling molecules during maturation. β-galactosidases have been characterized in fruit ripening, such as apple, strawberry, melon, and other plant species. Potato (*Solanum tuberosum*) is one of the most important crops in Mexico, and Sinaloa is the second largest producer during the fall-winter agricultural cycle. Although the β-gal gene family has been characterized in many plants, the knowledge about it in *S. tuberosum* is limited. In this study, the β-gal gene family was characterized using bioinformatics approaches. The results revealed that the β-gal family consists of 30 genes (*StBGAL*) through genome wide screening based on the PF00332.21 domain, which are closely related phylogenetically to Solanaceae species and *Arabidopsis thaliana*. This classification was also supported by gene structure and conserved motifs analysis. At the chromosomal level, we observed that the 30 β-galactosidases were heterogeneously distributed among all chromosomes across the potato genome. *In silico* expression profiles showed that the *β-gal* have differential expression patterns during pathogen attack, as well as under abiotic stress in roots and leaves. These results demonstrate that *StBGAL* genes are related to different stages of potato maturation and may be involved in defense processes to reduce susceptibility to pathogens by rearranging cell wall components.

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The role of *RKD* transcription factors in egg, zygote, and embryo identity in *Arabidopsis thaliana*

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Division of the zygote in angiosperms is asymmetric. After the zygote divides, the small apical daughter cell produces the spherical proembryo while the basal daughter cell forms the filamentous suspensor. A mitogen-activated protein kinase (MAP) cascade and a small number of transcription factors are known to promote elongation of the zygote and the first asymmetric division (Lukowitz et al., 2004; Bayer et al; 2009). One of these transcription factors is GROUNDED (GRD), a member of the RWP-RK family *(RKD)*. *GRD* acts downstream of the MAPKK Kinase *YODA*, and shows strong additive interactions with *wox8;wox9* transcription factor mutants, resulting in embryos that are arrest as zygotes or with only a few cells (Jeong et al., 2011). *GRD/RKD4* is one of five RKD transcription factors with transcripts in the egg cell, zygote, and early embryo. We will examine the expression of cell fate markers for early, mid, and late embryogenesis in *rkd* mutants, as well as generate novel CRISPR alleles of the *RKD* genes. Morphological and molecular analyses of higher order *rkd* mutants should broaden our knowledge of the role of these exciting transcription factors in regulating developmental programs in early embryogenesis.

Gene expression networks and auxin response during maize callus induction from explant tissues with contrasting embryogenic potential

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Embryogenic callus plays a crucial role in achieving successful indirect somatic embryogenesis (SE) in maize. The potential for embryogenesis depends on both the characteristics of the explant and the genotype. In order to gain a better understanding of the molecular mechanisms underlying these differences, this study investigated the transcriptomes of explants with varying embryogenic potential, specifically immature and mature zygotic embryos, as well as their corresponding induced calli. A particular focus was given to maize annotated transcription factors and functional enrichment analysis based on gene ontology annotations. The process of dedifferentiation in immature embryos exhibited enrichment in various pathways, including cell proliferation, oxidation/reduction, transcriptional regulation, metabolic processes, and plant hormone signal transduction pathways. Modules such as auxin response factors (ARFs), which are involved in auxin production and signal transduction (e.g., Homeobox; HB), as well as embryogenesis-related AP2-EREB, were particularly prominent in early embryos. On the other hand, stress-related factors governed the transcriptomes of mature embryos and their derived calli. An updated phylogenetic and structural analysis of maize, rice and Arabidopsis ARFs allowed to better correlate specific family members with the most contrasting expression patterns between embryogenic and non-embryogenic tissues. Notably, the accumulation pattern of activator and repressor ARFs during the initial stages of callus induction substantially differed between explants from immature and mature embryos. These data along with the global transcriptomic findings, contribute to unravel the molecular mechanisms underlying gene expression regulation in response to exogenous auxin during callus dedifferentiation from maize explants with varying embryogenic potential.

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Imprinted gene function in early *Arabidopsis* embryogenesis

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During double fertilization in Angiosperms, one sperm cell fuses with the egg cell resulting in a diploid embryo (one maternal and one paternal genome), while the second sperm fuses with a diploid central cell creating the triploid endosperm (two maternal and one paternal genomes). Imprinted genes are those genes which are primarily expressed from either the maternal or paternal allele, but not both. Hundreds of genes have been shown to be imprinted in the triploid endosperm. In the diploid embryo, few genes have been shown to be imprinted, and only one imprinted gene has been shown to have a function in the embryo.

We compared paternally biased genes in parent-of-origin sequencing experiments of early embryos from three different hybrid combinations: Col/Tsu, Col/L*er* and Col/Cvi. We reasoned that that genes which show paternal bias in two or all three of these hybrid combinations would be most likely to have biological functions that depend on imprinted expression. 11 genes were found to be paternally biased in zygotes and early embryos of at least two of these hybrids. These paternally biased genes are predicted to encode various biological functions. We are currently conducting phenotypic analysis of insertional mutants for these 11 genes, and validating their imprinted expression, with the aim of determining whether any of these putatively imprinted genes play important roles in early embryogenesis of Arabidopsis.

Exploration of the participation of *WOX5* pathway in the regulation of determinate root growth in Cactaceae.

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Most plant roots can grow indefinitely, i.e. present indeterminate growth, while cell proliferation occurs in the root apical meristem (RAM). However, the RAM of the primary root of most cacti is exhausted soon after germination and the root stops growing; this is, the root shows determinate growth (Dubrovsky, 1997, Shishkova *et al.,* 2013). RAM maintenance in *Arabidopsis thaliana* is controlled by different regulatory pathways, such as PLETHORA (PLT), SHORT ROOT-SCARECROW (SHR-SCR), and WUSCHEL-RELATED HOMEOBOX 5 (WOX5) transcription factor pathways. Loss-of-function *shr*, *scr* and double *plt1 plt2* mutants display quiescent center (QC) anomalies and RAM loss, that is, determinate growth of the primary and lateral roots. Furthermore, the introduction of *wox5-1* mutation on *shr*, *scr* and double *plt1 plt2* background accelerates RAM depletion (Sarkar *et al.,* 2007). *WOX5* is expressed specifically in the QC in *A. thaliana* and *Oryza sativa* roots (Kamiya *et al.,* 2003). To explore the participation of WOX5 ortholog(s) in determinate root growth in Cactaceae, we identified WOX-like sequences in the *de-novo* assembled transcriptomes of the primary root apex of two Cactaceae species, *Pachycereus pringlei* and *Carnegiea gigantea*; and extracted WOX protein sequences of *A. thaliana, O. sativa* and *Beta vulgaris*. The molecular phylogeny of the WOX proteins suggests that two possible *WOX5* paralogs, *WOX5a* and *WOX5b,* are present in Cactaceae genome. Transcriptomic data (Galván-Alcaraz *et al*., this meeting) reveal that both assembled *CgWOX5* paralogs include complete ORF and are expressed in *C. gigantea* root apex transcriptome. Genomic sequences of both *CgWOX5a* and *CgWOX5b* have one intron and two exons, similarly to that of *AtWOX5*. In addition, we identified two possible *WOX5* orthologues in *Lophocereus schottii, Selenicereus undatus,* and *Stenocereus thurberi* preliminary genomes.

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Transcriptional regulation of auxin and cell wall pathway genes by the BOL transcription factor

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Plants have two main stem cell niches, known as the shoot apical meristem (SAM) and the root apical meristem (RAM), where new organs are formed throughout their life. New organ formation is regulated and modulated by several factors, including phytohormones, transcription factors and the mechanical properties of the cell wall. In Arabidopsis, BOLITA (BOL), an AP2 transcription factor expressed in organ founder cells, transcriptomic analyses showed that, it affects bioprocesses such as hormone signaling, cell wall generation, expansion, and differentiation. One of the hormones that appears to be modulated by this transcription factor is auxin, which is responsible for cell wall acidification, leading to changes in cell wall structure and resulting in expansion and differentiation. How BOL regulates auxin and cell wall related genes, and what is their relevance in BOL function is unknown, in this work, we aim to qRT-PCR analyses confirm that alterations in the expression of this factor affect the accumulation of auxin and cell wall related transcripts, and to visualize the changes in auxin accumulation and cell wall changes in plants with increased or decreased expression. Moreover, we plan to analyze the genetic interactions between BOL and candidate auxin and cell wall target genes, and the effect of auxin and auxin transport inhibitors in the BOL over expressor and mutant.

CLE14 peptide impairs root tip regeneration and callogenesis in *Arabidopsis thaliana*

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CLE14 belongs into a family of plant secreted peptides that interact with leucine-rich repeat receptor-like kinase (LRR-RLK) receptors to orchestrate plant morphogenesis. Previous studies indicated that CLE14 plays an important role in cell division, phosphate homeostasis and senescence, but its specific involvement in cell fate determination and organogenesis remains largely unexplored. Here, through pharmacological, genetic and cell biology approaches, we show the critical roles for CLE14 in determining the balance between cell division and differentiation in root tip regeneration and callogenesis. Nanomolar concentrations of CLE14 or its overexpression in *Arabidopsis* represses primary root growth and triggers root branching and root hair formation. After resection of the primary root tip, *pCLE14:GUS-GFP* expression was located specifically at the cell layer adjacent to the cutting and at the outermost external cell layer of the root cap as the newly root cap formed. *cle14* mutants had comparable root tip regeneration when compared to WT seedlings, whereas *35S:CLE14* seedlings failed to regenerate the missing root tip after resection. The de-differentiation of tissue into proliferative growth was analyzed in WT, *cle14*, and *35S:CLE14* stem explants grown in callus-inducing media. The results showed comparable callus-biomass production for WT and *cle14*, but a dramatically reduced callogenesis for *35S:CLE14* explants. Our data show that CLE14 acts as a "brake" for root tip regeneration as well as callus formation.

Cell viability in *Arabidopsis* MEDIATOR 18 mutants increases under phosphate scarcity

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During their life cycle, plants experience various adverse conditions that affect the integrity of their genetic material, which could lead to the activation of programmed cell death. This phenomenon is manifested mainly in the apical meristems, highly proliferative areas flanked by the stem cell niche (SCN). Stress-induced cell death from DNA damage in the apical root meristem has been reported as a key factor for root growth and may be influenced by environmental factors. Recently, our group reported the function of the MED18 mediator complex subunit in root cell viability. In the present research, the effect of phosphate deficiency on cell death in the meristems of the *med18-1* mutant, deficient in the MED18 gene, under contrasting phosphate availability was studied. A comparative analysis based on the growth of the main root and the cell integrity of the meristem, shows that phosphate scarcity suppresses cell death in the *med18-1* roots. In conclusion, the results obtained in this research indicate that low phosphate concentrations influence viability processes and/or cell regeneration affecting in this manner the functioning of the root meristem.

Shedding light on mango (*Mangifera indica* L.) molecular ripening foundation by proteomics approach

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Mango is a tropical fruit of great economic importance worldwide even so, the knowledge about the molecular foundation of its maturation is scarce. Even though there are a wide variety of protein extraction methods, the characteristics of the recalcitrant tissues make it difficult to find the method to give us the greatest identification of proteins that would contribute to major information about the molecular and biochemical process of ripening. Thus, carried out a comparative scrutiny of two protein extraction methods, a phenol-based and a methanol/chloroform, and two peptide fractionation techniques the strong cation exchange and high-pH phase reverse. We conducted an SPS-MS3 and label free approach. We identified more than 3,000 proteins in the two ripening stages, from these we identified more than 1,000 differentially accumulated. Some of these proteins are associated with diverse biological processes such as response to oxidative stress, defense to bacteria, fatty acid biosynthetic process, and response to jasmonic acid; such as the proteins like coronatine-insensitive protein 1 (COI1, O04197) and mitogen-activated protein kinase (MPK4, Q39024), both involved with the jasmonate-regulated defense processes and the 12-oxophytodienoate reductase 2 (OPR2, Q8GYB8) which acts as an alternative and OPR3-independent pathway for jasmonic acid biosynthesis. The phenolic-based extraction method showed a better performance than the methanol/chloroform, likewise, the high-pH reverse phase peptide fractioning allowed the identification of a great number of proteins compared to the strong cation exchange. However, all methods tested in our study exhibited significant complementation, improving the protein identification coverage.

Proteomic analysis of mango fruit cuticle ripening by multiple search engines: The contribution of artificial intelligence-based algorithms

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Mango is one of the world's most cultivated tropical and subtropical fruits. However, there are losses of up to 30% in postharvest periods. During which time the fruit undergoes a series of molecular and structural changes, mainly in the cuticle, associated with critical physiological characteristics such as defence or response to physical, chemical, and biological stimuli, which impact shelf life. Unfortunately, limited information is available about the regulation and molecular dynamics that take place during the ripening of mango fruit, so this work analysed the proteomic profiles of the peel of two mango cultivars, Criollo and Tommy Atkins, the latter established as a high-quality cultivar for presenting better organoleptic characteristics and longer postharvest life. The samples were taken at zero and six days after harvest to extract total proteins using the phenol and methanol-chloroform methods; these extracts were analysed by isobaric labelling (TMTsixplex) and label-free quantitative proteomics, and the raw data generated by LC-MS/MS were processed with three algorithms for protein identification, SQUEST HT, INFERYS, and CHIMERYS via Proteome Discoverer v3.0. Our scrutiny allowed the identification of more than 6,000 proteins, representing ~25% of the protein-coding genes in the mango genome. Overall, CHIMERYS outperforms SQUEST HT and INFERYS in label-free quantification, but the opposite was observed with TMT. Amongst the differentially identified proteins between cultivars and maturation times, there are those related to the biosynthesis of jasmonic acid and cutin, fatty acids, and waxes, as well as those involved with alternative splicing processes and transcription regulators linked to the response to stress, these last two groups rarely reported in proteomic studies due to their low general abundance in different biological systems.

Protein-protein interactions between a tomato cofactor and transcription factors

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Multiprotein Bridging Factor 1 (MBF1) proteins are transcription cofactors that belong to the superfamily of helix-turn-helix proteins, present in all eukaryotes. MBF1 form a bridge between a transcription factor and the basal transcription machinery. In this work we focus on elucidating the role of MBF1 by evaluating protein-protein interactions with different transcription factors. We have demonstrated the interaction with at least three transcription factors by yeast two-hybrid analysis. In addition, we will characterize the transcription factors associated with MBF1 in plants. Bioinformatic analyzes suggest that these transcription factors are involved in development.

Identification of *PLETHORA* genes in *Mammillaria san-angelensis* (Cactaceae): from the novo whole genome sequencing to insights of root development evolution

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Plants in the subfamily Cactoideae exhibit determinate primary root growth and root apical meristem (RAM) exhaustion. Within this cactus subfamily, we propose the genus *Mammillaria* as a model system, given its relatively short life cycle, high diversity, and ease of reproduction. Yet, the exploration of primary root growth in *Mammillaria* is hindered by the lack of identified genes that could be regulating this developmental process. In this study we identified putative homologues of the *PLT* genes in *Mammillaria san-angelensis* using bioinformatic and molecular methods. Six PLT transcription factors have been described in *Arabidopsis thaliana* and play a key role in root development regulating processes including the maintenance of RAM. The whole genome of *M. san-angelensis* was sequenced using the PacBio long-read sequencing platform, and a bioinformatic pipeline was established through which we were able to isolate 19 candidate sequences. We used those sequences, as well as the putative homologues to the *A. thaliana PLT*s that could be found in *Selenicereus undatus* (Cactaceae), *Pachycereus pringlei* (Cactaceae), and *Beta vulgaris* (Amaranthaceae) to build gene trees. After discarding repeated sequences, we were able to identify the *M. san angelensis* homologues in the *PLT1/2, PLT3/7,* and *PLT5* subclades, but not *PLT4.* Using primers designed for these sequences we were able to validate by PCR 4 of these genes from *M. san-angelensis* gDNA and a total of 11 genes from cDNA belonging to different species of the *Mammillaria* genus. We discuss that the presence/absence of *PLT* genes as a biological phenomenon that could be important to explain root development and architecture in *M. san-angelensis*, thus providing the basic framework for the functional study of the *PLT* genes and their role in *M. san-angelensis* root development.

The transcription factor AIB/JAM1 regulates the sugar response during early development in *Arabidopsis thaliana*.

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Glucose (Glc) is able to modulate specific aspects of the growth and development of the plant, through its function as a signaling molecule. Global expression analyses have shown that Glc affects the expression of a large gene number. For the regulation at transcriptional level diverse *cis* regulatory elements (CREs), have been identified in promoters of sugar responsive genes. Through the analysis of the promoter region of the sugar regulated *STP1* (*SUGAR TRANSPORTER PROTEIN 1*) gene, it was found that a 310 bp region of its promoter contains the CREs for the Glc response. A closer analysis of this regulatory region of *STP1*, revealed the presence of CREs described in other sugar repressed genes; an AMY2BOX (*TATCCA*), a G-box-related element *ACGTG*, and the E-box variant *CACATG*. The transcription factors (TFs) that recognize the AMY2BOX are known, but not for the other two motifs. By a search in the literature, we found that the AIB/JAM1 (ABA INDUCIBLE bHLH/JASMONIC ASSOCIATED MYC2-LIKE1) TF recognizes the E-box variant *CACATG* in the jasmonic acid signaling and that acts as positive regulator in the abscisic acid (ABA) signaling. Due to the crosstalk between ABA and Glc signaling, we evaluated the participation of AIB/JAM1 in Glc response. We found that *jam1* mutants exhibited a Glc hypersensitivity phenotype, evidenced by a delayed germination and arrested development at low Glc concentrations, where the wild-type developed normally. A transcriptomic analysis of the *jam1-2* mutant vs wild-type at 4% Glc, demonstrated that 3,502 genes were differentially expressed in the mutant, including *STP1*. A chromatin immunoprecipitation assay evidenced that AIB/JAM1 binds to the promoter region of *STP1* in presence of Glc. These results suggest that AIB/JAM1 participate as a final effector in the sugar signaling pathway during early seedling establishment.

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A 200 bp deletion in *StEP* promoter leads to loss of self-pollen recognition in *Nicotiana plumbaginifolia*

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Pollen rejection in several species relies on the specific interaction of the *S*-locus products: The S-RNase (female determinant) and SLFs (male determinant). However, modifier genes (MG) are essential for a proper pollen rejection response. One MG deeply studied in our group is *NaStEP (Nicotiana alata Stigma Express Protein)*, a protease inhibitor with specific expression in the mature stigma of self-incompatible (SI) *Nicotiana* species. Self-compatible (SC) species, such as *N. plumbaginifolia,* encode this gene in their genome, but it is not expressed. The absence of *StEP* expression could be related to the loss of function mutations in the promoter region or any transcription factors (TFs). We cloned a sequence upstream of the *NaStEP* coding region (pNaStEP) from *N. alata* to test this hypothesis. We evaluated its promoter activity in *Arabidopsis thaliana* transformants with this putative promoter fused to *GUS* and *GFP*. Outcomes from T_3 transgenic *A. thaliana* lines indicate pNaStEP directs *GUS* expression in mature pistils. To evaluate if the lack of *StEP* expression in *N. plumbaginifolia* stigmas is because of mutations in TFs, we transformed *Nicotiana* hybrids with a *pStEP*::*GFP* construct. Results showed that this promoter correctly directs *GFP* expression to stigmas, indicating no alterations in TFs in this SC species. To determine if the *StEP* promoter from *N. plumbaginifolia* has loss of function mutations, we cloned this promoter and compared its sequences with that from *N. alata*. Results showed that the *NpStEP* promoter has a 200 bp deletion close to the transcription start site, which could account for *NaStEP* absence expression. Finally, to identify TFs regulating *NaStEP* expression, we conducted a yeast one-hybrid assay using different versions of the *NaStEP* promoter as a bait *vs. A. thaliana* TFs library from anther and pistil. We identified 18 TFs that bind to *pNaStEP* and direct reporter gene expression.

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Functional characterization of β-1,4-glucanases during gynoecium development

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Plant β-glucanases are enzymes involved in the synthesis, remodeling and turnover of cell wall components during multiple physiological processes. Notable examples are β-1,4,-glucanases, or cellulases, which degrade cellulose and other polysaccharides containing 1,4-glycosidic bonds to remodel and disassemble the wall during cell growth. Plant cellulases are classified in the GH9 family, which has been related with various physiological roles to plant growth, development, and interaction with the environment. During gynoecium development (the female reproductive structure of the flower), modifications occur in the cell wall that involve the synergistic action of hydrolase enzymes such as cellulases. In Arabidopsis and *Solanum lycopersicum* the expression patterns of two cellulases: *AtGH9B2* (Cel2) and *SlGH9B3* (TomCel4), have been described in early stages of gynoecium development. However, functional studies are still needed to verify the role of these cellulases during the development of the organs and tissues that make up the gynoecium. In this work, *Cel2* and *TomCel4* genes will be functionally characterized by generating loss of function and overexpression lines and together with their expression patters, we hope to elucidate the role of these cellulases during gynoecium development and whether there is redundancy or functional specificity between them.

Incidence and distribution of determinate growth of the primary root in species from the subfamily Cactoideae (Cactaceae).

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The family Cactaceae contains ~1,500 species distributed in America, mainly in arid zones. The Cactaceae family includes four subfamilies: Cactoideae, Maihuenioideae, Opuntioideae, and Pereskioideae; the first one concentrates approximately 80% of the total species of Cactaceae. Previous studies in the laboratory (Dubrovsky et al., 1997, Shishkova et al., 2013) revealed that most Cactaceae species exhibit determinate growth of the primary root, i.e. the root apical meristem (RAM) is active only for a short period after germination, and then all cells at the root tip differentiate. Consequently, the root stops growing. In this work we further extend the analysis of the type of primary-root growth, indeterminate or determinate, adding 107 analyzed species from the Cactoideae subfamily. For some of them primary-root growth kinetics were recorded. Our results show that all but one Cactoideae species analyzed until now exhibit determinate growth of the primary root. Furthermore, we mapped the natural distribution of the analyzed species to assess whether the determinate primary root growth is concomitant with arid and semiarid environments. Our results further suggest that the determinate growth of the primary root could represent an adaptative advantage in environments with severe water deficit. This hypothesis was supported even for epiphytic Cactaceae species, which despite growing in humid environments, have root systems exposed to air and therefore also experience water deficit. Together with previously analyzed species from the other three subfamilies, we have a record of primary-root growth type for 161 Cactaceae species, including 147 Cactoideae species.

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A comparative transcriptomics approach to uncover the genetic regulation of the determinate primary root growth in Cactaceae.

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Cactaceae is a plant family distributed along the American continent with high diversity and endemism in countries such as Mexico. Cactaceae is distinguished by remarkable anatomical and physiological adaptations; one of these is the determinate growth of the primary root, a developmental and genetically regulated program that implies root apical meristem (RAM) exhaustion. With the exception of a recently improved genome assembly for *Carnegiea gigantea,* Cactaceae genomic and transcriptomic resources are limited, with mostly low-coverage and highly fragmented genomes available. To explore the transcriptional regulation of root determinate growth, we sequenced and analyzed the transcriptome of the primary-root apex of *C. gigantea*, assessed differential gene expression in the initial (active RAM) vs terminal (exhausted RAM) stages of primary-root development, and found 499 differentially expressed genes. These results were compared to the root apex transcriptome of *Pachycereus pringlei*, a sister species of *C. gigantea*, sequenced at similar stages of root growth. Our results show that the transcriptional landscape of RAM exhaustion is conserved in *P. pringlei* and *C. gigantea*, exhibiting a correlation in gene expression pattern in more than half of the putative orthologs between these species. For the previously assembled root apex transcriptome of *P. pringlei*, around 40% of the contigs were not annotated. Therefore, we also explored the conservation of these sequences in *C. gigantea* to discard assembly artifacts and assess lineage specific transcripts. Our results showed that these sequences are present in Cactaceae genomes, and some of them are differentially expressed across root development, suggesting the involvement of lineage-specific transcripts in Cactaceae root determinate growth. Using comparative transcriptomics and genomics, we identified conserved genes, including putative lineage specific genes, presumably involved in the determinate growth of the primary root.

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Chromatin modification and the control of sexual reproduction in *Tagetes*.

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The genus *Tagetes* is widely valued for its ornamental, medicinal and industrial characteristics. In Mexico, the Cempasúchil is the best-known specie of the genus, it is cultivated mainly for its use in the celebration of the Day of the Dead, however, the high production of carotenoid compounds, as well as its secondary metabolites, have been a high value traits explored in the industry.

The reproduction of the majority of *Tagetes* species occurs through seed, constituting the most common way for its sowing and propagation. The commercial flowers of the Cempasúchil with high production of carotenoids and small-sized plants have been generated through domestication, selection and traditional genetic improvement, all strongly dependent on the sexual reproduction of the plants. However, in nature there exists varieties of Tagetes with reproductive problems, such as male sterility or seed abortion, such abnormalities can be acting as a barrier in the sexual reproductive cycle and a strong problem in obtaining hybrids. In Arabidopsis, several studies have related the role of chromatin modifications with the molecular control of reproductive development. In the genus tagetes, little is known about the reproductive system and seed development. In a preliminary cytological study in *Tagetes patula* and *Tagetes erecta* we found strong abnormalities in the formation of the female gametophyte, suggesting that sexual reproduction in these plants could be severely affected.

The objective of this project is to analyze the role of chromatin modification in the molecular control of reproductive development of *Tagetes patula* and *Tagetes erecta*. Based on cytological studies, immunolocalization analysis and gene expression we are seeking to improve the understanding about the sexual reproduction pathway of Tagetes, elucidating the origin of cytological abnormalities with deregulation at the molecular level, one of the largely unexplored research areas in this plant.

Which conformational changes are involved in S-RNase ribonuclease activity increase following the reduction of a conserved S–S bond?

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The RNase T2 family is one of three major groups of transferase-type RNases (endoribonucleases which cleave RNA through a 2'3'cyclic phosphate intermediate). T2 RNases are found on bacteria, protozoans, animals, plants, and viruses, and perform diverse biological roles, from housekeeping to tumor suppression. Studies on plant T2 RNases began with the discovery of S-RNases, a group of T2 RNases associated with self-incompatibility (SI) in flowering plants. Since then, many more plant T2 RNases have been identified. Phylogenetic studies divide plant T2 RNases into three classes: Classes I and II originated in green algae and angiosperms, respectively, while Class III (S-RNases) is exclusive of core dicots. All plant T2 RNases possess conserved structural features such as the conserved active-site segments (CAS I and CAS II) motifs, a core of hydrophobic residues in similar positions, and an $(\alpha + \beta)$ type structure, which in plants include 7-8 helixes and 7 strands. Plant T2 RNases conserve 8 half-cystine residues with 4 common to all T2 RNases and are thought as essential for structure-function. Previous studies in *Nicotiana alata* show that a thioredoxin involved in SI, NaTrxh, interacts with the S-RNase and reduces the conserved C155-C185 disulfide bond (numbered as in S_{C10}-RNase). This specific reduction results in a seven-fold increase in ribonuclease activity, which is essential for self-pollen rejection to occur. Since the activity increase in the S-RNase suggests conformational changes in the protein structure, we seek to uncover them by solving the structure of an S-RNase variant that simulates a reduced-by-NaTrxh state/conformation, i.e., S_{C10}-RNase(C155S/C185S). We are currently generating *N. tabacum* transgenic plants that constitutively express the S_{C10}-RNase(C155S/C185S) in order to purify and crystalize the protein for X-ray diffraction analysis. This will allow us to observe the conformational changes required for this activity increase that is essential for self-pollen rejection in *N. alata*.

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Transcription factors caracterization in *Utricularia gibba* trap development

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Utricularia gibba is an aquatic carnivorous plant that belongs to the Lentibulariaceae family. It is characterized by having a minute genome of approximately 100 megabases, the absence of roots, and its trap, which is capable of suctioning its prey. This mechanism is due to a release of stored elastic energy in the trap body, making it one of the fastest movements in nature and unique to the Utricularia genus, this adaptation has allowed the plant to thrive in diverse environments and develop new structures, such as the Bladderworts trap. Transcription factors play a crucial role as gene regulators, determining the physiology, morphology, and evolutionary trajectories in plants. While some studies have explored the genome and transcriptome, the specific transcription factors responsible for regulating the development of the trap in *Utricularia gibba* have yet to be identified. Thus, our focus in this research is to evaluate transcription factors that are exclusively expressed in the trap. Based on the annotation of *Arabidopsis thaliana*, we have selected four transcription factors known to be involved in development. Reporter lines were generated, along with overexpressing lines in Utricularia and Arabidopsis backgrounds. These lines allowed us to elucidate the role of these transcription factors, comprehensively assess their impact on trap development in Utricularia, and explore their potential implications for Arabidopsis development.

A tetratricopeptide-domain protein implicated in root elongation

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Arabidopsis thaliana root is a dynamic model for studying cell proliferation and differentiation processes. In this system, we can follow cells from origin to tissue-specific differentiation *in vivo*. Three longitudinal zones can be distinguished in the root: the meristem zone towards the tip, where there is a high cell proliferation rate, follows a zone of cell elongation where proliferation decreases and cell size increases, and finally there is a zone of cell differentiation where cells reach their final size and become specialized.
In this work, we characterized a mutant with a short root phenotype. Interestingly the mutant root has a meristem of the same size as the wild type (WT), but the elongation zone is shorter than WT. Cellular analysis revealed that cells elongate less than cells from WT plants. However, it produces the same number of cells in the meristem, indicating that cell elongation is specifically affected in this mutant.
The mutated gene is annotated in The Arabidopsis Information Resource (TAIR) database as a prenylyltransferase protein. However, after phylogenetic analysis, we established that it is, in fact, a *TETRATRICOPEPTIDE-REPEATED PROTEIN* (*TPR1*). Complementation analysis confirmed that the *TPR1* gene is responsible for the phenotype observed. This type of protein's function is unclear but is conserved in angiosperms. We also studied the possible involvement of TPR1 function in hormones or stress responses.

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ROS1 demethylase is involved in determining seed development of *Amaranthus hypochondriacus*

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Amaranth (*Amaranthus hypochondriacus*) is a genus of herbaceous plants and belongs annually to the *Amaranthaceae* family, which comprises more than 60 genera and approximately 80 species, it has been reported as a very versatile crop due to its short life cycle, it is cultivated in a wide range of agroclimatic conditions; It has great tolerance to drought, according to some studies, it requires up to 40 percent less water than wheat and barley, heat and pests, having a small, smooth, white seed as its fruit. This cereal is part of the 50 foods of the future, classified as such by the Global Fund, its characteristic is that they are nutritious, accessible, taste good, have a low impact on our planet, compared to meat foods, and are available in a wide variety of foods. variety of countries. The grain is distinguished by an interesting nutritional contribution that until now has been reported to have a 17% protein content, and high concentrations of the amino acid lysine, which according to the FAO is the ideal protein content for humans. One of the genes that has been shown to be important in the regulation of early seed development is *ROS1*, which is responsible for carrying out DNA demethylation in gene regions important for seed formation. In this project, the function of the *ROS1* gene was analyzed during seed development in Amaranth plants, a comparison of amino acid sequences belonging to ROS1 found *in Oryza sativa*, *Arabidopsis thaliana*, among other cereals, was made, and the presence of domains was identified. belonging to the DEMETER-LIKE family. The analysis of the function of *ROS1* in amaranth is being carried out by silencing mediated by microRNAs, the results obtained so far will be presented during the congress.

Optimization protocol to Isolation of protoplast from female gametophytic cells to genetic expression studies

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The female gametophyte is composed of seven cells which are differentiated in four distinct types: two sinergid cells, one egg cell, one central cell and three antipodal cells. Although, being a multicellular organ, it develops from a unique precursor-cell called Megaspore Mother Cell (MMC), which meiotically divides to form the Functional Megaspore (FM). Several genes have been associated to these early developmental stages, however, the essential molecular mechanisms involved FM selection remain still unknown. In this project, we optimized a protoplast isolation protocol for all female gametophytic cells, we confirm the presence of cells that express the specific molecular markers by confocal microscopy and flow cytometry. We will collect a single-cell type bulk by FACS to obtain the transcriptional profile of mRNAs and miRNAs, additionally the TRAP system will be implemented to isolate ribosomes and obtain the translational pattern. The acquiring of these expression profiles will give us more comprehension of the molecular mechanisms during the female gametogenesis in plants.

BOL as a transcriptional regulator of multiple bioprocesses during cellular reprogramming in callus and new organ formation in Arabidopsis

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Multiple bioprocesses associated with cell proliferation and differentiation are required for organ development in plants. These bioprocesses can be coordinated by transcription factors. *BOLITA (BOL)*, an AP2/ERF transcription factor, is expressed in organ founder cells. *BOL* loss of function causes cotyledons fusion and flower alterations in Arabidopsis. Interestingly, the mutation of the unique homologue of *BOL* in tomato just have an active meristem lacking aerial tissue (cotyledons and leaves). On the other hand, overexpression of BOL causes vitrification and reduction of aerial organs while presenting green callus in roots, indicating cell reprogramming without hormone addition. All these phenotypes suggests that *BOL* could regulate various processes involved in organogenesis. To elucidate these bioprocesses and genes, our research group obtained transcriptomes from a *BOL* inducible line. After a detailed bioinformatic analysis of differentially expressed genes, we selected some relevant genes and enriched bioprocesses related to cell proliferation and differentiation to study them. These bioprocesses include some well-studied categories like auxin transport, cell wall modification and cell cycle; also, production/scavenging of reactive oxygen species which have been described to be critical in plant development in the last few years. The objective of this project is to study the relationship between *BOL* and those selected bioprocesses to understand how it coordinates them. To this purpose, the effects of *BOL* induction and loss of function will be visualized and evaluated using different pharmacological techniques and histological staining, as well as crosses with marker lines. We are going to select at least one bioprocess and one gene to analyze its biological relevance, including the physical interaction of *BOL* with the regulatory sequences.

Exploring the genetic basis of photosynthetic plasticity in maize varieties adapted to diverse climates.

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México is considered the center of origin of maize, with 64 native varieties. Maize has become a model for studying C4 photosynthesis, as plants with this characteristic have a higher grain production rate, grow faster, and use less water compared to C3 plants. C4 plants have a special type of veins known as 'range 2 veins'. This anatomy is called Kranz anatomy and is responsible for increasing photosynthetic efficiency at high temperatures. What's even more interesting is that the density of range 2 veins is variable. Our research group has demonstrated that vein density is modulated by environmental factors, and this has been observed in different native maize varieties from Mexico that come from contrasting climates. Therefore, we are interested in elucidating the genetic mechanisms involved in the plasticity of vein density in maize. To achieve this, we conducted two transcriptomes. We evaluated the transcriptomes of these nine varieties under two different environmental conditions and at two stages of early leaf development, in order to correlate phenotypic changes in vein density with changes in gene expression during their formation, and thus identify the genes responsible for photosynthetic plasticity in maize. These findings could potentially help us in the future to modulate photosynthetic efficiency, impacting resistance and adaptation to high temperatures.

Transcriptomic analysis of differential expression during the flower to fruit transition in *Vanilla planifolia* Andrews (ORCHIDACEAE)

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The flower to fruit transition (FFT) has been described in model plants. However, plants with post-pollination syndrome (PPS) such as Vanilla planifolia Andrews (Orchidaceae) present a developmental modification characterized by the absence of ovules during anthesis, which has not been fully described in orchids. To study the main changes in the molecular phenology of ovaries during TFF in vanilla, a differential transcriptomic analysis, and an anatomical analysis were performed. Differential expression (DE) was identified among development stages, which defined three expression profiles. EPD-1 corresponded to the over-expression of the genes VpMYB05 and VpPIGA (floral development and heat stress memory) and the registration of placental proliferation during the Pre-pollination and Pollination stages; EPD-2 explained the induction of the genes VpYAB4, VpNFYA2 (ovum development) and VpBLH9 (organ development) and the formation of the embryonic sac during Post-pollination. EPD-3 correlated the over-expression of genes related to response to environmental stimuli, seed development (VpPR1, VpOLEO1, and VpCOS1), fruit yield (VpNAP2), and histological evidence of seed formation at the fecundation stage.

Transcriptome-wide analysis of microtubers of *Solanum tuberosum* L. induced in cytokinin containing medium and osmotic stress under darkness

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Potato *Solanum tuberosum* L. have emerged as a key non-grain crop for food security worldwide. Through *in vitro* techniques it is possible to obtain propagules for producing high quality potato seeds. We generated a rapid and efficient protocol for microtuber (MT) development in medium with sucrose (8% w/v), gelrite (6g/L), and 2iP as cytokinin under darkness.To understand the molecular mechanisms involved in MT development, we performed a transcriptome-wide analysis. We found that 1715 up- and 1624 down-regulated genes were involved in this biological process. Through the protein-protein interaction (PPI) network analyses performed in the STRING database (v11.5) with the highest confidence, we found 299 genes tightly associated in 14 clusters. Two major clusters of up-regulated proteins fundamental for life growth and development were found: 29 ribosomal proteins (RPs) interacting with 6 PEBP family members and 117 cell cycle (CC) proteins. The PPI network of up-regulated transcription factors (TFs) revealed that at least six TFs-MYB43, TSF, bZIP27, bZIP43, HAT4 and WOX9-may be involved during MTs development. RPs are essential during MTs development, and highlighted their interaction with PEBP family members as potential MT activators. The elucidation of the molecular biological mechanisms governing RPs and carbon metabolism, will help improve the resilience of potato crops in the face of today's changing climatic conditions.

Functional analyses of an uncharacterized member of the B-1 subfamily of ERF/AP2 transcription factor family in *Arabidopsis thaliana*

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At least 1533 transcriptional regulators have been reported in the *Arabidopsis thaliana* genome, representing ~5.9% of its estimated total number of genes. Particularly, proteins belonging to the APETALA2/Ethylene Response Factors (AP2/ERF) superfamily are involved in biological processes such as growth, development of new organs, and regulation of hormonal and stress response. Transcription factors such as DORNRÖSCHEN (DRN), PUCHI, LEAFY PETIOLE (LEP), BOLITA (BOL/DNRL/ESR2), etc. are in this family. Here, we studied a related gene (which contains an AP2 domain) that encodes a member of the B-1 subfamily of the ERF/AP2 transcription factor family. This integrase-type DNA-binding superfamily protein has not been previously studied or characterized; so, the aim of this study is, by reverse genetics, elucidate the function of the gene in the development of *Arabidopsis thaliana* (Col-0) plants. For this, an overexpressing (using the 35S promoter), loss-of-function (using CRISPR-Cas9) and a reporter line (fusing its promoter to GUS-GFP) mutant lines will be generated.

Genome-Wide Association Studies meta-analysis uncovers NOJO and SGS3 novel genes involved in *Arabidopsis thaliana* primary root development and plasticity

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Postembryonic primary root growth relies on meristems that harbour multipotent stem cells that produce new cells that will duplicate and provide all the different root cell types. *Arabidopsis thaliana* primary root growth has become a model for evo-devo studies due to its simplicity and facility to record cell proliferation and differentiation. To identify new genetic components relevant to primary root growth, we used a Genome-Wide Association Studies (GWAS) meta-analysis approach using data that have been published in the last decade. In this work, we performed intra and inter-studies analyses to discover new genetic components that could participate in primary root growth. We used 639 accessions from nine different studies and performed different GWAS tests ranging from single studies and pairwise analysis with high correlation associations, analyzing the same number of accessions in different studies to using the daily data of the root growth kinetic of the same research. We found that primary root growth changes were associated with 41 genomic loci, of which six (14.6%) have been previously described as inhibitors or promoters of primary root growth. The knockdown of genes associated with two of these loci: a gene that participates in Trans-acting siRNAs (tasiRNAs) processing *Suppressor of Gene Silencing* (*SGS3*) and a gene with a Sterile Alpha Motif (SAM) confirmed their participation as repressors of primary root growth. As none of them has been shown to participate in this developmental process before, our GWAS analysis identified new genes that participate in primary root growth. Overall, our findings provide novel insights into the genomic basis of root development and further demonstrate the usefulness of GWAS meta-analyses in non-human species.

The role of *MIR160* during somatic embryogenesis in Avocado (*Persea americana*)

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The microRNAs are small non-coding RNAs 20 to 22 nucleotides long, that play essential roles as negative regulators of gene expression during growth, development and in response to internal and external stimuli. Somatic embryogenesis (SE) refers to the formation of embryos from differentiated somatic cells and is one of the most employed techniques for mass production of economically relevant plant species. Establishment of SE in avocado has been hampered due to extremely low efficiencies of embryo induction and plant regeneration. Auxin plays an important role during SE as AUX/IAAs, ARF16 and ARF17 are associated with the modulation of the embryo-induction stage and are targets of the miR160 family of microRNAs. In this work, we profiled the expression pattern of miRNA160 and ARF17 by RT-PCR assays in zygotic embryos (immature fruits 1–2 mm) and three key stages of SE: early globular (EG), late globular (LG) and white-opaque (WO) from avocado variety "Hass". We observed a remarkably contrasting expression pattern of miR160 and its target *PaARF17* in zygotic embryos relative to the SE stages analyzed. While in zygotic embryos the expression of miR160 was low and PaARF17 was increased, in SE the expression of miR160 was increased in EG, LG and WO accompanied by a concomitant reduction in basal levels of PaARF17. Our results are consistent with a model where the expression of PaARF17 is negatively regulated by miR160. We believe that differential modulation of auxin signaling is one of the mechanisms that contribute to the low efficiency of SE in avocado.

Using CRISPR/Cas9 technology to study the *SPATULA* function in tomato flowers and fruits.

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In addition to their function in preserving angiosperm species, fruits are an invaluable component of the human diet. Since a fruit derives from a gynoecium, understanding the factors that guide its correct development is very important. One of the important transcription factors of gynoecium development in Arabidopsis is *SPATULA (SPT)*. The aim of this study is to determine the function of SPT in tomato flowers and fruits. To achieve this, we generated stable loss-of-function tomato lines using CRISPR-Cas9 technology. Here we demonstrate that *SlSPT* has a role in floral organ growth, particularly in stamen fusion, length and correct development of the style and stigma, and trichome formation on the carpels. Also, during fruit development, the lack of SPT function causes incorrect pigmentation of the exocarp in both developing and mature fruits. A metabolomic analysis of the exocarp showed that in the *SPT* mutants, there is a significant perturbation in several pathways: flavonoid biosynthesis, glycerophospholipid metabolism, and glycerolipid metabolism, that could affect the nutritional value of tomato. In summary, the results show various expected conserved functions during carpel formation and a diversity of novel functions that enrich the knowledge of the *SPT* gene in fleshy fruits.

The plasma membrane H^+-ATPase activity in *Capsicum annuum* roots in response to an avocado seed-derived biostimulant

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The activity of the plasma membrane H^+-ATPase activity in the cell is required for a variety of plant functions: it is involved in the opening of stomata, the growth of cells in stems and the nutrient uptake in the roots, among other. On the other hand, the use of biostimulants has led to agricultural benefits like an improved plant structure that allows increased crop yields and fertilizer savings. Thus, in this work, we are studying the effects of applying an avocado seed hydrolysate, which has been used as a biostimulant, on jalapeño chili pepper *(Capsicum annuum)* on the plasma membrane H^+-ATPase activity in roots. A method to prepare mixed-membrane fractions from these roots was developed, and the plasma membrane H^+-ATPase activity (vanadate-sensitive ATPase) was determined in these membrane fractions. This ATPase activity will be correlated with the agronomically interesting variables (plant height, number of branches, flowers and fruits, etc.) that could change as a result of the application of this biostimulant.

Expression of *PEROXIDASE 35*, a new player involved in the lateral root primordium morphogenesis, is regulated by ATX1

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In search of new genes involved in lateral root (LR) development, we performed transcriptomic analysis of the root in the *atx1setm* mutant deficient in methyltransferase activity of a histone methyltransferase ARABIDOPSIS HOMOLOG OF TRITHORAX1 (ATX1). ATX1 catalyzes the H3K4 trimethylation to maintain a transcriptionally active chromatin state and controls root growth by regulating cell cycle duration, maintaining stem cell niche, and patterning during primary and LR development (Napsucialy-Mendivil *et al*., 2014). We have found that *PEROXIDASE 35* (*PER35*) gene from a classical plant (class III) peroxidase subfamily was downregulated in the *atx1setm* root and confirmed it by RT-qPCR analysis. Moreover, the *PER35* transcript abundance was upregulated in the *35S::ATX1* overexpression line. The Reactive Oxygen Species (ROS) signaling is central to many processes in plant development, and catalase and peroxidase activities maintain their homeostasis. It is known that peroxidase activity is essential to promote cell wall remodeling during LRP development (Orman-Ligeza *et al*., 2016). Therefore, we explored the role of the *PER35* gene in root development. We found that by 8 days after germination (dag), the primary root of the loss-of-function *per35-1* mutant was longer than the Wt (Col-0) root. Although no LR primordium (LRP) density changes were found in the *per35-1* mutant, the LRP morphogenesis was abnormal in 8 dag *per35-1* seedlings compared to the Wt. These abnormalities were similar to those found in the *atx1setm* mutant; in both mutants, the LRPs formed had asymmetric and flat-shaped domes. The quantification of the abnormal LRPs after treatment of roots with 2mM H_2O_2 showed an increment of these abnormalities in the *per35-1* mutant (54%) compared to untreated seedlings (29%). Our data suggest that ATX1 regulates the *PER35* gene expression and uncover a new player involved in the LRP morphogenesis.

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Interaction of maize CDKs with the catalytic subunit of SnRK1

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The cell cycle (CC) plays a fundamental role in the growth and development of all organisms, which, together with cell differentiation, will lead to the a new organism. CC is a series of highly coordinated temporal, spatial and unidirectional molecular events whose basic purpose is to generate two identical daughter cells[1].
In plants, proper CC progression requires changes in enzyme complexes, including the kinase activity of heterodimeric protein complexes formed by the cyclin-dependent kinase (CDK) catalytic subunit and the cyclin regulatory subunit (Cyc). CDK/Cyc complex activity can be regulated by post-translational modifications such as activating or inhibitory phosphorylations. These complexes respond to intrinsic and extrinsic stress signals which influence the activity of central regulators to determine whether the cell advances or blocks CC progression[2].
A principal component of the stress response is SnRK1 complex (SNF1-related protein kinase 1), which drives extensive metabolic and transcriptional rearrangements that control processes for conserving energy and mobilizing reserves to promote survival. It is part of a highly conserved protein family in eukaryotes that includes yeast SNF1 (Sucrose non-fermenting 1) and mammalian AMPK (AMP-activated kinase). SNF1/AMPK/SnRK1 complexes are conserved in all eukaryotes and share a heterotrimeric structure, composed of a catalytic α-subunit and two regulatory subunits, β and γ [3].
SnRK1 has been described to have an important role in cell cycle regulation through different targets such as TOR, E2F1a/b, KRP6, KRP7, etc [4, 5, 6]. In this Project, we speculate that SnRK1 might target the CDK/Cyc complex to regulate its kinase activity, and therefore, control the progression of the CC.

Bibliography:
[1] Dewwitthe & Murray *et al.,* 2003.
[2] Magyar *et al*., 2013.
[3] Yujiao Wang *et al.,* 2019.
[4] Seungmin *et al.,* 2022.
[5] Baena-González *et al.,* 2017.
[6] Guérinier T. *et al.,* 2013.

An efficient protocol for extracting high-molecular-weight DNA from Cactaceae and other challenging species

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Most of the biomass on Earth is produced by plants, however, there is still an underrepresentation of green genomes assemblies within the reported eukaryotic sequenced genomes. In plants, priority has been given to crop species of economic significance. Although Cactaceae is a numerous family of ~1,851 accepted species with striking adaptations at the anatomical, physiological and molecular level, highly fragmented genome assemblies were reported for only seven species.Genomic studies in Cactaceae have been hindered by the difficulties to obtain DNA of high integrity and quality given the elevated content of polysaccharides and phenolic compounds in this family. Polysaccharides and phenolic compounds can bind DNA irreversibly, inhibiting further enzymatic reactions during library preparation and subsequent sequencing steps. Here, we propose a protocol for high-molecular-weight DNA extraction from Cactaceae and other challenging plant species. The spectrophotometric analysis of the isolated DNA shows little to no contamination, presenting A260/A230 and A260/A280 values ≥1.60 and ≥1.86, respectively, i.e. within optimal values for DNA purity. Agarose gel electrophoresis evidenced the integrity and high-molecular weight of the genomic DNA, which can be used for further applications such as long-read genome sequencing. Also, the DNA extracted with the present protocol can be applied in enzymatic reactions such as PCR amplification. Furthermore, the same procedure can be used for DNA extraction from different botanical families such as Asparagaceae (Yucca sp.) and Myrtaceae (Eucalyptus sp.).Our proposed protocol could be used to increase the availability of genomic resources for underrepresented plant families, and therefore advance and strengthen our efforts in understanding, assessing, and conserving plant biodiversity.

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Do *VIM* genes have a role in embryo and endosperm development?

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DNA methylation and histone modifications are known regulators of gene expression. In mammals, DNMT1 is the main responsible for CG DNA methylation maintenance and is recruited to hemi-methylated DNA by its cofactor UHRF1. UHFR1 recognizes silent histone marks and recruits effectors for chromatin condensation, providing a connection between DNA methylation and histone modification. In plants, DNMT1 and UHRF1 homologues are called MET and VIM, respectively. Arabidopsis has six *VIM* genes. *VIM1*, *VIM2* and *VIM3* are functionally redundant, and the *vim1/2/3* mutant presents alterations in chromatin modifications and DNA methylation, suggesting a similar role to their mammalian counterpart. In addition, several *VIM* genes may show paternally biased expression during seed development. Though imprinted genes are common in animals, their functional importance in plants is not understood.

Despite their potential imprinted expression and importance for gene regulation, a functional role for *VIM* genes during seed development has not been described. In this work, we test the hypothesis that *VIM* genes are preferentially expressed from the paternal allele and that this imprinted expression has a functional role during seed development. To begin to address this hypothesis, I examined seed development in single mutants of all *VIM* genes (*VIM1-6*). *vim1*, *vim2*, *vim3*, *vim4* and *vim6* showed embryo defects at low penetrance, while *vim5* showed endosperm defects with high penetrance. To better understand their role during development, I am generating higher order mutants. Preliminary analysis suggests that the *vim1/2/3* mutant shows more severe phenotypes than single mutants, which worsen when propagated as homozygotes. I am currently working on the generation of *VIM* reporter lines to verify the paternally imprinted expression seen in RNAseq studies. Our ultimate goal is to determine whether imprinted *VIM* expression is important for function by testing whether *vim* mutants show paternal effects on embryo and endosperm morphology and gene expression.

Analysis of the function of LGN and NOD maize proteins with a transcriptomic and proteomic approach

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Liguleless narrow (Lgn-R) and *narrow odd dwarf (nod)* are maize mutants with striking developmental phenotypes that affect most vegetative and reproductive organs, and additionally display autoimmune defects. NOD is a protein that spans the plasma membrane and participates in calcium import, and LGN encodes a kinase with a transmembrane domain and a small extracellular region. Transcriptional and phosphoproteomic evidence suggests that these mutants are autoimmune, however, little has been addressed on this issue. We decided to perform a detailed transcriptomic analysis of the *Lgn-R* (a kinase-dead mutant)*, nod* (a null protein mutant), and the double mutant *nod;Lgn-R.* Our results showed a generalized induction of genes related to biotic and abiotic stress. Functional enrichment analysis showed that biological processes related to defense against fungi, secondary metabolism, systemic acquired response, and oxidative stress are strongly overrepresented in the mutants. Consistent with these results, experiments to detect reactive oxygen species showed that there is an exacerbated accumulation in these mutants. In addition, through an immunoprecipitation analysis followed by mass spectrometry of LGN in WT and *Lgn-R* tissue, we found abundant interaction of mutated LGN with proteins in the MAP kinase signaling pathway. These findings suggest that together NOD and LGN proteins play an important role in stress response in maize. Next experiments will be carried out to determine the level of resistance to pathogenic fungi in these mutants compared to wild type plants.

Solving the structure of the NaTrxh-S-RNase protein interaction

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Thioredoxins (Trxs) are widely distributed oxidoreductases among all taxa. The conserved active site of these proteins, Trp-Cys-Gly-Pro-Cys, allows to reduce disulfide bonds on target proteins. The plants exhibit a complex Trx system from which eight types have been identified. Type *h* Trxs (Trxh) form the largest and most heterogenous group that is subdivided into three subgroups.
NaTrxh is a *Nicotiana alata* Trxh clustered within subgroup 2, which is characterized by possessing extensions towards the N- and/or C-terminus. The NaTrxh extensions play a critical role in its interaction with its target protein from *N. alata* styles, the S-RNase. While the N-terminus comprises two motifs, Nα (Met1-Ala17) and Nβ (Ala17-Pro27), the C-terminus contains only one (C: Glu136-Gln159). While both N- and C-termini participate in its S-RNase interaction, only the N-terminus is also needed to reduce S-RNase.
Although the tertiary structure of all Trxs is highly conserved, they exhibit different specificity towards the corresponding target proteins. Plant Trx system complexity also rises the question regarding the balance between specificity and redundancy. In the case of NaTrxh, for instance, it reduces insulin disulfide bonds as the *Escherichia coli* Trx, but the latter is unable to reduce S-RNase. The major difference between these two Trxs is indeed the N- and C-termini extensions in NaTrxh.
This project aims to analyze the structure of the NaTrxh-S-RNase complex to understand how NaTrxh extensions participate in this specific protein-protein interaction. To stabilize this complex by a covalent bond, we generated the NaTrxh(C65S), which replaces the second Cys residue of its active site, preventing the release of the reduced S-RNase and the oxidized NaTrxh. Solving the structure of this "dimer" will provide biochemical evidence to explain the specificity between both proteins, the role of NaTrxh extensions, and contribute to understanding the functional diversity that exists among Trxs.

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Structure-function analysis of DNA Ligase VI and its role in non-homologous end joining (NHEJ) in plant organelles

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Plants, as sessile photosynthetic organisms, are necessarily exposed to high levels of environmental stress, including UV-B rays, gamma radiation and heavy metals, which have required the evolution of highly effective DNA repair response to counteract the damage ongoing damage to the nuclear and organellar genomes. The main damages are divided into two groups: 1. Single strand damage, and 2. Double strand damage, which include double-strand breaks, this being lethal damage since this alteration can lead the system to cell death. Repair of double-strand break occurs in mainly through three ways: Homologous recombination (HR), Non-homologous end joining (NHEJ) and Microhomology-mediated end joining (MMEJ). Non-homologous end joining phrase is original used to describe an illegitimate repair way that uses little or non-homology. This repair pathway in plant organelles are currently unknown because no homologues of the enzymes involved in this mechanism have been found. However, an uncharacterized DNA ligase with signal peptide to mitochondria was identified in the *A. thaliana* genome, called AtLIG6, which encodes a protein with a domain structure unique to plant species because it contains a nuclease domain at its N-terminus and a highly conserved ligase domain at its C-terminus. In this project I will evaluate the role played by AtLIG6 DNA ligase in the repair of double-strand breaks and its role in the non-homologous end recombination (NHEJ) pathway in *A. thaliana* organelles.

In search of *ARABIDOPSIS HOMOLOG OF TRITHORAX1* targets involved in root development

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ARABIDOPSIS HOMOLOG OF TRITHORAX1 (*ATX1*) is a histone methyltransferase (H3K4me3), associated with a transcriptionally active chromatin state (Avramova, 2009). Our previous study showed that ATX1 is required to cell production, stem cell niche maintenance, and lateral root development (Napsucialy-Mendivil *et al*., 2014). In search of ATX1 targets involved in root development, we performed transcriptomic analysis of the root tissues of the *atx1-setm mutant, deficient in SET domain activity. Next, we selected* those transcription factors whose expression was downregulated in the *atx1-setm mutant. Among candidates of the ATX target genes involved in root development we identified KIDARI (KDR) and ATHB21, and this work presents the results of our study of these transcription factors*. *KDR* encodes a non-DNA binding Helix-Loop-Helix (bHLH) transcription factor involved in brassinosteroid signaling (Wang *et al*., 2009), and *ATHB21* is an *Arabidopsis thaliana* zinc finger homeodomain protein 21. We genotyped the *kdr-1* mutant and obtained homozygous F3 generation. The primary root growth was slower in the *kdr-1* mutant compared to the Wt (Col-0), suggesting the role of KDR in root growth. In plants growing in the soil, rosette, and inflorescence growth were also slower than in the Wt. Analysis of *the ATHB21* promoter showed the presence of auxin response elements suggesting a link with auxin signaling. We analyzed the relative *ATHB21* transcript abundance after auxin treatment and, surprisingly, we found that exogenous auxin repressed *ATHB21* expression. We will discuss the implications of these results. This preliminary study suggests that *KIDARI* and *ATHB21* genes, which are putative ATX1 targets, could have an important role in root development. The work on the *athb21* mutant isolation and further *kdr-1* mutant phenotype characterization is in progress.

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Role of XAL1 in the regulation of flowering in response to light and increased temperature in *Arabidopsis thaliana*

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Environmental cues such as light quality, photoperiod (light hours per day) and temperature regulate flowering in *A. thaliana*. Detection of long-day (LD) photoperiod relies on CONSTANS (CO) transcription factor. During day hours, phytochrome B (PHYB) promotes the degradation of CO, while during the afternoon CO accumulates and induces *FT.* The latter, translates in the leaf's phloem and travels to the apical meristem, where it binds to FD transcription factor to induce other flowering genes. Traditionally, PHYB has been considered a negative regulator of flowering, however, our work demonstrates that in the apex, PHYB mediates light induction of *XAANTAL1* (*XAL1*) and other genes. Genetic analysis showed that *XAL1* and *FD* participate in the same signaling pathway when plants are grown under LD photoperiod at 22°C. Also, we found that XAL1 and FD regulate downstream factors in a dependent and independent manner. Through ChIP assays, we identified direct binding of XAL1 to key flowering genes like *FD, SOC1*, *LFY* and *AP1*. Interestingly, we also observed that elevated temperatures of 27°C, XAL1 and FD function independently in the flowering process. Therefore, XAL1 role is relevant on flowering transition in response to light and high temperatures. Our findings lead us to propose that light and temperature signaling at the shoot apical meristem is partially independent of the signaling derived from the leaves.

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The PLETHORA transcription factors regulate RNApol-I transcription to orchestrate cell proliferation and differentiation

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The root apical meristem (RAM) consists of pluripotent, mitotically active cells at the root apex, this includes the quiescent center important for the RAM maintenance and the stem cells adjacent to the quiescent center. The PLETHORA (PLT) transcription factors play a crucial role in maintaining the RAM in *Arabidopsis thaliana*. In *Arabidopsis*, 4 out of 6 PLTs are strongly expressed in the RAM, act as master regulators of RAM initiation and maintenance, and their function is partially redundant. Here we assess the specific pathways by which PLTs shape the gene expression programs in the RAM. From publicly available data, we modeled a gene regulatory network (GRN) for the PLTs in the RAM, by integrating gene co-expression, transcription factor binding site (TFBS) validation, expression profile, and single cell transcriptomics data. We built a GRN with 534 putative PLT target genes and 1,444 interactions in *Arabidopsis* with PLTs acting as hub nodes. To address GRN plasticity, we performed a cross-species network preservation analysis, including monocot and dicot species. Our results suggest that PLTs strongly regulate RNApol-I transcription of ribosomal RNA genes (rDNA) and expression of genes involved in RNA metabolism, thus suggesting the role of rDNA transcription and RNA modifications in the RAM maintenance. Furthermore, PLT homolog-mediated interactions found in *Arabidopsis* are also conserved in other dicots, namely, tomato, cucumber and soybean; and even in the monocots rice and maize. Therefore, we revealed a functional conservation of these interactions that correlates with the high mitotic rate needed for RAM maintenance, and a significant preservation of the connectivity and density of the PLT-mediated co-expression module. Overall, our study provides new insights into the complex interactions mediated by PLTs in maintaining RAM, and how this GRN is conserved across dicots and monocots species.

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MBF1 proteins and their characterization

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The Multiprotein bridging factor 1 (MBF1) family of proteins are transcription cofactors that form a bridge between transcription factors and the basal transcription machinery in eukaryotes. In plants, this family is related to development. MBF1s have been studied at the level of the expression of their transcripts, but there is lack of information on the corresponding proteins. In this work we obtained specific antibodies for the MBF1 proteins, which will be used in the identification of the protein complexes. In addition, from inferred coexpression subnetworks we identified possible protein-protein interactions that we tested experimentally by the bimolecular fluorescence complementation technique. Finally, the subcellular localization was determined by fluorescence microscopy.

In-plant localization of NTT interactions with A-type ARRs

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Plant development and growth are a highly controlled processes that depend on a plethora of interactions between biomolecules such as DNA-protein and protein-protein interactions, as well as the recognition of signaling molecules. Among the proteins capable of interacting with DNA, transcription factors (TFs) play an important role in gene regulation, modulating gene expression by activating or repressing their transcription. In the regulatory pathway, phytohormones play a central role as signaling molecules. One of the most studied hormones in Arabidopsis are cytokinins (CKs), adenine-derived molecules that play various roles in the plant, such as cell division, meristem maintenance, plant growth, among others. CK signaling occurs in Arabidopsis through a Two Component System (TCS). Within the TCS, Arabidopsis Response Regulators (ARRs) play a crucial role by receiving the translocated phosphate group from the TCS and regulating the transcription of CK-response genes. Previous reports suggest that ARR4 and ARR16, both A-type ARRs, are able to interact with NO TRANSMITTING TRACT (NTT). NTT is a C2H2 zinc finger-type TF that has been found to be important for proper development of the transmitting tract and replum in Arabidopsis, as well as being a regulator of several related genes mainly in hormone pathways. To further gain knowledge about the biological function of NTT interactions with type-A ARRs, it is necessary to know the *in planta* localization of these interactions, for which, *in planta* BiFC assays will be performed using the promoter-gene genomic sequences of the interactors, to know the cellular localization of the protein-protein interactions of NTT-ARR4 and NTT-ARR16 dimers. The results obtained in this research will provide knowledge to understand the NTT regulatory network in TCS activity and its biological relevance.

Regulation between members of the MADS-box and SPL transcription factors families during flowering transition in *Arabidopsis*

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Flowering transition is controlled by a genetic network that integrates endogenous and environmental cues and culminates in flower formation and seed production. Therefore, flowering time can influence the fitness of the offspring. In *Arabidopsis,* this transition is promoted by long-day (LD) photoperiod (16 light / 8 dark hours). However, under non-inductive conditions like in short-day (SD) (8 light / 16 dark hours), gibberellins (GAs) and the age-mediated signaling become essential for flowering transition.
Flowering is regulated by many transcriptional factors including members of the MADS-box and the SQUAMOSA BINDING PROTEIN LIKE (SPL) families. Previous studies showed that the MADS-domain proteins AGAMOUS-like 19 (AGL19), XAANTAL1 (XAL1), and XAANTAL2 (XAL2) function as flowering regulators under specific photoperiod and temperature conditions. In this work, we analyze the transcriptional regulation between these MADS-box and SPL3, SPL5, SPL9 and SPL15. For this, semi or quantitative RT-PCR assays were performed in leaves and apices of plants grown under LD and SD conditions. The results on LD show that SPL transcription factors differentially regulate *AGL19*, *XAL1* and *XAL2* in both organs. Most of the SPL factors repress the expression of the MADS-box in the leaves, except for SPL3 and SPL9, which induce *AGL19*. Likewise, XAL1 and XAL2 induce *SPL9* and repress *SPL5* in the leaves. Interestingly, XAL1 induces all *SPL* tested at the apex. Under SD, *XAL2* mRNA is induced in the shoot apex by SPL9 and SPL15, which accumulate in response to age. We also found that SPL9 and SPL15 partially mediate *XAL2* induction by GA_3, integrating hormonal and aging signals. Furthermore, XAL2 partially mediates *SOC1, LFY*, and *AP1* induction in response to GA_3. These findings constitute a start point to understand the complex regulation between *AGL19*, *XAL1* and *XAL2* and the *SPL*s.

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Identification and characterization of *PLETHORA* genes in five Cactaceae species

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During determinate primary-root growth the root apical meristem (RAM) is exhausted, and all the cells of the root apex differentiate soon after germination. When this happens the root stops growing. Almost all species reported to exhibit determinate primary-root growth in wild type plants belong to the family Cactaceae. The genetic regulation underlying the RAM exhaustion in Cactaceae is poorly understood. Here we explore a possible role of the PLETHORA (PLT) transcription factors in this process. PLTs are master regulators of the primary root development in *Arabidopsis thaliana*. Four out of six *PLT* genes are strongly expressed in the *A. thaliana* RAM, a zone of frequent cell divisions, and are not expressed in the root zone where cells are differentiated. These four PLT proteins redundantly keep the *A. thaliana* RAM present and active. Analysis of the *de novo* assembled transcriptome from the primary root apex of the cactus *Pachycereus pringlei* (Rodriguez-Alonso *et al.*,2018) indicate that *PLT* genes are expressed even when the RAM is exhausted, and all root-apex cells are differentiated. In this work, orthologs of five *PLTs* were identified and characterized in five Cactaceae species with public preliminary genomes, namely, *P. pringlei*, *Carnegiea gigantea*, *Lophocereus schottii*, *Stenocereus thurberi* and *Hylocereus undatus*. We found that the coding region of the *PLT* orthologs in these cacti species have a conservation above 94%; and the UTRs, and possible promoters have a conservation above 31% and 50%, respectively. Furthermore, the number of exons and introns, as well as their position, is similar between the orthologs of the five cacti and *A. thaliana*. A preliminary analysis of the regulatory elements indicates that the putative promoters of the cactus *PLT* genes share regulatory elements present in *A. thaliana*.

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Functional analysis of long non-coding RNAs (lncRNAs) involved in genome topology of *Arabidopsis thaliana*.

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Mechanistic links between gene expression regulation and genome topology are beginning to uncover functional implications of the arrangement of chromatin in the nuclear space. In mammals, insects, and plants it is known that long non-coding RNAs (lncRNAs) participate in the regulation of the three-dimensional organization of chromatin, but only very few have been functionally characterized. In order to identify lncRNAs involved in genome topology of *Arabidopsis thaliana*, we used a whole-genome approach to identify 62 chromatin-associated lncRNAs. Based on their RNA-DNA interactions, expression patterns, and putative target genes, four candidate lncRNAs were selected for functional analyses. T-DNA insertional mutants for these lncRNAs show phenotypic differences in shoots and roots when compared to wild-type Columbia (Col-0) plants. These results suggest a relationship between these chromatin-associated lncRNAs and the regulation of gene expression in the development of both organs in *Arabidopsis*.

In vitro Pharmacological Screening and Metabolomic Profile of Ten *Amaranthus cruentus* L.: A Survey of Amaranth - A Functional Food

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Amaranth, a well-known pseudo-cereal, is highly valued for its exceptional nutritional composition, particularly its protein content, which often surpasses milk. Moreover, numerous health benefits have been attributed to amaranth, including its potential role in managing diabetes, hypertension, bacterial infections, and even cancer. Therefore, this work aimed to conduct a comprehensive pharmacological screening to study the inhibitory effects of amaranth extracts on critical enzymes associated with diabetes and hypertension. Furthermore, we sought to explore the antioxidant properties of amaranth extracts. We found that it ranged from 27% to 78% of activity, potentially contributing to treating these globally prevalent diseases.

The pharmacological screening revealed that aqueous methanolic extracts exhibited significant enzyme inhibition of α-glucosidase (32% to 65%) and α-amylase (37% to 84%), which are crucial enzymes involved in the breakdown and hydrolysis of complex carbohydrates in diabetes management. Additionally, the extracts demonstrated angiotensin-converting enzyme (ACE) inhibition ranging from 36% to 78%. ACE is a critical enzyme implicated in hypertension. Elevated levels of Angiotensin II, originating from reduced ACE activity, contribute to hypertension.

In parallel, we studied and analyzed the phytochemical profile of ten *A. cruentus* L. accessions using HPLC-DAD to identify and quantify betalains, flavonoids, and organic acids. Furthermore, molecular docking techniques were used to predict the binding energies and types of interactions between the identified metabolites and the enzymes α-glucosidase, α-amylase, and ACE.

This study shows the antioxidant properties of *A. cruentrus* accessions and their potential role in promoting human health. Moreover, the pharmacological screen was correlated with the presence of main betalains, flavonoids, and organic acids in *A. cruentrus* extracts. Our findings show that amaranth can be considered a functional food with promising therapeutic implications.

Unveiling Metabolic Crosstalks in Plants: Ascorbic acid, Folates and One Carbon (1C) pathways in *Arabidopsis thaliana*

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Plants have developed intricate metabolic networks to efficiently respond to environmental challenges and maintain cellular homeostasis. Among these networks, ascorbic acid (AsA, vitamin C), folates, and one-carbon (1C) metabolic pathways play crucial roles in plant growth, development, and stress tolerance. AsA is a major metabolite in plants, primarily functioning as an antioxidant, regulating a wide spectrum of cellular mechanisms against environmental stresses. Folates (vitamin B9) serve as cofactors in 1C metabolism, facilitating the transfer of 1C units and participating in the biosynthesis of essential components including amino acids, nucleotides, and methyl donors in the metabolism of all living organisms. The objective of this study is to investigate the relationship between these metabolisms in plants. For this purpose, we evaluated free amino acids (aa) content in *Arabidopsis* mutant lines involved in ascorbic acid metabolism, namely *MIOX4* (myo-inositol oxygenase overexpression), *vtc1-2* (GDP-D-mannose phosphorylase mutation), and *vtc2-1* (GDP-L-galactose phosphorylase mutation) using plants of 36 days (Stage 6.1); to which folates and total protein content will also be measured. Results indicate that the total aa content in the *vtc2-1* mutant was significantly higher compared to the other analyzed lines. Particularly, arginine, known for its involvement in preventing oxidative stress through the production of nitric oxide, was approximately 15-times higher in *vtc2-1* than in the wild type (wt) Arabidopsis. Moreover, both mutants accumulated between 1.5 and 3 times more methionine, serine, and glycine (aa involved in 1C-metabolism), and vtc1-2 exhibited a lower Ser/Gly ratio compared to wt (0.44-fold). Interestingly, *MIOX4* did not exhibit significant changes in aa accumulation compared to wt. These findings suggest a potential crosstalk between ascorbic acid and 1C metabolism, warranting further investigation to uncover the underlying molecular mechanisms. Understanding these relationships could lead to valuable insights into enhancing plant stress tolerance and overall growth performance.

Interaction between the mitochondrial maize hexokinase 4 and the beta-glucosidase aggregating factor 1 and its role in the plant physiology.

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Hexokinases (HXKs) the first glycolytic enzyme are also moonlighting proteins. The HXKs can sense glucose concentration and act either as a repressor protein or protein kinase. It has been discovered that most of the mitochondrial HXKs are glucose sensor proteins, for example AtHXK1 a mitochondrial protein, can sense glucose content in plants and move to the nucleus to interact with two other proteins to create a photosynthetic gene repressor complex. In this context, it would be interesting to find out if the HXKs have any other protein partners or roles that affect plant physiology.
We have identified putative interactors between ZmHXK4 and several biotic stress proteins using pull-down assays, including the Beta Glucosidase Aggregating Factor 1 (ZmBGAF1). We corroborated that ZmBGAF1 is interacting with the mitochondrial glucose sensor protein ZmHXK4 by Far-Western Blot and bimolecular fluorescence complementation (BiFC) in transient and stable plants from Nicotiana and maize. Plant expressing both recombinant proteins show wilted leaves and experience cell death over time.
ZmBGAF1 is a protein with two domains, the jasmonate-inducible dirigent domain and the jacalin-related lectin domain. Treatment of maize plants with Methyl-JA (MeJA) increased the expression of ZmHXK4 and ZmBGAF1. We also noticed that MeJA treatment or overexpression of ZmHXK4 and ZmBGAF1 together adversely impact photosynthesis, decreases turgor, and promotes cell death in leaves. We suggest that MeJA affects the ZmHXK4-mitochondria interaction in plants as occurs in mammals, detaching the enzyme from the mitochondria. Such process may also play a role in the interaction ZmBGAF1-ZmHXK4, an impact on the plant physiology of the plant.

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Bases of metabolic engineering of anthocyanins for the modification of bract color in poinsettia (*Euphorbia pulcherrima* Willd. ex Klotzsch)

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The poinsettia or nochebuena (*Euphorbia pulcherrima* Willd. ex Klotzsch), native to Mexico, is the ornamental plant symbol of Christmas. Its popularity is the result of the diversity of colors exhibited by the bracts, a kind of modified leaves that accumulate anthocyanins based on cyanidin and pelargonidin pigments, providing the typical reddish colors. The commercial success of poinsettia lies in the development of new varieties with novel colors; however, the attractive bluish color range is absent due to the lack of delphinidin-based anthocyanins. This deficiency is caused by the lack of the flavonoid 3′,5′-hydroxylase (*F3'5'H*) gene, encoding a key enzyme for delphinidin biosynthesis. The present study is focused on determining the bases of the manipulation of the anthocyanin's biosynthesis in poinsettia, to set the preferential production of delphinidin. To favor a bluish pigmentation, the pH of the bracts from fourteen varieties of poinsettia was determined, and the content and type of different flavonoid compounds as potential copigments were quantified and identified by High-Performance Liquid Chromatography. In addition, eleven *F3'5'H* gene coding sequences from different species of bluish flowers were isolated and used for *in silico* three-dimensional structure modeling (AlphaFold) and molecular docking (AutoDock Vina), using dihydroquercetin (DHQ) and dihydrokaempferol (DHK) as ligands. The comparative analyses allowed us to identify that poinsettia varieties 'Prestige Red' and 'Cortez Burgundy' were the most suitable to contain and display a bluish pigment, due to the less acidic pH of the bract and its high content of copigments. Furthermore, the *Lobelia erinus* and *Torenia fournieri F3'5'H* genes showed a higher affinity for DHQ and DHK substrates. Therefore, these plants seem to be potential candidates to provide the genes to effectively induce delphinidin production. These results represent a promising first step for the effective modification of blue poinsettia.

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Protoplast isolation and characterization from *Argemone Mexicana* L.

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Argemone Mexicana L., commonly known as chicalote, produces a variety of benzylisoquinoline alkaloids (BIAs), mainly, sanguinarine and berberine that exhibit important medical and industrial applications. The biosynthesis of BIAs is a complex process, commonly associated to plant tissue organization. Tissues involved in BIA biosynthesis and accumulation often present specialized cell types for each one of these processes. In mature *A. mexicana* plants, sanguinarine is restricted to roots and mature seeds, whereas berberine is found in both roots and aerial tissues. However, little is known about how sanguinarine and berberine are allocated in the chicalote cells. Both berberine and sanguinarine present protonable N2 atoms in their structure, resulting in distinctive autofluorescent properties. Sanguinarine and berberine exhibit orange/blue-green fluorescence, respectively, when exposed to $\lambda_{385\ nm}$ UV radiation. This allows their detection in cells accumulating them by fluorescent microscopy.

To understand the mechanism of distribution and accumulation of these BIA's in cells of this plant, we have implemented the use of protoplasts isolated from different organs. This strategy allows the dissemination of the cell types in the tissue, facilitating its characterization. In addition, better images of the interior of the cell can be obtained, compared to whole tissue, due to the absence of a cell wall. Once protoplast have been released, microscopic observation under fluorescence will allow the identification of the specific cell types involved in the accumulation of the BIA berberine and sanguinarine. Until now, a protocol for protoplast isolation from leaves, roots, and *in vitro* cultures of *A. mexicana* has been developed. Incubation of tissues with cellulose and pectinase for periods under 6 h released protoplast that remained viable for at least 72 h. Protoplast peparation of each tissue produced cells with different characteristics which, when exposed to different wavelengths emitted distinctive pattern of fluorescence, suggesting a differential chemical composition.

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Metabolites and proteins in mesquite pods (*Prosopis laevigata*)

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Prosopis laevigata (mesquite) is a wild legume tree that grows in semiarid regions of 17 Mexican States, where agriculture is a difficult activity due to the lack of water and poor soil fertility. Mesquite tree produces an indehiscent fruit with excellent nutritional properties, highlighting the content of sugars, high-quality protein, and phenolic compounds. Despite these important nutritional properties, the mesquite tree is an underutilized natural resource, mainly used for feed and charcoal production. In the present work, pods of mesquite trees were collected at the Mezquital Valley in Hidalgo, México. Pods were ground to obtain mesquite pod integral flour (MIF) and mesquite seed flour (MSF), both fractions were used for proteome and metabolome analysis. For metabolome analysis, both samples were separated using an HPLC system followed by a triple quadrupole mass spectrometer. For proteome analysis, both samples were analyzed using nanoflow liquid chromatography tandem mass spectrometry system (Q Exactive Plus). More than 100 metabolites were identified in mesquite integral flour and mesquite seed flour. The main five compounds by quantity found in mesquite pod integral flour and mesquite seed flour were syringaresinol, gallocatechin, ferulic acid, sinapic acid, and vitexin. In the mesquite pod integral flour were found 371 proteins and 131 in mesquite seed flour, 446 proteins were found in both mesquite pods fractions. Some identified proteins in both samples were beta conglycinin, vicilin-like, provicilin-like, 2 albumin-like and kunitz-type trypsin inhibitor. Metabolome evaluation shows that, in comparison with other legume seeds, mesquite pod has specific secondary metabolites like syringaresinol and that isoflavones are absent. On the other hand, the proteome evaluation shows that mesquite pods share similarities in proteins profile with the presence of storage proteins and enzyme inhibitors. This study corroborated the nutritional value of the mesquite pods and their similarity with other legume seeds used in human nutrition.

In silico analysis of maize VDAC sequences and production of the ZmVDAC1b recombinant isoform

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Voltage-dependent anion channels (VDACs) are the most abundant proteins in the mitochondrial outer membrane. They fulfill a variety of functions ranging from the exchange of high-energy molecules and metabolites to debatable functions such as cell death, having an important role between cytoplasmic and mitochondrial signaling events.
In plants, the study of VDAC is limited, what is known is that they are multigene families that code for several isoforms, which have been shown to participate in different types of biotic and abiotic stresses. However, to date there is scarce information about the VDAC family in important agronomic plants such as *Zea mays*. Here we present the identification and the *in silico* characterization of the VDAC family in maize (ZmVDAC), which is the starting point to contribute to the understanding of the ZmVDAC and their participation in stress responses and in the physiology of maize.
We identified 9 genes that comprise the *ZmVDAC* family through an *in silico* analysis, that have high potential for coding functional VDAC proteins. In addition, a nomenclature was proposed in accordance with the phylogenetic relations, naming the 9 isoforms of this family from ZmVDAC1a to ZmVDAC5b. Also, by obtaining the transcript levels along the germination time and in response to *F. verticillioides* infection was determined that ZmVDAC1b is one of the potential isoforms with an important function in maize. ZmVDAC1b was cloned and the recombinant protein in bacteria was produced. We continue to explore the participation of this isoform in the stress responses in maize and also to search protein interactors to search its role in cell death.

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Characterization of Plant Methionine Synthase: Highly Conserved Protein

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Folates are cofactors that donate one-carbon units for the biosynthesis of amino acids, nucleic acids, and other metabolites, such as S-adenosyl-methionine (SAM). Folate derivatives exist in nature in a variety of polyglutamyl forms (PG), and several folate-utilizing enzymes have more affinity to the PG forms than to the monoglutamyl forms (Rebeille et al., 1994). One such enzyme is Methionine Synthase (MS), which utilizes 5-CH_3-THF-PG as a cofactor for methionine (Met) synthesis. Subsequently, this residue donates one-carbon units to SAM synthesis, a precursor of other metabolites in different plant development processes. For instance, ethylene is produced during postharvest ripening, and polyamines and nicotianamine are formed in nodules during symbiosis. Arabidopsis contains three *MS* genes: *MS1* and *MS2*, which produce Met through the salvage pathway, and *MS3,* which synthesizes Met *de novo*. Our previous analysis suggests that *MS* gene expression and protein activity could be regulated by metabolites produced downstream, such as ethylene in ripening and Met or SAM during symbiosis between *Rhizobium* and legumes. The role of the interaction between MS enzymes and substrates-products in the development process has yet to be fully understood. This work aims to characterize the MS. We retrieved and compared the deduced MS sequences from various plants, predicted their subcellular localization, and analyzed their phylogenetic relationship and primary sequence. Additionally, we conducted a docking analysis between the protein and folate-PG. Furthermore, we determined the free amino acid involved in Met synthesis in fixing and non-fixing nodules during symbiosis between *Rhizobium* and *Phaseolus vulgaris.* We found that other plants encode at least two MS proteins, one cytoplasmic and one plastid isoform, similar to Arabidopsis. The plant MS protein sequences contained essential and highly conserved domains, including the Zinc binding domain, HCy/Met binding domain, and folate binding domain, indicating the crucial role of this in plants development.

Avocado Lipid Composition: Fatty Acid and Acetogenin profiles in seed and leaves.

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Avocado lipids are widely studied in the pulp; however, their full characterization in other tissues, such as seeds and leaves, remains incomplete. The present work characterized fatty acid and aliphatic acetogenin profiles in the germinating embryonic axis, cotyledon, and leaves from seedling and trees. Embryonic axis contained the highest amounts of fatty acids and acetogenins, and had the biggest fatty acid profile with 22 different species. Interestingly, during leaf development, carbon units were predominantly allocated in acetogenin pools, while seeds consistently had more carbon units in fatty acid pools. Thus, we hypothesized the use of the acetogenin acyl chain for energy production toward β-oxidation. Furthermore, correlations among all acetogenin and fatty acid species revealed negative association of acetogenin with unsaturated fatty acids such as linoleic, α-linolenic and docosahexanoic acid. Fatty acid extracts were also fractionated to obtain triglycerides (TAG), monoglycerides (MAG) + diglycerides (DAG) and polar lipids (phospholipids). Intriguingly, acetogenin backbones were found to be bound to polar lipids in all the analyzed avocado tissues. TAGs were widely consumed in cotyledon and polar lipids were mostly accumulated in leaves as they developed. In conclusion, this study provides valuable insights into the distribution and allocation of fatty acids and acetogenins in various avocado tissues, shedding light on their potential roles in energy metabolism and lipid regulation.

Association of maize cell cycle-related proteins with the repair protein RAD51A2

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Crop productivity is directly linked to the genome stability of cells that make up the seed. Genotoxic agents such as chemical molecules or radiation, as well as metabolic processeses during seed formation, can cause DNA damage. In some cases, the integrity of the genome is severely damaged, affecting metabolic processes that may trigger cell death.
Multiple DNA damage repair mechanisms exist, allowing eukaryotes to contend with the different types of damage. Most of the DNA repair mechanisms reported in mammals are conserved in plants, implying the presence of homologous proteins or functional analogues in eukaryotic organisms, and ATM and ATR kinases are examples of this.
Proliferation Cell Nuclear Antigen (PCNA) is a "platform" protein that promotes interaction between different proteins. PCNA is involved in cellular processes such as DNA replication, cell cycle regulation and DNA damage repair. PCNA appears to play a significant role in several repair mechanisms, particularly in Homologous Recombination (HR), in which the activity of RAD51 recombinase is essential.
Cyc/CDK protein complexes are crucial for cell cycle progression, a process that is inhibited upon detection of DNA damage. CycB1/CDKB1 complexes in plants are specifically involved in the repair of double-strand breaks by homologous recombination.
In this project, both *in silico* and *in vitro* studies in maize show the existence of protein complexes composed of cell cycle proteins such as PCNA and CycB1;2, and the repair protein RAD51A. In addition, it was found that the kinase activity associated to CDKB1;1 or CycB1;2 phosphorylate RAD51A2 suggesting the participation of CycB/CDKB complexes during the response to DNA damage.

How does the fusaric acid affect plant metabolism?: an approach in forestry and agronomic species

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Fusaric acid (FA) is a characteristic virulence factor of filamentous fungi of Fusarium genus, including the emerging species *Fusarium kuroshium*, which maintains a symbiosis with the ambrosia beetle *Euwallacea kuroshio*, allowing it to infect a wide range of tree species of agricultural and forestry importance. In this work, the *in vitro* effect of FA on hosts of *E. kuroshio* were evaluated, two species of agroeconomic importance: *Persea americana* and *Citrus sinensis*, and three species of forestry importance: *Salix lasiolepis*, *Liquidambar styraciflua* and *Populus nigra*. The leaves were exposed to FA (5 mM) for 72 h, later the evaluation of parameters related to cell death was carried out, in order to know the damage caused by FA. In addition, the plant samples were extracted with methanol and analyzed by liquid chromatography coupled with high resolution mass spectrometry (UHPLC-QTOF) to identify the metabolic pathways and metabolites altered by FA. The species most susceptible to damage was *L. styraciflua* followed by *S. lasiolepis*, *P. nigra* and *P. americana*, in *C. sinensis* no cell death was observed. The metabolomic analysis showed that the pathways altered by FA were fatty acids and chlorophyll metabolism, as well as the biosynthetic pathways of phenols and flavonoids, leading the latter to a specific study of the phenolic compounds present in the tested plants. A total of 40 phenolics were detected, the majority decreased their concentration after exposure to FA, however some others increased their presence and be related to their antioxidant (scopoletin and luteolin) or antifungal (ferulic acid, hesperidin and vanillin) activities, inhibition fungal secondary metabolism (kaempferol and quercetin) or cell walls reinforcement (coumaric acid, sinapic acid, *p*-coumaric acid). All above mentioned showed how each species has different response capacity when exposed to FA and contributes to the knowledge of the plant susceptibility to the ambrosia beetle attack.

Expression patterns of glycosyl hydrolases family 32 genes and their relationship with carbohydrate accumulation during the CAM cycle in *Agave tequilana*

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Agave tequilana is a succulent plant with great economic importance due to its ability to store high levels of fructans, which are used in the production of tequila. Fructans are the main storage polysaccharides in this plant, and their synthesis is carried out by four different catalytic enzymes: sucrose 1-fructosyltransferase (1-SST), fructan: fructan 1-fructosyltransferase (1-FFT), sucrose: fructan 6-fructosyltransferase (6-SFT), and fructan: fructan 6G-fructosyltransferase (6G-FFT). On the other hand, the degradation of fructans is performed by fructan exohydrolases (FEH) enzymes. These synthesis and degradation enzymes belong to the glycosyl hydrolase family 32 (PGHF32), which also includes vacuolar and cell wall invertases. *A. tequilana* is one of the few plants that combines fructan metabolism with crassulacean acid metabolism (CAM). However, the factors that regulate fructan metabolism in these species and how this metabolism integrates with CAM are not yet fully understood. Studying this would provide an important model for understanding the coordinated regulation of these processes.
Therefore, it is of great interest to investigate in detail the mechanisms of light and darkness regulation in *A. tequilana*. In order to identify key genes that regulate fructan and CAM metabolism, a transcriptomic analysis was carried out using RNA-seq under light and dark conditions. Additionally, gene expression of PGHF32 genes in the four specific phases of the CAM cycle was quantified using qPCR. Lastly, a carbohydrate profile analysis was performed to evaluate fructan accumulation during different stages of the CAM cycle and gain a better understanding of its relationship with PGHF32 gene expression.

Nβ motif from NaTrxh is a protein localization signal

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Protein localization is essential for activity protein regulation and overall cellular physiology. Each cellular localization is usually achieved by a transit peptide –when it is an organelle– or by a signal peptide (SP) if the protein means to be secreted. Secretory proteins containing an SP are co-translationally translocated to the endoplasmic reticulum (ER) to continue the well-known conventional secretory pathway, which involves the Golgi apparatus and secretory vesicles. However, the evidence of SP-lacking proteins has increased, and different secretion routes have been proposed –unconventional secretory pathways–.

In this work, we described new features of the secretion signal called Nβ motif, previously identified in the extracellular thioredoxin from *Nicotiana alata* NaTrxh. Unlike conventional SPs, the Nβ motif is a negatively charged hydrophilic short sequence (eleven residues long). However, the Nβ directed secretion utilizes the conventional secretory pathway elements (ER and Golgi). In addition, when the Nβ motif was fused to the C-terminus of GFP, it directed protein secretion through the ER (tested by adding the ER retention signal KDEL), suggesting that this motif acts as an ER-transit peptide rather than an SP.

Similar sequences to Nβ were found in other SP-lacking secretory proteins, suggesting a possible widespread usage of this motif as a secretion signal. Bioinformatic and experimental data indicated that Ser-8 and Ser-9 are essential for Nβ to act as a secretion signal at least in plant cells. When different thioredoxins with similar Nβ motifs where compared, two major groups were identified: (1) clustered together with NaTrxh, suggesting an extracellular localization; and (2) clustered with *At*Trxh2, which is known to be a mitochondrial protein. An interesting feature of this latter group is that they all contain Glu-8 instead of Ser-8, among other variations that possibly account for this atypical localization of this type of plant thioredoxins.

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Central carbon metabolism and Cell Cycle inter-relationship. Interactome map in maize.

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The machinery regulation of the cell cycle (CC) controls the progression of the cellular events taking place during proliferation. The Cyclin (Cyc) and Cyclin-Dependent kinases (CDKs) protein families are their main controllers. Cyc and CDKs form heterodimer complexes with serine/threonine kinase activity. Some well-recognized Cyc/CDK´s targets are RBR and Histone H1, two nuclear proteins that participate also in the regulation of the CC events.

Recently, unexpected molecular targets for Cyc/CDK complexes have been recognized. Among those phosphorylation targets are proteins involved in the control of carbon central metabolism such as PFK and PK, reported on cancer cell lines.

To address whether Cyc/CDK complexes were also able to be recognized and phosphorylate metabolic regulators controlling glycolysis in maize we initiated this work, analyzing pairwise protein-protein interactions by Pull-Down: one CC protein-one metabolic regulator.

After finding that some glycolysis enzymes were able to interact with CycD, CycB, CDKA, and CDKB we extended the analysis in search for more putative Cyc/CDK interacting and phosphorylation targets.

This work gives an insight into the CC interactome with metabolic regulators from different pathways: glycolysis, Krebs cycle, oxidative pentose phosphate pathway, anaplerotic reactions, and even the catalytic domain of SnRK.

We found that not all the metabolic proteins were able to interact with all the CC proteins analyzed, but in general, all metabolic proteins were able to interact with either a Cyc or a CDK, suggesting a probability that they are phosphorylation targets. Almost all metabolic proteins showed one or more CDK-phosphorylation motifs (full or minimal) in their amino acid sequences, but even glyceraldehyde 3 phosphate dehydrogenase, lacking any of those was phosphorylated by CycD2/CDKA or CycD2/CDKA in vitro and in vivo.

Results suggest that Cyc/CDK complexes have an important regulatory role in carbon metabolism whose scope we are only beginning to understand in maize.

Metabolic changes in bean fruit pericarp could contribute to seed development under severe restriction of nutrients

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The large demand for metabolic resources during seed development makes this stage very susceptible to adverse environmental conditions. The certainty that the negative effects of a harsh environment on plant productivity will worsen in the coming years needs a deep understanding of the mechanisms that plants use to cope with stressful conditions. Bean fruit pericarp not only protects developing seeds. This structure distributes nutrients and stores surplus sucrose to buffer changes in nutrient supply to developing seeds. The bean fruit pericarp is also photosynthetically active. The small contribution of the CO_2 fixed in the pericarp to developing seeds could be essential when the nutrient supply is restricted. All these facts together suggest that the metabolism of bean fruit pericarp might have a decisive influence on seed development. Our objective was to investigate whether pericarp metabolism changes to favor seed development in response to severe nutrient restriction. To do so, we interrupted the phloem continuity in fruits that were about to start the fast-growth seed stage. We used the incorporation of $^{14}CO_2$ by the fruit pericarp photosynthetic activity to assess how fruit metabolism responded to nutrient restriction. Our results show that the fruit photosynthetic activity did not increase; however, there was a dramatic change in how the photosynthetic products were distributed: the amount of the label found in starch was reduced, while the amount in the soluble fraction was increased. This change was accompanied by an increase in the amount of label detected in seeds. We are currently investigating if the synthesis of sucrose is stimulated in bean fruits affected by the reduction in nutrient supply.

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The Plant Mitochondrial Genome Repair by Homologous Recombination Mechanism

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Plant mitochondrial DNA (mtDNA) is exposed to a variety of physical and chemical agents that cause damage such as double-strand breaks. Repair of this type of damage is necessary to maintain the integrity of the mitochondrial genome, the function of mitochondria and therefore, cell viability. The homologous recombination (HR) pathway is the main double-strand break repair mechanism at plant mitochondria.
In *Arabidopsis thaliana*, a group of nuclear genes that code for proteins with mitochondrial localization signals have been identified, and their potential role in mitochondrial DNA repair via the homologous recombination pathway has been proposed. However, it has not been described a biochemical mechanism of homologous recombination. Understanding how homologous recombination occurs in the plant mitochondrial genome is important for understanding other phenomena characteristic of plant mitochondria, such as the low nucleotide substitution rate, as a replication pathway, the evolution of the mitochondrial genome, and the repair of double strand breaks.
In the present work, the heterologous expression and purification of *A. thaliana* proteins was carried out, such as the AtRecA2 and AtRecA3 recombinases, an AtRecX regulatory protein, single-stranded DNA-binding proteins (SSBs), helicases and a potential Holliday Junction mitochondrial resolvase AtYqgFi. In vitro biochemical assays for ATPase activity were carried out using thin layer chromatography, EMSA assays for DNA binding evaluation, D-loop formation assays, as well as assays with the potential resolvase AtYqgFi for binding to DNA substrates and cutting assays. The results obtained show that *Arabidposis* mitochondrial recombinases bind to DNA of non-specific sequence, of different length and structure, form D-loops, and have DNA-dependent ATPase activity, which is inhibited by the AtRecX protein. On the other hand, the AtYqgFi protein binds preferentially to DNA substrates that emulate Holliday Junctions and cuts these substrates, demonstrating its potential role as a possible resolvase.

Two Multidrug And Toxic Compound Extrusion (MATE) proteins putatively involved in alkaloid transport in *Argemone mexicana*.

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Argemone mexicana produces the benzylisoquinoline alkaloids (BIA) berberine, sanguinarine and chelerythrine. These alkaloids participate in plant chemical defense and also have pharmacological interest. Tissues involved in alkaloid biosynthesis and accumulation differ in this plant and these discrepancies suggest the participation of long-distance mobilization mechanisms, mediated by transporters. AmABCB1, a B-subtype of ATP Binding Cassette protein (ABC) from roots and immature seeds participates in berberine and sanguinarine mobilization (1). In addition to, BIA can also be transported by Multidrug and Toxic Compound Extrusion proteins (MATE). A MATE transporter; CjMATE1, was suggested to import berberine into vacuoles in *Coptis japonica* elicited cell cultures (2).

Using MATE signatures from the Pfam PF01554, we searched for putative MATE's sequences in a seedling *A. mexicana* transcriptome. 16 candidates were retrieved and 5 were selected for a phylogenetic tree (neighbor-joining algorithm; 1000 bootstraps). Other well characterized MATE transporters, such as the NtJAT1 from *N. tabacum* were also include. Two clades were formed, one of them associated to the *Coptis* CjMATE1, whereas the other was closer to the *Nicotiana* NtJAT1. Clade 1 and 2 grouped 3 (AmMATE-1 to –3), and 2 (AmMATE-4 and –5) *A. mexicana* MATE candidates respectively. One member of each group was picked for isolation and characterization. The *Coptis* related AmMATE-1 sequence displayed the typical RXSNELGA and CGQA domains and it was mainly expressed in leaves and roots. Moreover, transient expression of CFP-fusion proteins in *N. bentamiana* suggested localization in tonoplast. On the other hand, the *Nicotiana* related AmMATE-5 expression has been detected mainly in roots.

The role of these transporters on alkaloid mobilization in this plant will be discussed.

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References

[1] Loza-Muller et al. 2021. Planta, 65:4.
[2] Takanashi et al. 2017. Phytochemistry, 138: 76.

Identification of transcription factor genes possibly involved in the regulation of the short branched-chain fatty acid pathway of capsaicinoid biosynthesis in chili pepper fruit (*Capsicum annum* L.)

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Chili pepper fruits contain a very peculiar group of substances, the capsaicinoids, which are derived from the condensation of vanillylamine, derived from the phenylpropanoid pathway, and 8-methyl-6-nonenoyl-CoA, produced from the branched-chain fatty acid pathway. This capsaicinoid is found in high concentrations in the placental septum of the fruits, although other studies describe their presence in other tissues of the plant, albeit in low concentrations. Capsaicinoids are known to serve as a defense mechanism against pathogens and have been demonstrated to have medicinal effects. The branched-chain fatty acid biosynthesis pathway is regulated at the transcriptional level, but the specific transcription factors (TF) involved in this pathway are unknown. Potential candidates were identified using an algorithm in the R package called "*Salsa*", which was determined based on the expression profiles (SEPs) of structural biosynthetic target genes in fruits at different developmental stages (DPA). Four candidates were selected, which belong to the WHY, bHLH, WRKY families, and the SCARECROW subfamily. Subsequently, to test each TF gene function, virus-induced gene silencing (VIGS) will be performed, using the TRV1/TRV2 viral vector bearing a partial sequence of each selected candidate TF gene, will be carried out by *Agrobacterium tumefaciens infiltration* on the underside of the leaves and in the apical meristem to achieve a more efficient infection. RT-qPCR analyses will be performed to observe the absence of gene expression in genes involved in the fatty acid biosynthesis pathway. HPLC assays will be conducted to quantify the capsaicin content in control and silenced fruits. Chili pepper fruits from silenced plants with some of the candidate genes will exhibit lower capsaicin levels than the control fruits. These analyses represent an important lead in the study of transcription factors and could provide insights into obtaining chili pepper varieties with high or low levels of pungency.

TOR signaling in the oil-producing green microalga *Botryococcus braunii*

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The colony-forming green microalga *Botryococcus braunii* produces large amounts of petroleum-like hydrocarbons that can be used as renewable feedstock for producing combustion engine fuels. These hydrocarbons are mainly stored in an extracellular matrix in contrast to most other oleaginous microalgae, which accumulate lipids in the cytoplasm. *B. braunii* is grouped into at least three chemical races (A, B, and L) based on the type of hydrocarbon produced. *B. braunii* hydrocarbons are derived from fatty acids in A race, or isoprenoid precursors generated by the plastidial methylerythritol phosphate (MEP) pathway in B and L races. However, its potential use for biofuel feedstock production is hindered by its characteristically slow growth rate. The target of rapamycin (TOR) is a conserved eukaryotic kinase that regulates the cell growth and metabolism in coordination with the energy and nutrient status. Manipulation of TOR signaling pathway in microalgae may help to overcome the metabolic trade-off between biomass production and accumulation of energy storage molecules. In this work, we inhibited the TOR kinase in *B. braunii* race A (Yamanaka strain) and race B (Showa strain) cultures with AZD-8055 in order to analyze its effect on algal cell growth, photosynthetic pigments and extracellular hydrocarbon content. Both *B. braunii* races A and B, showed inhibition of cell growth and enhanced carotenoid accumulation in response to TOR inhibition. Under AZD-8055 treatment, no significant differences in hydrocarbon content were observed in *B. braunii* race B cells, whereas *B. braunii* race A cells showed an increased in hydrocarbon accumulation. These results indicate that TOR modulates the carotenoid accumulation and fatty-acid derived hydrocarbon production, but not triterpene hydrocarbon, in *B. braunii* cells.

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Insights into metabolome changes in *Carica papaya* (L.) fruit at different ripening stages under artificial maturation.

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Carica papaya (L.) belongs to the Caricaceae family and is cultivated in tropical and subtropical regions worldwide. The ripe fruit is the most consumed part of the plant. During growth, the pericarp is green, shiny, and firm (pre-climacteric state). As the papayas reach physiological maturity, they undergo an ethylene burst (climacteric state), turning from yellow to orange/red and reducing skin rigidity. Metabolomics has been utilized to profile papaya fruit metabolites, identifying differences between varieties and assessing fruit quality during the supply chain. Commercially, the transition to the climacteric state is induced by agents like calcium carbide (CAC_2). In this study, we describe significant metabolome changes in three ripening stages of papaya fruit (cv maradol) ripened with CAC_2. We use MS-based metabolomics and chemometrics to analyze the peel, pulp, and seed to understand metabolite turnover under this condition. Our findings reveal decreasing mass-to-charge ratio (m/z) signals over time, showing a correlation between flesh and peel but not the seed. This suggests the appearance of climacteric compounds in the peel and pulp, distinct from the seed. The study demonstrates CAC_2's role in producing metabolites in papaya fruit and sheds light on the speed of metabolic pathway readjustments at different tissue levels. Moreover, it enables the detection of metabolites as molecular markers of artificial maturation during ripening stages. In summary, our study elucidates the effects of CAC_2-induced ripening on the metabolome of papaya fruit, providing valuable insights into the underlying processes and facilitating the identification of molecular markers for different ripening stages.

Phosphorylation of DPE2 at S786 by SnRK1 kinase partially regulates starch degradation.

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In plants, transitory starch is synthesized during the day and degraded at night to provide the carbon needed for growth and development. Starch metabolism is a highly coordinated process that requires metabolic reprogramming to adjust to changes in environmental stimuli. Within the process of starch degradation, maltose is exported to the cytosol, where disproportionating enzyme-2 (DPE2) is responsible for its metabolism. We found that the amount of DPE2 remained unchanged, but its activity increased at the end of the day and during the night, suggesting that posttranslational modification drives one mechanism underlying the regulatory activity of this enzyme. Sucrose nonfermenting-related kinase-1 (SnRK1) is a metabolic sensor that induces catabolic processes and represses anabolic processes by directly phosphorylating key metabolic enzymes to maintain energy homeostasis. We showed that SnRK1 was able to interact and phosphorylate DPE2 at three different residues located in the a-glucanotransferase domain. Complementation of *dpe2*-deficient mutants with the wild-type (WT) and S786A forms of DPE2 showed that the latter only partially restored starch degradation, suggesting that in addition to S786, phosphorylation of other residues is also involved in enzyme regulation. We are currently complemented *dpe2* mutant plants with a triple nonphosphorylatable form of DPE2 to characterize plant growth and sugar metabolism.

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Germination of four materials of common bean (*Phaseolus vulgaris*)

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To improve the global food system the consume of legumes must increase, due to their capacity to fix nitrogen and its dense content of nutrients, mainly minerals, fiber and protein and active compounds, legumes are an important group of food to configurate healthy and sustainable diets. Common bean (*Phaseolus vulgaris*) as other legume foods are an extraordinary source of nutrients, however, the presence of antinutrients such as phytic acid, oligosaccharides and protease inhibitors can reduce their nutritional quality. The germination of common bean seed can be used to decrease the content of these negative components and to improve the content of active compounds and essential amino acids. The germination process is a complex biochemical activation, where the seed changes their composition in order to produce a new plant. Imbibition or water uptake by the seed is the first step that led to enzymatic and metabolic activation, respiration and the metabolic pathways involved are the main drivers of the composition change. In order to describe the imbibition and germination process of common bean, in the present work we evaluate four materials of common bean in its water uptake velocity (2, 4, 6 and 8 h) and germination response. The water uptake for the four materials (8025, Otomi, Eugenia and Dalia) after two hours of imbibition were 9, 7, 7 and 7 %, respectively, meanwhile at the hour eight were 38, 29, 23 and 29 %, respectively. Once the imbibition process was ended, the seed were stored in darkness and the germination was evaluated after 48 h. After 8 hours of imbibition and 48 h of germination the percentage of germination for the four materials were 35, 25, 26 and 11 %, respectively. The water uptake is different in the four material and it has impact in the seed activation and germination.

Novel insights into plant G-protein structure, biochemistry, and regulation.

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Heterotrimeric GTP-binding proteins are membrane-bound signal transducers that regulate various cell signaling pathways. The heterotrimeric complex, comprising the α, β, and γ subunits, functions via bimodal active versus non-active states, and the transition between these states relies on the biochemical properties of the Gα subunit. Signaling begins when GTP is exchanged for GDP on Gα, leading to the dissociation of the heterotrimer into two functional entities, the GTP-bound Gα and the Gβγ dimer. GTP hydrolysis by Gα, which is also a GTPase, deactivates the signal allowing the heterotrimer's regeneration. The plant Gα proteins have relatively slow intrinsic GTPase activity and require a GTPase-Activating Protein (GAP), such as Regulator of G-protein signaling (RGS), to promote catalysis and have an effective deactivation.

We are studying two interrelated aspects of the regulation of G-protein signaling. Our first approach focuses on studying the posttranslational modifications (PTMs) of the Gα subunit. Arabidopsis AtGPA1 undergoes several PTMs, such as phosphorylation, oxidation, N-glycosylation, sumoylation, and ubiquitination. However, the precise influence of these PTMs on the biochemical properties of AtGPA1 and its interaction with regulatory proteins has yet to be explored. In our study, we performed site-directed mutagenesis on specific amino acids involved in different PTMs, generating multiple variant proteins of AtGPA1. We subsequently assessed the effects of these variants on the biochemistry of AtGPA1 using BODIPY-based fluorescent assays and found that specific PTMs affect the GTPase activity of Gα.

Our second approach focuses on the regulation of the GTPase activity of Gα. We obtained crystallographic structures for two plant species: *Selaginella moellendorffi* (SmGα) and *Oryza sativa* (rice, RGA1), and found conserved structural features of the plant Gα subunits. Using molecular dynamic simulations, we examined the SmGα-SmRGS interaction and identified crucial residues in Gα that interact with RGS. These interactions and their contribution to the RGS-mediated GTPase activity of Gα were further validated using yeast-two hybrid and GTPase activity assays. We found that the recognition between plant RGS and Gα proteins is conserved across plant species. Together, our data provide valuable insights into the regulatory mechanisms of plant G-proteins.

uORFs in the polyamine oxidases of *Arabidopsis thaliana*: a mechanism of translational regulation

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Polyamines (PAs) are polycationic derivatives of amino acids that are essential for development and growth in living organisms. Under stress conditions, plant cells modulate PA levels by regulating the enzymes responsible for their biosynthesis and catabolism through various elements, one of which are the upstream open reading frames (uORFs) found in the 5' UTR region of mRNAs. The translation of the biosynthesis enzyme SAMDC is regulated by two uORFs: at high PA concentrations, the small-uORF represses the translation of the main open reading frame (mORF) by inducing ribosomal stalling, while at low concentrations, the tiny-uORF stimulates ribosomal reinitiation in the mORF. Polyamine oxidases (PAOs), catabolic enzymes, are also regulated by uORFs. We are currently performing the characterization of the uORFs found in *At*PAO2, *At*PAO3, and *At*PAO4. Using *Arabidopsis* GUS reporter lines, we found that the uORF present in *At*PAO2 exerts a repressive effect on the translation of its mORF. This repression is PA-dependent, and we show that translation of the uORF is essential to exert the regulatory effect. Moreover, the N-terminal region of the uORF-*At*PAO2 peptide displays a high degree of conservation. To analyze their role in translational represión, we generated several reporter constructs that included specific point mutations in conserved amino acids of the uORF-PAO2 peptide (Leu-7, Ser-14, Leu-21-22). In addition, we are functionally characterizing the *At*PAO3 and *At*PAO4 uORFs using wild-type (WT) and mutant (without ATG) constructs.
Collectively, the data obtained from these uORFs will offer compelling evidence regarding the uORF-mediated regulation of polyamines in their respective metabolic genes.

Glycolytic enzymes interact with cell cycle proteins in maize

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Cyclin/cyclin-dependent kinase (CDK) heterodimers have multiple phosphorylation targets and may alter the activity of these targets. Enzymes from different metabolic processes are among the phosphorylation targets, that is, central carbon metabolism enzymes. This work explores the interaction of Cyc/CDK complex members with the maize glycolytic enzymes hexokinase 7 (HXK7) and glyceraldehyde-3-phosphate dehydrogenase (GAP), phosphofructokinase (PFK) and pyruvate kinase (PK).
HXK7, GAP, PFK and PK interacted with several cell cycle proteins, including CycB1;2, CycB2;1, CycD2;2, CDKA;1, and CDKB1;1. However, in the pull-down assay, only PFK interacted with CDKB1;1. However, Cyc/CDKB1;1 recombinant complexes phosphorylated the four enzymes and decreased the enzymatic activity of HXK7, GAP, and PFK. After kinase assay, treatment with a CDK-specific inhibitor (RO-3306) or lambda phosphatase restored total HXK7 activity but not GAP activity. In enzymatic assays, increasing concentrations of CDKB1;1, but not of CycD2;2, CycB2;1 or CycD2;2/CDKB1;1 complex, decreased GAP activity. Cell cycle regulators may modulate carbon channeling in glycolysis by two different mechanisms: Cyc/CDK-mediated phosphorylation of targets (e.g., HXK7; canonical mechanism) or by direct and transient interaction of the metabolic enzyme (e.g., GAP) with CDKB1;1 without a Cyc partner (alternative mechanism). The Cyc/CDK complexes effect over PFK and PK is still in work, but preliminary results on PFK show a similar decreasing enzymatic activity.

In situ localization of enzymes involved in fructan metabolism in *Agave tequilana* Weber var. azul.

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Fructans are fructose polymers found in 15% of angiosperm species which function not only as reserve carbohydrates but also as osmotic regulators, are strongly associated with succulence and are involved in signaling responses to different types of stress and stages of vegetative and reproductive development. Fructan metabolism is controlled by the action of various enzymes. In *Agave*, fructans are synthesized from sucrose molecules initially by the action of the 1-SST enzyme and are elongated at the fructose or glucose residues by the catalytic action of several types of fructosyltransferases (FTs) such as 1-FFT, 6G-FFT, and 6-SFT that add fructose units. Fructan exohydrolases (FEHs) are the enzymes in charge of degrading fructans and releasing fructose residues. Due to these functions, it is likely that the balance between fructan synthesis enzymes (SSTs and FFTs) and degradation enzymes (FEHs) play a key role in modulating the structure and degree of polymerization (DP) of fructans, as well as determining their storage in vacuoles, mobilization, and distribution in different tissues in response to plant needs during vegetative or reproductive development. Although evidence exists for the presence of fructans in the vacuoles and apoplast, the precise localization of the associated enzymes in *Agave tequilana* tissues is unknown. Identifying their localization at the tissue and subcellular level would provide valuable information on their roles in the overall regulation of fructan metabolism.

Transcriptome-based identification and functional assays of three transcription factor genes possibly involved in the regulation of carotenoid biosynthesis in chili pepper fruits (*Capsicum annuum* L.)

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Chili peppers (*Capsicum* spp.) synthesize and accumulate carotenoids, the pigments responsible for the yellow, orange, and red colors of fruits. Carotenoids are important complementary pigments for photosynthesis and function as protectants against ROS. The interest in chili pepper fruits has increased due to the nutraceutical properties of carotenoids because they have antioxidant and anti-inflammatory activities and increase the immune response preventing cardiovascular diseases and some types of cancer. The biosynthetic pathway of carotenoids in chili pepper fruits has been extensively investigated; however, its transcriptional regulation is still unknown. In this study, we identified 3 transcription factors (TF) (ERF113, WRKY31 and WRKY20) possibly involved in the regulation of carotenoid biosynthesis by RNA-Seq co-expression analysis of profiles from the *β*-carotenoid hydroxylase (*β-CHX*) and capsanthin-capsorubin (*CCS*) structural genes with those of all transcription factor sequences of our chili database ("Salsa"), and also through the identification of putative binding sequences of the TF candidates in the promoters of *β-CHX* and *CCS* genes. Function analysis of the selected TF candidate genes was carried out by virus-induced gene silencing (VIGS) using a modified agroinfiltration protocol, with *Tobacco rattle virus* (TRV)-derived constructs bearing partial sequences from *ERF113*, *WRKY31 and WRKY20*. Molecular analysis and phenotypification help us to corroborate the efficiency and persistence of the silencing process in 1, 3 and 12-week-old plants grown in a room with controlled temperature at 24 ± 2 °C. A positive correlation between virus-induced silencing of *ERF113*, *WRKY31, WRKY20* genes (a reduction in gene expression) and a decrease in the total carotenoid levels was observed indicating their participation in the transcriptional regulation of carotenoid biosynthesis in chili pepper fruits. Chemical analysis by HPLC will be performed to confirm the concentration and accumulation of specific carotenoids in chili pepper fruit at different developmental stages.

New method to identify lncRNA that interacts with lipids in plants

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Research on long non-coding RNAs has described new interactions, such as lncRNAs that interact with lipids that have shown biological functions. In plants, the lncRNAs have an important role in growth, development, and their response to abiotic stress [1]. Phospholipids also have relevant roles in signaling, development, and stress, among other functions [2]. Research on phospholipids with biologically relevant processes outside membranes, as well as RNA-lipid interactions, is a growing field of research that shows the need for new methods to explore the identity of these RNAs. Most research on lncRNAs, mostly about the interaction with phospholipids, has been conducted in animals or for medical applications, and lncRNA research in plants is relatively lacking. In this work, we describe a method for the selection of RNAs that can interact with lipids in plant (*Stenocereus queretaroensis).* Our results evidence a population of RNAs enriched in the lipid-coated beads and control beads, of which 22,348 were bound genes with PA and PIP2 interaction, and 44 lncRNAs were enriched in this lipid interaction. Then this analysis, four lncRNA were selected, and using an RT-PCR analysis was identified that XR_002284730 and XR_707783 showed PIP2 and PA interaction, respectively.

Bayona-Hernandez, A., Guerra, S., Jiménez-Ramirez, I. A., Sztacho, M., Hozak, P., Rodriguez-Zapata, L. C., Pereira-Santana, A., & Castaño, E. (2023). LIPRNAseq: a method to discover lipid interacting RNAs by sequencing. *Molecular Biology Reports*. https://doi.org/10.1007/s11033-023-08548-5

Wang, X., Fan, H., Wang, B., & Yuan, F. (2023). Research progress on the roles of lncRNAs in plant development and stress responses. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1138901

Yao, H. Y., & Xue, H. W. (2018). Phosphatidic acid plays key roles regulating plant development and stress responses. In *Journal of Integrative Plant Biology* (Vol. 60, Issue 9, pp. 851–863). Blackwell Publishing Ltd. https://doi.org/10.1111/jipb.12655

The application of a synthetic dsRNA based on miR1917 triggers constitutive triple response in tomato seedlings.

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The field of RNA interference-based technology for crop improvement is rapidly emerging, with a principal focus on resistance against pests and pathogens. The mechanisms of RNA interference for posttranscriptional gene silencing bear a resemblance to those involved in microRNA silencing. As the most extensively studied non-coding RNA, microRNA has numerous databases dedicated to it, which provide valuable insights into their sequences and targets. In this study, our goal was to investigate the expression of an endogenous gene triggered by the application of a synthetic dsRNA molecule. This molecule was predicated on the microRNA and target interaction pair miR1917-CTR4sv3, which is linked to ethylene perception in tomato plants. The experiment was carried out on young tomato seedlings, which were treated through germinated seed soaking in solutions of dsRNA at 10 and 100 pmol/µL concentrations, using water as a control. The results demonstrated the distinctive triple response, characterized by a reduction in both root and hypocotyl lengths by up to 81% and 60% respectively at a concentration of 100 pmol/µL, accompanied by noticeable hook formation observed at 10 pmol/µL. Additionally, the expression of CTR4sv3 was reduced by up to 81% at the highest concentration of dsRNA. These results serve as a proof of concept that knowledge of microRNA can indeed be instrumental in developing strategies pertinent to RNA interference-based technology in plant species.

Physicochemical properties of corn and ayocote bean tortilla chips

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Current food industry research trends emphasize the need for designing high-value food products that can balance convenience and health benefits, including higher amounts of protein. This research was aimed at preparing snacks using corn and ayocote bean flour. The baked tortillas were formulated according to a central composite design with two variables: bean/corn flour ratio (3/7, 5/5, 7/3) and particle size (420-177 µm). Textural analysis, viscosity analysis, Fourier Transform Infrared (FTIR) spectroscopy, and proximate analysis were performed to evaluate the physicochemical properties and protein increase of the snacks. Higher bean/corn flour ratios (7/3) lead to a decrease in maximum viscosity of 50% in baked tortillas compared to lower bean/flour ratio samples (3/7). The hardness values found in our study (13.52 ± 0.81 N) in bean/corn flour ratio of 3/7 are higher than results in bean/corn flour ratio of 7/3 (12.145 ± 0.61 N) and less brittle 1.44 ± 0.43 mm and 2.99 ± 0.32 mm, respectively. FTIR spectra and protein determination studies showed an increase in protein content with the bean flour addition. Samples with higher bean/corn flour ratio (7/3) snacks showed the development of α-helix protein structures on the 1650 cm-1 wavelength and molecular inter (1625 cm-1) and intra (1616 cm-1) associations within the Amide III bands compared to corn flour snacks (control). Furthermore, the addition of bean flour leads to an increase in protein levels (18.926±0.8). These results demonstrate that the addition of bean flour improves the protein quality of snacks, and the mixture with corn flour as a source of starch and its well-known textural properties can be balanced to produce a functional product.

Genetic transformation of the oleaginous microalga *Neochloris oleoabundans* by biolistic using glass particles

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Microalgae are unicellular photosynthetic organisms that exhibit a great biological diversity estimated in more than 200,000 species, each with a different ecological role. They constitute an attractive organism for application in industry with advantages over other systems such as low production costs and ability to no need of arable land. The green microalgae *Neochloris oleoabundans* produces a high amount of triacylglycerols (21-80% of total lipids) which may be converted into biodiesel through a methylation reaction, as an interesting alternative to replace petroleum-derived fuel. To exploit the potential of *N. oleoabundans* its necessary the establishment of genetic transformation protocols to improve its characteristics. More than 30 types of seaweed have been reported to have been successfully transformed by various transformation methods. Up to now, *N. oleoabundans* has been transformed just by electroporation. So, the purpose of this work is to establish a protocol for the genetic transformation of *N. oleoabundans* through biolistic using glass particles. These were coated with plasmid DNA (pChlamy_4: GFP), and different parameters were evaluated such as the shooting distance, bombardment pressure, osmotic pre-treatment, plasmid concentration, and the use of linear or circular plasmid. Microscopy test shows that transformation of *N. oleoabundans* was possible by biolistic, and the use of glass particles instead the commonly used gold or tungsten particles represents an advantage because of the low costs of the process. The procedure will be confirmed by PCR detection of the *GFP* gene insertion.

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Extractability of soluble solids from integral flour and hard coat of mesquite pods (*Prosopis laevigata*)

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Legumes are plants that can fix nitrogen, this property improves their capacity to produce biomass. Mesquite (*Prosopis laevigata*) is a legume tree that grows in arid land and produce a fruit. Mesquite fruit is a pod with a developed mesocarp and a hard coat that protect the seed. Mesquite pod is an important source of sugars (45%), mainly sucrose, glucose and fructose, substrates for biotechnological process. With the aim of use these materials for the formulation of grow media for fermentation process, in the present work the integral flour of the mesquite pod and the residual hard coat were used for the evaluation of the extractability of sugars as soluble solids. Both fractions were extracted with different levels of water (solids:water - 1:2, 1:2.5, 1:3 and 1:3.5 ratio) and the soluble solids of the mix were measured as Brix degrees. For the integral flour the Brix degrees were 25.4, 20.5, 17.3 and 15.1, respectively. For the hard coat fraction the Brix degrees were 9.3, 7.3, 6.1 and 5.6, respectively. When the dilution was expressed as percentage, the soluble solids extractability for integral flour was expressed by the equation $Y = 0.701 X + 0.25$ and for the hard coat fraction was expressed by the equation $Y = 0.3125 X – 2.23$, where Y was Brix degrees and X was percentage of dilution, with a correlation factor of 0.935 and 0.858, respectively. A dilution of 33 % of integral flour in water produce a solution with 25.4 Brix degrees and a dilution of 33 % of hard coat in water produce a solution with 9.3 Brix degrees. This evaluation highlights the potential of mesquite fraction for the formulation of grow media for biotechnological process.

EVALUATION OF THE INSECTICIDAL EFFECT OF ESSENTIAL OIL OF *Pelargonium citrosum* FOR THE CONTROL OF *Pseudococcus longispinus*

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Insects are very important in ecosystems, however when their population grows to the point of causing economic losses they are considered a pest, this is the case of *Pseudococcus*, a hemiptera that decreases the yield of agricultural crops and impacts ornamental gardening. Although there is a wide range of organophosphorus insecticides to control these organisms, they have deleterious effects on the environment and human health. On the other hand, essential oils, given their complex chemical composition, could be the basis for formulating biopesticides that present a lower degree of Therefore, the objective of this research is to evaluate the insecticidal activity of the essential oil of *Pelargonium citrosum*, estimating the mortality index of nymphs and adults of *Pseudococcus longispinus* under laboratory conditions. The method consisted of 4 phases; a) the obtaining, identification and maintenance of *P. longispinus*, b) the obtaining of the oil by hydrodistillation, c) the toxicity bioassays, where 5 concentrations were evaluated, 1 control and 1 blank, in a total of 240 insects, whose mortality was recorded at 6, 12 and 24 hours and d) the chemical characterization of the oil by high performance liquid chromatography coupled to masses. The extraction yield by hydrodistillation for 1 kg of vegetable matter was 2.99%. The lethal concentrations (LC50) obtained were; for 6 hour LC50 0.91 mL/L, 12 hour LC50 0.33 mL/L, and 24 hour LC50 0.002 mL/L. Regarding the chemical composition, the oil presented 60 components, among which geraniol stands out. Regarding the insecticidal effect, it was shown that the more time elapses, the concentration of the oil in the standard solution decreases. Finally, *P. citrosum* oil has the potential to develop botanical insecticides and could even be an alternative to organophosphate insecticides such as Diazinon.

Flocculation methods for *Haematococcus pluvialis* to extract astaxanthin.

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Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a red pigment that belongs to a group of chemicals called carotenoids. The natural sources of astaxanthin are algae, yeast, salmon, trout, krill, shrimp, and crayfish. It is an antioxidant and has been used to protect cells from damage. Astaxanthin has been proposed to improve the way the immune system functions and for many other purposes, including Alzheimer disease, athletic performance, aging skin, muscle soreness from exercise, among others. We are interested to produce astaxanthin from the microalgae *Haematococcus pluvialis*. It is accumulated as astaxanthin monoesters in specific hydrophobic deposits in the cytoplasm composed of (neutral) lipid droplets. Harvesting microalgae from liquid medium is known to be energy-intensive, and finding cost-effective methods is crucial for the processing of microalgae. While centrifugation is the most used method, it requires a significant initial investment and operational cost. On the other hand, flocculation has been identified as a promising alternative due to its lower initial investment and operational cost, as well as its ease of use. However, there is limited knowledge regarding the effectiveness of flocculants for *H. pluvialis*. Our study presents a wide arrangement of flocculants tested on *H. pluvialis* cultures along with their influence on the disruption-less astaxanthin extraction. We found the optimal concentrations for the different flocculants presented on a range of pH, along with the effect upon the extraction of astaxanthin after the cyst germination of *H. pluvialis*. We expect the present results promote the usage of an environmentally friendly flocculant such as chitosan or cationic polysaccharides, to replace the centrifugation step in the harvesting of microalgae. It can be coupled with cell disruption-less methods of astaxanthin extraction, reducing upfront and operational costs of two of the crucial steps to successfully exploit microalgae products.

Rapid genotyping of plant samples using TaqMan™ assays

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Insertional mutagenesis is a routine method used in reverse genetics, but commonly-used end-point PCR molecular screening methods are flawed because they rely on negative results and require labor intensive gDNA extractions. During our work with maize transposon insertion mutants, we developed TaqMan™ genotyping assays that allow us to compare the copy number of specific alleles of a gene of interest to an internal control. The method is effective with 'on-the-fly' template prep with no purification that allows for streamlined genotyping of up to 95 samples in ~2 hours. TaqMan™ genotyping has been effective in both identifying all possible genotypes (wild type, heterozygous, and homozygous) for insertional mutants and for determining transgene copy numbers of individual transformation events in transgenic lines. Expression of maize transposon-insertion alleles was determined and the correlation between the transposon's insertion location and its ability to affect gene expression was examined.

The chitin-binding domain from the chitinase ChiA74 presents antimicrobial activity against plant pathogens.

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The development of alternatives to control plant pathogens safer for the environment has been of great interest in the last years. In this sense, peptides have become a popular research subject in plant protection as antimicrobial inducers. Plant lectins and defensins are proteins with chitin-binding activity that exhibit antibacterial and/or antifungal activity, nevertheless little is known about similar proteins from bacteria. The chitinase ChiA74 is synthesized by a Mexican strain of *Bacillus thuringiensis*, an enzyme with a modular organization and a chitin-binding domain in the N-terminal. The chitin-binding domain (ChiA74's CBD) has not been characterized as a single domain separated from the enzyme, and it is unknown whether it has antimicrobial activity, as has been reported for other carbohydrate-binding modules and proteins with chitin-binding activity. This study aimed to evaluate the antimicrobial activity against bacterial and fungal plant pathogens. To study the antimicrobial properties, we produced the ChiA74's CBD as a single domain separated from the enzyme in *E. coli* BL21 and purified it as a protein of ~14 kDa. To evaluate its antimicrobial activity, both, the agar well diffusion and turbidity method have been used. For the antifungal activity, the inhibition of conidia germination was evaluated against *F. oxysporum*. The ChiA74's CBD possesses antibacterial activity against *Pseudomonas syringae* with a MIC of 230 µg/mL. Besides, it inhibits the total germination of conidia of *Fusarium oxysporum* (MIC=192 µg/mL).
These results demonstrate that the ChiA74's shows potential for utility as a biological agent for the control of various economically important pathogens of plants.

Comparative evaluation of different extraction methods for identification and quantification of glyphosate in fortified corn flour

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Over the past decade, the use of glyphosate, and molecules related to its catabolism as the active principle of many herbicides in crops have significantly risen despite scientific evidence which suggested harmful effects on human health. This pesticide is difficult to detect in foods and related matrices because of its chemical features (amphoterism, high polarity, non-volatility, low molecular weight, and hydrophilicity). Therefore, establishing reliable and scalable extraction and quantification pipelines for glyphosate in crops and food-associated products is of great interest. In this work, we tested different concentrations of solvents and various extraction protocols for recovering this pesticide without the use of derivatization steps in corn flour as an experimental extraction matrix. We used dynamic multiple reaction monitoring methods with ultra-high resolution liquid chromatography coupled to a triple quadrupole mass spectrometer for quantification. The results show that water with 20% (v/v) methanol was the best solvent for extraction. Accelerated solvent extraction (ASE) and ultrasonication approaches allowed better recovery values, however, the extraction with the energized dispersive extraction system (EDGE) exhibited an efficient result in half of the time compared to the other automated protocol tested in our study. This investigation provides valuable information for the extraction, identification, and quantification of glyphosate, which will contribute to monitoring the level of this pesticide in corn flour.

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ABSTRACT

Optimization of an HCR-RNA FISH method to rapidly determine gene expression patterns in C4 leaves.

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The function of a gene depends to a great extent on its specific spatiotemporal expression patterns. Defining these patterns accurately is crucial to not only determine transcript localization, but also to infer genetic organization and regulatory dynamics. For years, the main methodology to study transcript localization has been *in situ* hybridization (ISH), which relies on complementary binding of a nucleotide probe to the target sequence without losing the spatial context. The relatively recent implementation of fluorescent *in situ* hybridization (FISH), and in particular hybridization chain reaction (HCR) technology, has allowed the simplification of transcript detection methods. It magnifies the localization signal while reducing background noise, making it possible to detect low abundant transcripts. But despite these advances, we still lack protocols to work with photosynthetic tissues such as leaves. In this work, we standardized a method for whole mount HCR-RNA FISH in sectioned leaves of the C4 grass *Setaria viridis*. Good sample preservation and high signal to noise ratio was achieved, leading to discovery of new spatial patterns for relevant developmental genes and to confirm transcript localization of putative marker genes identified in previous studies. These include the novel identification of *Short-Root* gene expression (SvSHR1 and SvSHR2) in the *bundle sheath* of C4 leaves and the validation of cell populations defined by scRNAseq experiments. Overall, the implementation of this protocol allows efficient investigation of spatial gene expression patterns in leaf tissues, with consistent and meaningful results.

Functional validation of enhancer-based synthetic promoters in *Arabidopsis thaliana*

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Enhancers are one of the most important distal *cis*-regulatory elements (CREs) in eukaryotic genes and are responsible for mediating gene expression in different tissues, developmental stages, and in response to environmental cues. Despite the potential application of these CREs for direct gene expression, there have been few studies for this purpose and few information is disponible about how the enhancers regulate gene expression in plants. In this work, we used the database PlantDHS to identify predicted enhancers in the *Arabidopsis thaliana* genome. After designing the constructions, using the golden gate modular cloning system we manually constructed synthetic promoters composed of the sequence of each predicted enhancer ligated with the 35S minimal promoter from the cauliflower mosaic virus (CaMV). The reporter module is composed of a synthetic promoter, the reporter gene GFP-β-Glucuronidase and the 35S terminator. The synthetic promoters and reporter modules described here are artificial sequences that do not occur naturally. An enhancer functions independently of its positions and orientation in relation the target core promoter, therefore, the functional validation of each predicted enhancer (and each synthetic promoter) was performed by evaluating them individually in both senses forward and reverse orientation, using transient expression on heterologous system (*N. benthamiana and P. vulgaris*) and stable expression on the homologous system (*A. thaliana*). Finally, we analyzed their expression pattern in the stable transformation to determine whether the predicted enhancer sequence participate in the tissue-specific development.

Technological Breakthroughs

An efficient and reproducible localized vacuum-based agroinfiltration method to expand *in-planta* transformation

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In-vivo analysis of gene function relies primarily on transient gene expression in model plant systems, however overexpression in heterologous systems precludes functional studies of unique plant pathways that require a homologous background for functional analysis. We have developed a method to vacuum infiltrate parts of a plant instead of the whole plant to extend *in-vivo* functional characterization of genes to a wider range of plants, especially those that are excluded from the vacuum pump forced infiltration method due to their large size. Using localized vacuum infiltration, we achieved the first transient *in-planta* transformation of avocado and cacao, two agronomically important horticultural tree crops that are recalcitrant to transformation. *Agrobacterium*-mediated transient expression of RUBY vector via vacuum infiltration of selected group of attached of avocado and cacao leaves demonstrated the versatility of this method for application to other large plants. Tissue culture-free *Agrobacterium*-mediated transformation of attached leaves by localized vacuum infiltration can be performed in a rapid, straightforward, and cost-effective manner by laboratories that already use vacuum agroinfiltration on plants that fit inside a laboratory desiccator, since the same pump and vacuum vessel can be used, with no need to add costly equipment. This is an efficient and reproducible method of localized vacuum infiltration that not only provides the genomic research community with a timely tool to rapidly assess *in-planta* gene function in avocado and cacao, but also opens the *Agrobacterium*-mediated vacuum infiltration method for functional genomics and metabolic pathway research to a greater number of plant species, regardless of size or advanced stage of development.

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Expression of the *Bacillus thuringiensis* Cry10Aa Protein in *Coffea arabica* L. for resistance to Coffee Berry Borer (*Hypothenemus hampei*).

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Somatic embryogenesis (SE) is the most important plant biotechnology process for plant regeneration, propagation, genetic transformation and genome editing. We developed a highly competent SE line of *C. arabica* with a high rate of secondary embryogenesis and conversion to plants in 8-month plant regeneration period. To validate this capability, gene expression analysis of master regulators of SE, such as *BBM, FUS3*, and *LEC1*, embryo development, such as *EMB2757*, and cell cycle progression, such as *ETG1* and *MCM4*, were analyzed during induction and propagation of non-competent and highly competent embryogenic lines. This protocol was applied in genetic transformation with the plasmid pMDC85 containing the *Cry10Aa* gene in Typica cultivar of *C. arabica* L. by biobalistic. Transformation efficiency of 16.7% was achieved. Stable transformation was proven by qualitative and quantitative molecular analyses of Cry10Aa with a variable in the different transformation events expression levels from 3.25 to 13.88 µg/g fresh tissue, with ELISA. Somatic embryo (SEs) conversion to plantlets is the principal bottleneck for basic and applied use of this process. In this study we focus on the induction and maturation of SEs of *C. arabica* var. Typica. SEs conversion to plantlet up to 95.9% was achieved under osmotic stress, using 9 g/L gelrite, as compared with only 39.34% in non-osmotic stress. This is the first report about the stable transformation and expression of the Cry10Aa protein in coffee plants. Trough of Bioassays with transgenic fruits on Coffee Berry Borer (CBB) first instar larvae and adults induced mortalities between 85 and 100% after 10 days. In addition, transgenic fruits showed a seed damage lower than 9% compared to 100% of control fruits and adult mortality. This is the first report of stable transformation and expression of the Cry10Aa protein in coffee plants with efficient control of CBB.

Naturally occurring phenolic compounds from *Sechium* spp. (Chayote) genotypes as potential inhibitors of dipeptidyl peptidase-IV

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Dipeptidyl peptidase-IV (DPP-IV) has become an effective target in the treatment of type 2 diabetes mellitus (T2DM). Identifying natural DPP-IV inhibitors can thus have a significant impact in developing antidiabetic therapies. This study aimed to determine the efficacy of natural compounds from different chayote genotypes as potential inhibitors of DPP-IV, which plays a fundamental role in glucose metabolism and has been related to blood glucose regulation in patients with T2DM.

To determine the inhibitory capacity of DPP-IV, a total of 40 methanolic chayote extracts were subjected to *in vitro* enzymatic assays. Results showed that these extracts had percentages of enzyme inhibition ca. 30%. Based on these findings, the genotypes with the highest inhibitory activity were selected for directed analyses to identify and quantify up to 60 phenolic compounds using a high-resolution liquid chromatography system coupled to a triple quadrupole mass spectrometer (UPLC/MS-MS). A total of 26 phenolic compounds were identified and quantified, with the most abundant ones being L-phenylalanine, quercetin-3,4'-di-O-glucoside and kaempferol-3-O-glucoside.

Finally, virtual screening - ensemble docking findings suggested that among the experimentally identified phenolic acids in *Sechium* genotypes, penta-O-galloyl-β-D-glucose and quercetin-3,4′-di-O-glucoside had the highest negative binding energy (-15.37 kcal/mol and -11.02 kcal/mol respectively), showing a higher affinity for DPP-IV relative to the drug reference, sitagliptin (−7.97 kcal/mol), explaining in part the *in vitro* bioactivity observed at extract level. Overall, our study highlights that natural compounds, particularly chayote-derived phenolic compounds, are potential alternatives to synthetic drugs in the effective management of T2DM.

Effect of β-glucosidases from Chayote (*Sechium edule*) on the release of aromatic compounds in the preparation of *blonde ale*-style craft beer

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Craft beer, a fermented alcoholic beverage attractive for the organoleptic attributes such as aroma and flavor that it provides, consumer acceptability and the constant search to highlight these characteristics, Xiaoyu Han et al, 2023[1)] reported the participation of β-glucosidase enzymes released by non-*Sacharomyces* yeasts and their relationship with the increase of terpenes such as linalol and geraniol in the final product, contributing fruity and floral aromas and flavors.
The β-glucosidases (E.C.3.2.1.21) are also found in plants such as chayote (*Sechium edule*), isoform II[2)] of this enzyme has been purified by ourresearch. The objective was to evaluate clarified crude chayote extract (CCE) in the production process of a blonde ale style craft beer. Two hundred ml of must were distributed per fermentation flask (in triplicate), at 72 h five doses of CCE were added (250 µl, 500 µl, 1 ml, 2 ml and 4 ml), with 5.06 U/mg specific activity, similar to that of the maturation stage. The results indicate that some qualitative characteristics are modified when 4 ml of CCE are added during fermentation, with respect to the control these were; high gas production, good aftertaste and caramel flavor, in addition, they improve notably when they are added during maturation where it is appreciated, high foam and creamy appearance, characteristic color of the beer style evaluated, tearing, longer retention time of the foam, floral flavor. Quantitative analysis of terpenes is in progress.

1.- Han, X., Et al. (2023). Application of non-*Saccharomyce*s yeasts with high β-glucosidase activity to enhance terpene-related floral flavor in craft beer. Food Chemistry, 404, 134726.
2.-Cruz Rodríguez, A., Et al (2020). Aggregation and molecular properties of β-glucosidase isoform II in chayote (*Sechium edule*). Molecules, 25(7), 1699.

Bayesian Inference Estimates the Parameters of the Farquhar, Von Caemmerer & Berry Model for C3 Photosynthesis Better than Traditional Regression Approaches

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The rate at which a leaf fixes carbon dioxide via photosynthesis is known as assimilation rate, and it is the result of numerous physiological processes that take place from the biochemical to the organ level. To understand why some plants of the same species have higher assimilation rates than others, it is not enough to just measure the leaf assimilation rate. Instead, we need to estimate the contribution of each individual step; to do this, assimilation rate measurements are fitted to the equations of the Farquhar, Von Caemmerer and Berry (FvCB) biochemical model. Most parameters used in these equations are a numerical estimation of individual steps of the C3 photosynthesis pathway. Traditionally, sequential least squares regressions fit experimentally collected measurements to the equations in order to estimate the parameters. This approach has a fundamental drawback. The consecutive least squares regressions imply that each step is conditioned by previous optimizations; this could result in suboptimal or completely biased results. Here, we present a better mathematical method to estimate the parameters by using Bayesian inference and a full model posterior sampling resorting to Markov Chain Monte Carlo algorithms. Using 12 CIMMYT wheat lines with contrasting Radiation Use Efficiency, we show that the use of Bayesian inference in the full FvCB model can properly fit the model and prevent suboptimal solutions. We show that the posterior distribution of each estimated parameter is more informative than the single estimate obtained with the least square regression approach. The posterior distribution not only represents an estimate of the variability of each parameter in the population, but also can be used to assess if the model and/or data set are informative about each parameter. That is, the approach can estimate the parameters and the uncertainty of its estimation.

A Modular Toolbox for Studying the Dynamics of Volatile Organic Compounds (VOCs) in Biological Systems

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Volatile organic compounds (VOCs) are central in biological systems as mediators, signals, and bioactive molecules. For example, the fungal metabolite 6-pentyl-α-pyrone (6-PP) stimulates lateral plant root formation by modulating the transporters of the auxin signaling pathway. VOC profiles can change in seconds in some situations, such as plant wounding by predators.

However, conventional techniques for studying the dynamics of VOCs are slow (GC-MS: minutes to hours/sample), expensive (PTR-MS: ~1 million USD), and selective for certain molecule classes (PTR-MS, SPME). Therefore, we develop comparably inexpensive analytical tools and software for studying VOCs in their natural context and in biologically relevant time scales. To further promote the general accessibility of the tools, we follow the open development paradigm, and all components we develop are highly modular:

Our 'Modular Biological Mass Spectrometer' (MoBiMS) can detect a broad range of VOCs and provides spectra compatible with the NIST database. Depending on the experimental set-up, a time resolution of less than 1 second is possible; the device is relatively low cost (~60,000 USD) and semi-portable (~15 kg). As example applications, we demonstrated the identification of isoamyl acetate from bananas and the online monitoring of photosynthesis in tomatoes [1].

We developed a low-temperature plasma probe to investigate biological surfaces gently. Monitoring the 6-pentyl-α-pyrone (6-PP) signal during the interaction between *Trichoderma atroviride* and *Arabidopsis thaliana* for ten days, we noted a regular pattern correlating with the day-night cycle. Time series analyses with Julia indicate that 6-PP is a physiological variable promoting the homeostasis of the plant-fungal interaction [2].

Our 'Open LabBot,' also allows the high-throughput analysis of essential oils and the imaging of volatile and semi-volatile compounds in fresh plant tissue [3].

Altogether, we built a modular toolbox for studying VOCs in biological systems under realistic conditions and provided open hardware and software to the research community.

Funding: Conacyt-DFG 2016/277854

1. Alcalde-Vázquez, R. et al. Microchemical Journal 175, 107090 (2022).
2. Torres-Ortega, R. et al. Metabolites 12, 1231 (2022).
3. Rosas-Román, I. et al. Microchemical Journal 152, 104343 (2020).

Functional characterization of group 6 LEA proteins in *Arabidopsis thaliana*

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The seeds are among the most complex structures of plants. When they are facing water deficit, plants activate complex signaling networks to enhance their tolerance and adaptability. A pivotal component in this process is the Late Embryogenesis Abundant (LEA) proteins, which surge in response to limited water availability during plant development. Our study specifically delves into the group-6 LEA proteins and unveils their critical role in the plant's adaptive response to low water availability and salt-induced stress. Within this study, we present compelling data indicating that the absence of the AtLEA6-2.1 protein results in heightened sensitivity to osmotic stress (mannitol) and salt stress (NaCl). Additionally, we have established that this heightened sensitivity persists during seedling establishment. To confirm that the absence of AtLEA6-2.1 is responsible for this sensitivity, we conducted complementation assays using the wild-type gene, and we confirm that the phenotype is resulted from the absence of AtLEA6-2.1. It is noteworthy that mutant seeds lacking AtLEA6-2.1 also display lower germination rates than their wild-type counterparts under non-stress conditions when seeds collected over different years were examined, suggesting that the absence of this protein may influence seed aging. In summation, these findings underscore the pivotal role played by the AtLEA6-2.1 protein in the plant's adaptive response to limited water availability and its long-term viability. Lastly, we carried out protective protein assays under dehydration conditions, shedding light on the potential molecular function of LEA6 proteins as osmoprotectors.

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“XX National Plant Biochemistry and Molecular Biology Congress, 3rd Meeting of the Mexico Section of the American Society of Plant Biologists, 13th Mexico-USA Plant Biology Symposium”

Academic Program

Monday Oct 16		Tuesday Oct 17		Wednesday Oct 18		Thursday Oct 19		Friday Oct 20	
11:00 - 13:00	Plants&Python workshop		Moderator: **Alejandra Covarrubias**		Moderator: **Sue Rhee**		Moderator: **Dan Chitwood**	9:00	Departure
15:00 - 18:00	Registration	9:00 - 9:30	Abiotic stress - **John Cushman**	9:00 - 9:30	Plant biotic interactions - **Alejandra Rougon-Cardoso**	9:00 - 9:30	Plant Development - **Felipe Cruz-García**		
18:00 - 18:15	Welcome & Opening	9:30 - 10:00	Abiotic stress - **Christa Testerink**	9:30 - 10:00	Plant biotic interactions - **Sarah Lebeis**	9:30 - 10:00	Plant Development - **Daphné Autran**		
18:15 - 19:00	Keynote talk - **Ivette Perfecto**	10:00 - 10:30	Abiotic stress - **José Luis Reyes**	10:00 - 10:30	Plant biotic interactions - **Edel Pérez-López**	10:00 - 10:30	Plant Development - **Mary Gehring**		
19:00 - 21:00	Welcome cocktail	10:30 - 11:00	Coffee break	10:30 - 11:00	Coffee break	10:30 - 11:00	Coffee break		
		11:00 - 11:30	Ecology and evolution - **Tania Hernández**	11:00 - 11:30	Plant metabolism - **Karolyna Heyduk**	11:00 - 11:30	Plant technologies - **Cecilia Mayo-Montor**		
		11:30 - 12:00	Ecology and evolution - **Zsuzsanna Merai**	11:30 - 12:00	Plant metabolism - **Jing Ke Weng**	11:30 - 12:00	Plant technologies - **Anabel Romero**		
		12:00 - 12:30	Ecology and evolution - **Carlos Ortiz-Ramirez**	12:00 - 12:30	Plant metabolism - **Elizabeth Sattely**	12:00 - 12:30	Plant technologies - **Luis Díaz-García**		
		12:30 - 13:00	Lightning talks (for posters)	12:30 - 13:00	Lightning talks (for posters)	12:30 - 14:30	Lunch		
		13:00 - 14:30	Lunch	13:00 - 14:30	Lunch		Moderator: **Gustavo Rodríguez-Alonso**		
			Moderator: **Cesar Cuevas-Velazquez**		Moderator: **Stewart Gillmor**	14:30-14:45	Short talk - Arely Viridiana Perez-Lopez		
		14:30-14:45	Short talk - Irving Jair García-López	14:30-14:45	Short talk - V. Miguel Palomar	14:45-15:00	Short talk - Ana Laura Alonso-Nieves		
		14:45-15:00	Short talk - Svetlana Shishkova	14:45-15:00	Short talk - Nuria De Diego	15:00-15:15	Short talk - Margarita Rodríguez y Domínguez Kessler		
		15:00-15:15	Short talk - José Luis Coyac-Rodríguez	15:00-15:15	Short talk - Daniel Oswaldo Camo-Escobar	15:15-15:30	Short talk - Andrea Romero-Reyes		
		15:15-15:30	Short talk - Marcela Hernández-Coronado	15:15-15:30	Short talk - Guadalupe Itzel Meneses-Reyes	15:30-15:45	Short talk - Miguel Angel Villalobos-López		
		15:30-15:45	Short talk - Sandra Rios-Carrasco	15:30-15:45	Short talk - Carlos E. Rodríguez-López	15:45-16:00	Short talk - Michel Pale-Rivas		
		15:45-16:00	Short talk - Ronald Pierik	15:45-16:00	Short talk - Rodrigo Muñoz-Javier	16:00-16:15	Short talk - Gladys I. Cassab		
		16:00-16:15	Short talk - Gerardo del Toro de León	16:00-16:15	Short talk - Nelly Jazmín Pacheco-Cruz	16:15-16:30	Short talk - Sandra Isabel González-Morales		
		16:15-16:30	ASPB	16:15-16:30	Short talk - Andrea Tovar-Aguilar	16:30 - 17:00	Coffee break		
		16:30 - 16:45	Coffee break	16:30 - 17:00	Coffee break	17:00 - 17:45	Business meeting		
		16:45 - 19:00	Poster session 1	17:00 - 19:00	Poster session 2	17:45 - 18:30	Keynote Talk - **Sean Cutler**		
		19:00 - 19:45	Keynote Talk - **Katie Dehesh**	19:00 - 19:45	Keynote Talk - **Ruairidh Sawers**	18:30 - 18:45	Closing remarks		
						21:00 - 23:59	Gala Dinner		