







XII Symposium Mexico-USA / 2nd ASPB Mexico Section

# November 8 -11 • 2021

BOOK OF



First, we are deeply grateful for your enthusiasm, participation, and commitment to this scientific meeting in these difficult times during a pandemic. It is evident that there is great scientific interest to share research advances with the international science community. The abstracts in the following pages indicate that the flash talks and the science represented by the participants in the meeting will be exceptional.

This time the Congress will be online, but no less successful. For this XIX National Plant Biochemistry and Molecular Biology Congress, the XII Symposium Mexico-USA Symposium and 2nd ASPB Mexico Section Meeting, the participants — students, postdocs, and researchers — come from 49 countries. We also have 36 speakers, all international leaders in their respective fields of research. More than 100 flash talks will be presented, and more than 1,300 people will be attending this Congress.

The successful participation in this meeting is unprecedented, it is an important milestone, which will make our international plant science community stronger.

The organization of this Congress could not have been carried out without the enthusiastic participation of the organizing committee and the technical team, which have provided exemplary professional service.

> Yes, we can! iSí se puede!

Felipe Cruz-García Chair of the Conference



#### ORGANIZERS

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#### ACADEMIC PROGRAM

#### DAY 1 • MONDAY 8 NOV INAUGURATION PLANT DEVELOPMENT

Moderators: Dan Chitwood & Mario Arteaga

9:00 - 9:15 Inauguration 9:15 - 10:00 KEYNOTE Rob Martienssen Cold Spring Harbor Laboratory, USA Germline reprogramming and epigenetic inheritance in Arabidopsis

10:10 - 10:40 Marisa Otegui University of Wisconsin-Madison, USA Endosomes in plant development

10:40 - 11:10 Yoselin Benitez-Alfonso University of Leeds, UK PDWallMech: dissecting cell wall properties and cell-to-cell signaling via plasmodesmata

11:10 – 11:40 Stewart Gillmor Langebio-CINVESTAV, México Zygotic genome activation in Arabidopsis

**11:40 - 12:00** BREAK

12:00 - 12:30 María Jazmín Abraham Juárez IPICYT, México Novel protein interactions reveal a crosstalk between development and immunity in maize

12:30 - 13:00 José López Bucio UMSNH, México Cell death and regeneration within the root meristem

13:00 - 13:30 Nayelli Marsch Martinez Unidad Irapuato-CINVESTAV, México Dual cytokinin regulation by transcription factors that control development

13:30 - 14:15 KEYNOTE Roberto Solano CNB- CSIC, Madrid, Spain Evolution of Jasmonates in land plants and their role in thermotolerance

14:15 - 14:45 LUNCH

14:45 - 15:15 FLASH TALK SESSION 1

15:15 - 15:45 Przemysław Prusinkiewicz University of Calgary, Canada Phyllotaxis: beyond the standard theory

15:45 - 17:00 FLASH TALK SESSION 2

#### DAY 2 • TUESDAY 9 NOV ABIOTIC STRESS AND PLANT METABOLISM

Moderators: Felipe Cruz García & Alejandra Covarrubias

9:00 - 9:45 KEYNOTE Julia Baliey-Serres, University of California, Riverside, USA At the root of cellular responses to water extremes

9:45 – 10:15 José Dinneny Stanford U, USA Discovering innovations in stress reponse through comparative genomics

10:15 - 10:45 Beronda Montgomery Michigan State University, USA Lessons About and From Plants: Plant Color Vision and Developmental Acclimation

10:45 - 11:15 Jorge Nieto IB-UNAM, México Analysis of mesocotyl length variation in maize by combined QTL, transcriptomic, and Genome-Wide Association Studies

11:15 - 11:45 BREAK

11:45 – 12:15 Caspar Chater Kew Gardens, UK/IBt-UNAM, México Epidermal Patterning FActor signalling and legume nodulation

12:15 - 12:45 Patricia León Bt-UNAM, México Plastid retrograde signals and their impact in plant development

12:45 - 13:15 Marina Gavilanes FQ-UNAM, México Functional diversity on the plasma membrane sphingolipidome

13:15 - 13:45 Rodrigo Gutiérrez U Católica de Chile, Chile Plant life at the extreme in the Atacama Desert

1**3:45 - 14:15** LUNCH

14:15 - 14:45 FLASH TALK SESSION 3

14:45 - 15:30 **KEYNOTE** Xuemei Chen University of California, Riverside, USA **RNA NAD+ capping** 

15:30 - 17:00 FLASH TALK SESSION 4

#### Day 3 • Wednesday 10 Nov PLANT-MICROORGANISM AND BIOTIC INTERACTIONS

Moderators: Dan Chitwood & Stefan De Folter

9:00 - 9:45 KEYNOTE Hailing Jin University of California, Riverside, USA Cross-Kingdom RNAi and extracellular vesicle-mediated small RNA trafficking between plants and funcal pathogens

9:45 - 10:15 Oswaldo Valdés López FES Iztacala-UNAM, México Modulation of the root nodule symbiosis by plant-host Pi status

10:15 - 10:45 Laila Partida Martinez Unidad Irapuato-CINVESTAV, México Deciphering the secrets of the plant microbiome in drylands

10:45 - 11:15 Georgina Hernández CCG-UNAM, México Deciphering novel common bean regulators for the rhizobium N2-fixing symbiosis

11:15 - 11:45 BREAK

11:45 - 12:15 Mario Serrano CCG-UNAM, México Differential defense response induced by changes in the plant cuticle

12.15 - 12:45 Saskia Hogenhout JIC, UK How bacterial phytoplasma pathogens change plant architecture and plant defense to insect vectors

12:45 - 13:15 Alfredo Herrera-Estrella Langebio-CINVESTAV, México The Trichoderma-Plant Dialogue

13:15 - 13:45 Neelima Sinha University of California, Davis, USA Interactions between the parasitic Cuscuta campestris and its host tomato

1**3:45 - 14:15** LUNCH

14:15 - 14:45 FLASH TALK SESSION 5

14:45 - 15:30 KEYNOTE Sophien Kamoun Sainsbury Lab, UK How to trick a plant pathogen

15:30 - 17:00 FLASH TALK SESSION 6

17:00 - 19:00 Business Meeting

#### DAY 4 • Thursday 11 Nov ECOLOGY AND EVOLUTION AND TECHNICAL BREAKTHROUGHS

#### Moderators: Alejandra Covarrubias & Felipe Cruz García

9:00 - 9:45 KEYNOTE Michael Purugganan New York University, USA Natural selection on plant gene expression

9:45 - 10:15 Tatiana Arias Tecnológico de Antioquia, Medellin, Colombia Towards a solid orchid phylogeny: providing a solid timeframe for biogeography and macroevolution studies

10:15 - 10:45 Oscar Pérez-Escobar Royal Botanic Gardens, Kew, UK Delving into the evolution of the date palm through aDNA and molecular clocks

10:45 - 11:15 Susana Magallón IB-UNAM, México Thirty clues to angiosperm exceptional diversification

11:15 - 11:45 BREAK

11:45 - 12:15 Natalia Pabón Mora U de Antioquia, Colombia Genetic mechanisms controlling flower and fruit diversity in non-model neotropical angiosperms

12.15 - 12:45 Yiliang Ding JIC, UK RNA structure, a hidden regulator in vivo

12:45 - 13:15 Luis Herrera-Estrella Langebio-CINVESTAV, México Genome accessibility dynamics in response to phosphate limitation is controlled by the PHR1 family of transcription factors in Arabidopsis

13:15 - 13:45 Jim Haseloff University of Cambridge, UK Open tools for engineering biology

13:45 - 14:15 LUNCH

14:15 - 14:45 FLASH TALK SESSION 7

14:45 - 15:30 **KEYNOTE** Paul Gepts University of California, Davis, USA Molecular and environmental aspects of domesticated Middle American common bean

15:30 - 16:00 Awards ceremony and closing remarks



#### XII Symposium Mexico-USA / 2nd ASPB Mexico Section

#### SESSION 1

- 1. Granados Aguilar
- 2. Ortega Pérez
- 3. Sierra Sarabia
- 4. Cornejo Corona
- 5. Peña Castro
- 6. García Coronado
- 7. Magaña Rodríguez
- 8. Frias Muñoz
- 9. Xoca Orozco
- 10. Morales Elías

#### **SESSION 2**

- 1. Jara Servín
- 2. Cruz Mireles
- 3. Colchado López
- 4. Sánchez Villarreal
- 5. Aguilar Cruz
- 6. Angulo Ross
- 7. Li
- 8. Cortez Fonseca
- 9. Rosiles Ortega
- 10. Romero Reyes
- 11. Ortega Amaro
- 12. Pulido Torres
- 13. Pascual Morales
- 14. Madrigal Ortiz
- 15. Ortiz Martínez
- 16. Lujan Soto
- 17. Vázquez Chimalhua
- 18. Ruiz Aguilar
- 19. Sotelo Silveira
- 20. Amezquita
- 21. Colín Oviedo
- 22. Hernández Soriano

#### SESSION 3

- 1. Maldonado
- 2. Solís Miranda
- 3. Romero Gutiérrez
- 4. González Lemes
- 5. García Cárdenas
- 6. Gregorio Jorge

#### **FLASH TALKS**

- 7. Lara Mondragón
- 8. Enríquez Toledo
- 9. Flores Hernández
- 10. Gómez Hernández

#### **SESSION 4**

- 1. Cruz López
- 2. Raya González
- 3. Buendía Monreal
- 4. Palomar
- 5. Jiménez Chávez
- 6. Ramírez Hernández
- 7. Gómez Díaz
- 8. López Bucio
- 9. Jiménez Vázquez
- 10. Castro Bustos
- 11. Dipp Álvarez
- 12. León Ruiz
- 13. Maldonado Mendoza
- 14. Ruiz Ortega
- 15. Reyes Aguilar
- 16. Flores Martínez
- 17. Soberanes Gutiérrez
- 18. Piñón Simental
- 19. Bravo Rodríguez
- 20. Mendoza Galindo
- Video One
- 21. Cárdenas Torres
- 22. Cerbantez Bueno
- 23. Trillo Video Two

#### SESSION 5

- 1. Petrella
- 2. Huerta Pérez
- 3. García Reynoso
- 4. Zaragoza Gómez
- 5. Cazares Álvarez
- 6. Hernández León
- 7. Rodríguez Gandarilla
- 8. López García
- 9. González Vázquez

#### 10. Ortiz Luevano

#### **SESSION 6**

- Mendoza Galindo Video Two
- 2. Rosas Reinhold
- 3. Hernández Miranda
- 4. Morán Yáñez
- 5. Rodríguez Cisneros
- 6. Ayala Ruiz
- 7. Mancera Lara
- 8. Mejía Vázquez
- 9. Pacheco Cruz
- 10. Solano García
- 11. Conejo Dávila
- 12. González Pérez
- 13. Fonseca García

15. Esparza Reynoso

17. Sarmiento López

18. Parra Aguilar

20. Letts

21. Trillo

19. Méndez Gómez

22. Olivares Grajales

1. Martínez Martínez

3. Montalvo Guevara

2. Ravelo Ortega

4. Cruz García

5. Zilli Gutiérrez

6. Quero Hostos

9. Cucinotta

7. Pérez Hernández

10. Esquivel Aguilar

5

8. Hernández Álvarez

**SESSION 7** 

16. Martínez Hernández

14. López Pérez

# **DICER-LIKE1 controls cell fate specification during** gemma development in *Marchantia polymorpha*

Aguilar-Cruz, A<sup>1\*</sup>., Flores-Sandoval, E<sup>2</sup>., Oltehua-López, O<sup>1</sup>., Gutiérrez-Ramos, X<sup>3</sup>.,
Dorantes-Acosta, A.E<sup>1</sup>., Trujillo, J<sup>4</sup>., Ishizaki, K<sup>5</sup>., Kohchi, T<sup>6</sup>., Chen, X<sup>7</sup>., Mosher,
R<sup>4</sup>., Grimanelli, D<sup>8</sup>., Haseloff, J<sup>9</sup>., Bowman, J<sup>2</sup>., Arteaga-Vazquez, M.A.<sup>1</sup>

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MicroRNAs (miRNAs) are essential regulators of gene expression during development and in the responses to environmental stimuli. In plants, *DICER-LIKE 1 (DCL1)* is the master regulator of miJRNA biogenesis and its function is conserved across streptophytes. In this work, we employed a reverse genetics approach using the CRISPR-Cas9 genome editing system to isolate loss-of-function mutants in the *Marchantia polymorpha DICER-LIKE1a* (MpDCL1a) gene. Mpdcl1a mutant gemmae exhibit the formation of supernumerary meristems and ectopic mucilage papillae but most strikingly we observed the direct reprogramming of epidermal cells into ectopic gemmae. At the reproductive level, Mpdcl1 mutants display feminized antheridiophores and abnormal archegonia with supernumerary egg cells. Transcriptomic analysis of Mpdcl1a gemmae revealed that levels of both miRNAs precursors and miRNA targets are increased. We also found upregulation of genes involved in auxin biosynthesis and signaling. Our results indicate that MpDCL1a primarily functions in the biogenesis of miRNAs in *M. polymorpha* and regulates cell fate specification during gemmae development through a mechanism dependent on auxin.



#### Measuring hidden phenotype: quantifying the shape of barley seeds using the euler characteristic transform

Amézquita, E<sup>1\*,</sup>, Quigley, M<sup>2</sup>, Ophelders, T<sup>4</sup>, Landis, J<sup>5,6,7</sup>, Koenig, D<sup>7</sup>, Munch, E<sup>1,3</sup>, Chitwood, D.<sup>1,2</sup>.

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Shape plays a fundamental role in biology. Traditional phenotypic analysis methods measure some features but fail to measure the information embedded in shape comprehensively. To extract, compare, and analyze this information embedded in a robust and concise way, we turn to Topological Data Analysis (TDA), specifically the Euler Characteristic Transform (ECT). TDA measures shape comprehensively using mathematical terms based on algebraic topology features. To study its use, we compute both traditional and topological shape descriptors to quantify the morphology of 3121 barley seeds scanned with X-ray Computed Tomography (CT) technology at 127 micron resolution. The ECT measures shape by analyzing topological features of an object at thresholds across a number of directional axes. A qualitative analysis of the information encoded by the topological signature reveals that the ECT picks up successfully the shape of the crease and bottom of the seeds. Moreover, while traditional shape descriptors can cluster the seeds based on their accession, topological shape descriptors can cluster them further based on their panicle. We then successfully train a support vector machine (SVM) to classify 28 different accessions of barley based on the shape of their grains. We observe that combining both traditional and topological descriptors classifies barley seeds better than using just traditional descriptors alone. This improvement suggests that TDA is thus a powerful complement to traditional morphometrics to comprehensively describe a multitude of "hidden" shape nuances which are otherwise not picked up.



# Expression Analysis of sugar transporter genes induced by mycorrhiza colonization in tomato

Angulo-Ross, A.G<sup>1\*,</sup> Castro-Martínez, C<sup>-1,</sup>, Ramírez-Douriet, C.M<sup>-1,</sup>, Maldonado-Mendoza, I.E<sup>-1,</sup>, López-Meyer, M.<sup>-1</sup>

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Arbuscular mycorrhiza is a symbiosis between roots of most plants and arbuscular mycorrhizal fungi. The plant provides the fungus with carbon, whereas the fungus helps the plant to improve mineral nutrition. Little is known about the sugar transport mechanism in this symbiosis. Therefore, we studied the expression of some sugar transporter genes, such as the integral monosaccharide membrane transporters SISTP1, SISTP2, SISTP16 and SISFP7 in roots and leaves of tomato plants. These genes were previously described as induced in leaves of mycorrhiza-colonized tomato plants. The genes encoding for sucrose transportes SISUT1, SISUT2 and SISUT4 were also analyzed. As results, in mature leaf tissiue (LT3) and young leaves (LT1) of non-colonized plants, the monosaccharide transporter SISTP2 showed higher expression compared to colonized plants. The sucrose transporter SISUT1 showed higher expression in mature leaves (LT3) with respect to other tissues analyzed, irrespectively of their symbiotic status. In addition, the sucrose transporter gene SISUT4, which encoded a protein that has been located in vacuolar membranes, was repressed in leaf tissue LT1 and LT2 of mycorrhiza-colonized plants. The differential expression observed in sugar transporters in leaves of colonized and non-colonized plants, suggests that these genes could be involved in the regulation of the amount of sugars that move towards the mycorrhiza roots. In addition, the glucose content was also determined. A significantly lower content of this sugar was found in mycorrhiza colonized roots with respect to non-colonized, which supports the idea that glucose is the preferential transported sugar from the plant to the fungus.

# Antioxidant activity of the major terpenes of *Callistemon citrinus* in rats fed a high-fat diet

Ayala-Ruiz, L.A1\*, Magaña-Rodríguez, O.R1, Godines-Hernández, D2, Ríos-Chávez, P1

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High-fat diets increased risk of developing obesity, type 2 diabetes mellitus, and cardiovascular disease. The increase in saturated fat activates various signaling pathways that promote an increase in reactive oxygen species and inflammation in different cells, affecting cell function. *Callistemon citrinus* is a plant to have been reported biological activities. In this study, the antioxidant activity of the major terpenes of *Callistemon citrinus* (1-8-cineol, limonene, and  $\alpha$ -terpineol) in rats fed a high-fat diet model. 36 male albino Wistar rats were randomly divided into six groups (n=6). Group I was control; group II was high-fat diet (HFD) it contains 41.66% of the Purina® Rodent Chow food, 20.83% of INCA® vegetable fat, 20.83% of lard, and 16.68% of sucrose. The group III, IV, V, and VI were administered orally with terpenes at different concentrations (1-8-cineol at 0.88 mg/kg, limonene at 0.43 mg/kg,  $\alpha$ -terpineol at 0.32 mg/kg, and terpenic mixture of those), plus HFD daily for 15 weeks.

High levels of oxidative stress biomarkers: Malondialdehyde (MDA), 4-hydroxynonenal (HNE), and advanced protein oxidation products (AOPP) were found in the liver of the HFD group. Conversely, the levels of the biomarkers in the groups with 1,8-cineole,  $\alpha$ -terpineol, and limonene plus the HFD showed a decrease in these parameters similar to the control group. Reduced glutathione (GSH) showed low levels in all the groups compared to control group. The activity of paraoxonase (PON1) significantly decreases in HFD, limonene and 1,8-cineol groups when compared with  $\alpha$ -terpineol, terpenic mixture and, control groups. These results demonstrate that  $\alpha$ -terpineol was the best to improve the damage caused by oxidative stress as a result of a HFD for 13 weeks. On the other hand, limonene and 1,8-cineol also showed protection against certain biomarkers of oxidative stress.



# Allergenicity profiling of *Ligustrum lucidum* pollen IgE binding proteins associated with its geographical location in Mexico City

Bravo-Rodríguez, R.N<sup>1,2\*,</sup>Montero-Vargas, J.M<sup>2,</sup>, Porras-Gutiérrez de Velasco, R<sup>2</sup>., Vizuet de-Rueda, J.C<sup>2,</sup>, Terán-Juárez, L.M.<sup>1,2</sup>

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Pollen is the male reproductive unit of plants that insects and the wind disperse. Pollen inhalation causes allergic respiratory diseases, a growing health problem in Mexico that affects people's quality of life. The chemical composition of pollen is determined genetically and environmentally. Still, it has been proposed that location factors such as CO2 concentration, the incidence of UV light, water availability, and environmental pollution directly influence the allergenicity of pollen. This study focuses on knowing if there are differences in the allergenic protein profile of pollen from Ligustrum lucidum, a tree widely distributed in Mexico City. An immunoproteomics approach (slot blot and 2DE) will be used to evaluate the IgE binding proteins and their allergenicity according to different collected sites. At this time, our results showed high variability in morphological traits of L. lucidum pollen by location. We presume significant differences in the allergenicity pattern due to environmental and pollution conditions determined by its location.



# The evolution of *LMI1* and *RCO* in shaping leaves

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The amazing diversity of leaf shapes that we can find in nature, even within the same family as in Brassicaceae, makes it a good model to study how organismal forms have evolved. The REDUCED COMPLEXITY (RCO) gene was shown to be necessary for producing compound leaves in Cardamine hirsuta and sufficient to increase leaf complexity in Arabidopsis. RCO arose from a tandem duplication of LATE MERISTEM IDENTITY 1 (LMI1) after the split of the core Brassicaceae family from the basal Aethionema group, and it has been subsequently lost in some species (e.g., Arabidopsi s thaliana) leading to leaf simplification. Despite being duplicates, LMI1 and RCO have divergent functions during leaf development because they have completely opposite expression patterns. The recent resolution of the Brassicaceae phylogeny allows to study the evolution of gene families. I analyzed the sequences of the LMI1 and RCO genes from species of all the evolutionary lineages, finding interesting features in both their regulatory and coding regions. Based on these findings, and comparing the leaf shape of each species, we chose Euclidium syriacum and Arabis alpina to study in detail the early evolutionary divergence of LMI1 and RCO. Both species belong to the earliest divergent lineages and have both LMI1 and RCO genes, but they still produce simple leaves. I am analyzing transgenic plants to test whether the genes from these species show an evolutionary intermediate expression domain and function in order to elucidate the early steps of evolutionary divergence after gene duplication.



# **Crispr/Cas9 mutation of all NADPH oxidases from** *Physcomitrella patens* **and its ROS responses to chitin oligomers**

Luis Cardenas<sup>1\*,</sup> Samantha E. Ryken<sup>2</sup> and Magdalena Bezanilla<sup>2</sup>

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Many responses in animal and plant cells depend from reactive oxygen species (ROS). Reactive oxygen species (ROS) in plant cells play an important role in several physiological processes, for example: in plant development, hormonal signaling, polar growth, biotic and abiotic interactions, gravitropism and stress responses, etc. These ROS can also activate calcium channels and receptors involved in signaling processes (Mittler and Berkowitz, 2001). In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS levels generate and maintain an apical calcium gradient. This ROS accumulation also plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes (Pei et al., 2000; Foreman et al., 2003). Herein we report a new molecular probe to depict the ROS dynamic during apical growth in the moss model *Physcomitrella patens*. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. With this genetic probe in the WT background of *P. patens* we were able to visualize the apical ROS distribution. Furthermore, we used Crispr/Cas9 to edit the four NADPH oxidases and immediately determine the effect on intracellular ROS levels. In addition, we were able to determine the intracellular ROS responses to chitin oligomers. Our results demonstrate that chitin oligomers-induced ROS generation is dependent of the NADPH oxidase activity.



# Arabidopsis Glycine Rich Domain Protein (AtGRDP2) interacts with proteins associated with RNA processing and translation

Castro-Bustos, S1\*., Maruri-López, I 2., Ortega-Amaro, M.A1., Serrano, M3., Jiménez-Bremont, J.F.1

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*AtGRDP2* gene is expressed during the development and response to salt stress in *Arabidopsis thaliana* plants. The overexpression of the *AtGRDP2* in plants accelerated their growth, flowered earlier, and had increased tolerance to abiotic stress, while *Atgrdp2* mutant had an opposite phenotype. AtGRDP2 protein is constituted by three domains: a DUF1399 located at the N-terminal, a potential RRM in the central region, and a Glycine-rich at the C-terminal. Although AtGRDP2 has been reported to be involved in plant growth and stress response, the mechanism of action is still unknown. In this study, we showed that AtGRDP2 protein has a dual cytosol-nucleus localization in tobacco leaves cells. We also determined that the regions that include the DUF1399 domain or the RRM domain are also located in the nucleus by themselves. Using a yeast two-hybrid split ubiquitin assay, we identified potential interactors for AtGRDP2 protein. Three candidates encoding for proteins associated with RNA processing functions: PABN3, EF-1a and CL15, were selected and confirmed to interact with AtGRDP2 by the Bimolecular Fluorescence Complementation (BiFC) approach. Finally, functional characterization of the protein-protein interactors. These data suggest that AtGRDP2 might perform in multiple protein complexes placed at the nucleus with PABN3, cytosol with EF-1a and chloroplast with CL15 regulating RNA processing and translation.



# Fusarium verticillioides chitinase-modifying proteins may play a role on fungal invasion in the tripartite interaction maize-F. *verticillioides-Bacillus* cereus

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Maize is susceptible to various diseases caused by phytopathogenic fungi, one of them is Fusarium verticillioides (Fv), which causes stem and root rot. Bacillus cereus B25 has an antagonistic effect against Fv through the production of antifungal compounds such as siderophores, antibiotics and chitinases, these enzymes hydrolyze the chitin in the fungal cell wall. Likewise, maize can produce chitinases (ZmChitA and ZmChitB) and induce plant defense when invaded by fungal pathogens. However, Fv produces chitinase modifying proteins (fungalysin and subtilisin), which separate the chitin-binding domain and the catalytic domain of the plant chitinases causing to lose their ability to bind chitin and hydrolyze it. In our proposed model of tripartite interaction (maize-B25-Fv), B25 chitinases may not be modified by fungalysin because they don't have the protein domain that fungalysin recognizes. It has been postulated that the bacterial chitinases may degrade chitin in the fungal cell wall and elicit plant defense response. We found that maize chitinases were modified in the presence of a protein extract of hybrid maize seed inoculated with Fv conidia while bacterial chitinases remained intact. We also found that Fv may produce another protease, possibly a subtilisin, which acts together with fungalysin to modify the maize chitinases. A tripartite maize-B25-Fv interaction was established and we observed the accumulation of both maize chitinases in seedling tissues (root and shoot) with different levels of accumulation between treatments. This could lead to a greater tolerance or resistance to infection by fungal pathogens.

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# Analyzing the function of SHOOT MERISTEMLESS (STM) related to cytokinin during gynoecium development in *Arabidopsis thaliana*

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The flower is a complex and beautiful structure, exclusive for the angiosperm group, and is formed by different floral organs including the reproductive organs. The gynoecium is the female reproductive structure, which once fully developed and fertilized forms a mature ovary or fruit. In Arabidopsis, the phytohormone cytokinin plays an important role in the early development of the gynoecium, mostly because it regulates cellular division in the meristematic region of the carpels; however, it has also functions during later development. Cytokinins are produced by an enzymatic pathway that uses ATP or ADP to convert them in a free and active base. The enzymes ISOPENTENYL TRANSFERASE (IPT) and LONELY GUY (LOG) play a very important role in this biosynthetic process. Each of the IPT and LOG enzyme family has nine genes coding for enzymes of their kind. When cytokinin is produced, it has to be sensed by the cytokinin receptors (AHK) to trigger its genetic response. It has been reported that the transcription factor SHOOTMERISTEMLESS (STM) plays a role in cytokinin production and perception by activating the expression of some of the IPT and AHK genes that produce and perceive cytokinin, respectively. However, this function has been attributed to STM based on studies in seedlings. Since cytokinin and STM are very important for gynoecium development; and all the genes for biosynthesis (IPT; LOG) and perception (AHK) seem to be expressed in this structure, we want to know if STM regulates biosynthesis and perception of cytokinin in the gynoecium. In this study, we used genetic and molecular tools to determine the relation between STM and cytokinin production and perception, and to know if this regulation is direct or indirect. The latest results will be presented.

# Community composition patterns of halophyte and xerophyte root associated bacteria

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In plant roots, bacteria-plant interactions have been extensively studied for numerous plant species across the World. Thus, we have realized the importance of these interactions for plant survival and growth, through exploitation of the metabolic byproducts of bacterial biochemical processes, such as nitrogen fixation and Extracellular Polymeric Substances (EPS). As halophyte and xerophyte plants are subject to extreme environmental conditions in the substrate, bacteria-root interactions should have a strong relevance for plant establishment and survival. Despite the imminent scenario of climate change and increased water scarcity, we have little understanding on whether there are world-wide patterns of plant microbial interactions in desert plants. For that reason, here we compare the patterns of bacterial community composition associated with halophyte and xerophytes through a meta-analysis based on currently available data on NCBI. Using alpha and beta diversity analyses, we aim to elucidate if there are any global patterns in these bacterial communities and if a relationship exists between microbial community composition, and the environmental or host species' traits.



# Lipid dynamics during seed germination and leaf development in Avocado *(Persea americana* Mill.)

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Avocado fruit is characterized by a high lipid content, including various unsaturated fatty acids with nutritional value. A large part of the lipid profile in avocado fruit seeds is taken by Acetogenins, which are fatty acid derivatives containing acetoxy, oxo, and hydroxy groups in their acyl chains. Understanding the metabolism that leads to acetogenin synthesis is necessary, as they possess desirable biological activities. To establish possible substrate-product relations between fatty acids (FAS) and acetogenins, we determined their profiles in seeds and leaves from different developmental stages of avocado fruits and plants. Acetogenins were found in all tissues analyzed, they reach their maximum values in seeds during imbibition at day 14 for the embryonic axis and day 21 in cotyledons (24.78 and 18.43 mg/g DW, respectively). Also, seedling leaves increase their acetogenin pool as they develop, reaching a maximum of 18.43 mg/g. In comparison, tree leaves harbor the smallest acetogenin pool reaching only 6.18 mg/g. The embryonic axis showed complex acyl editing capacity; we determined up to 22 different FAS with different lengths and unsaturation levels, while FAS accumulation increased in cotyledons at the last stages of germination. Leaves showed contrasting tendencies for lipid accumulation. Seedling FAS decreased down to half with a simultaneous 2-fold acetogenin increase during development. We also found significant correlations between 17C, 19C and 21C acetogenins and 18, 20 and 22C fatty acids respectively. When analyzing carbon partitioning between acetogenins and FAS, grown leaves had half of the carbons within each lipid pool, while seed tissues accumulated more than two thirds of the carbons within FAS.



# Molecular effectors involved in the germination process of the mistletoe *P. calyculatus*

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Psittacanthus calyculatus is a hemiparasitic mistletoe that infects mesquite trees. The success of the infection process depends on different mechanisms that synthesize molecular signals known as haustorium inducing factors (HIF) and also has been reported that host volatile organic compounds (VOC's) served as cues recognition factors to induce the seed germination of parasitic plant. Additionally, some phenolic compounds derived from bark degradation can stimulate germination and haustorium development. During the infection process, the host cells can be damaged and cellular fragments are released, those can be recognized by the host and induce an immune response to the stress, those molecules are known as damageassociated molecular patterns. To visualize if there are molecular effectors involved in the seed germination and haustorium development in the mistletoe, we evaluated in vitro seedle germination and the VOCs emitted during the germination process. Additionally, we analyzed the hydrogen peroxide, phenolic compound contents, bark degrading enzyme activities, and performed metabolic signatures from the phenotypic stages during the infection process on the host tree. The results show a higher lignocellulolytic enzyme activities related to the host bark degradation. The hydrogen peroxide and phenolic compounds presented a decrease in the haustorium formation stage, this can be associated with the formation of HIF that can stimulate the haustorium development. The metabolic profile presented high differences between the mistletoe infection stages and the host. This is the first study focused on the chemical signals and their response involved in the germination of the mistletoe P. calyculatus.



# Antifungal activity of Mexican mistletoe (*Psittacanthus calyculatus*) leaf and flower extracts against fusarium spp

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Plant extracts are a very important part of agroecology, because they have a great beneficial impact on the environment since some can be used to combat phytopathogenic organisms. The Mexican mistletoe (*Psittacanthus calyculatus*) is a parasitic plant that various endemic trees of the Bajío region of Mexico cause serious damage to their hosts 1the interaction with its hosts and dispersers are directly related with its distribution and community dynamics. For the last decade, the infested areas in central México have increased, becoming a potential plant health problem. The aim of this study was to investigate three main biotic factors associated with the distribution of P. calyculatus in a tropical deciduous forest: (1. Due to its chemical components, this species of mistletoe could be an important source of compounds that confer antimicrobial activity which has been tested on microorganisms pathogenic to humans. 2,3. The phytopathogenic fungus *Fusarium* spp causes economic losses in different agricultural crops of economic importance 2. That is why we aimed to evaluate the antifungal activity of extracts of mistletoe obtained from the host "palo dulce" (Eysenhardtia polystachya) against the inhibition of Fusarium spp. Firstly, our plant materials were dried (50 °C, up to constant humidity) and were ground in an industrial blender (80 mesh). A solid/liquid extraction was carried out using methanol/acetone After it, we removed the organic phase, using a rotary evaporator at 65°C for 4h. Subsequently, we mix PDA medium and the extract at 3 different concentrations and we placed the inoculum in the center of the petri dish. The control was petri dishes with PDA medium and with the phytopathogen without extract. All treatments and control were incubated at 28±2 °C in darkness. Our evaluations were inhibition percentage of mycelial growth in a petri dish (%IMG), final sporulation (FS) and, germination (G). The extracts show an inhibition greater than 87% for both types of extracts, likewise, a decrease in sporulation and germination was observed. These results show the potential of using mistletoe extracts for the inhibition of phytopathogens.



# Study of the participation of the AIB/JAM1 transcription factor in gene regulation by glucose in *Arabidopsis thaliana*

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Sugars serve as energy source, carbon skeletons, and signaling molecules. Plants have developed the ability to sense sugars and elicit the appropriate response through signaling pathways; by this plants control the nutrient status, sugar homeostasis, growth and development. Various studies have been carried out to identify the elements that participate in sugar signaling, but the current knowledge of these elements and the molecular mechanisms is still limited, such is the case of the transcription factors. Previously, in order to establish novel components in sugar signaling pathways, we conducted a DNA pull-down assay, using the regulatory region of the sugar responsive STP1 (SUGAR TRANSPORTER PROTEINI) gene, and nuclear protein extracts from plants treated with glucose (Glc). As a result we identified the AIB/JAM1 (ABA INDUCIBLE bHLH/JASMONIC ASSOCIATED MYC2-LIKE1) protein as a possible regulator of the STP1 expression. To determine the role of this transcription factor, we evaluated the phenotype of AIB/ JAM1 allelic mutants (jam1-1 and jam1-2) in presence of different Glc concentrations. It was evidenced that the *jam1* mutant presented an altered development in low (3-4%) concentrations, showing a *glo* (glucose oversensitive) phenotype, in comparison to wild-type plants. This approach suggested that AIB/ JAM1 participates in Glc signaling, regulating early developmental processes. To further explore the role of AIB/JAM1 in the gene regulation by sugar, we conducted a global transcriptomic analysis by RNAseq of the *jam1-2* mutant vs. wild-type plant, in presence of 4% Glc. The transcriptome analysis provides novel and integrated insights into sugar signaling, where AIB/JAM1 modulates the expression of certain genes, including STP1.



# Understanding the Pmk1 MAP kinase signalling pathway during plant colonisation by the rice blast fungus *Magnaporthe oryzae*

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Rice blast is among the most devastating diseases affecting global agriculture. It is caused by the fungal pathogen Magnaporthe oryzae. This fungus enters the plant by forming a dome-shaped infection structure called an appressorium. The Pmk1 MAP kinase (MAPK) signalling pathway plays a key role during appressorium formation, plant penetration and host colonisation. However, how Pmk1 regulates such physiological processes remains unclear. Here, we report a quantitative phosphoproteomic analysis to identify direct downstream targets of the Pmk1 MAPK during plant infection. By using discovery phosphoproteomics followed by Parallel Reaction Monitoring (PRM) we have identified 30 putative direct downstream targets of Pmk1 from an early time course study of appressorium samples. Some of those putative targets are proteins related to cellular processes such as cytoskeleton remodelling, vesicular trafficking and cell cycle control. There are also some targets with unknown function, like the SAM domaincontaining protein named Vts1. Interestingly, we found that Vts1 associates to Pmk1 by yeast-two-hybrid and co-immunoprecipitation experiments during early infection. By in vivo and in vitro studies, we have also demonstrated that Vts1 phosphorylation is Pmk1 dependent and occurs in two proline-directed sites (S175 and S420). We also discovered that Vts1 is necessary for appressorium development and pathogenicity. We are currently validating the role of Vts1 during plant infection by the rice blast fungus. Taken together, using a phosphoproteomic approach, we have found that Vts1 is a novel component of the Pmk1 signalling pathway.



# The VACUOLAR PROTEIN SORTING 13 (VPS13) affects female germline establishment and progression in *Arabidopsis thaliana*.

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Ovules are the precursors of seeds and arise from the placenta, a meristematic tissue inside the ovary. In the primordium of the ovule, one of the subepidermal cells of the nucellus, the archespore cell, differentiates into the megaspore mother cell (MMC), that undergoes meiosis to form four spores, in a process named megasporogenesis. The three most apical spores degenerate, while the remaining one, the functional megaspore (FM), enters megagametogenesis to ultimately form the mature female gametophyte, or embryo sac. Recent findings suggested that several molecular pathways are involved in the correct establishment and progression of the female germline. Here we reported a novel role for *VACUOLAR PROTEIN SORTING 13* (*VPS13*) in ovule development. Using different marker lines, we showed that alteration of *VPS13* expression affects MMC identity, thus affecting the correct progression of megasporogenesis. Our results provide insights into the molecular mechanisms that determine the correct differentiation and development of the female germline and pave the way for a better understanding of the hub factors determining this process.



# Study of the Auxin – AINTEGUMENTA-LIKE/ PLETHORA positive feedback loop conservation in the liverwort *Marchantia polymorpha*

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It has been predicted that diversification, co-option and reassembly of gene regulatory networks implicated in development is related to morphological innovations that led to more complex land plant bodies. AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) genes are considered part of the ancestral developmental toolkit of land plants. In Arabidopsis thaliana these transcription factors (TFs) are induced by auxin and are mainly expressed in dividing tissues where they play significant roles such as the maintenance of stem cell niches, the correct development of the embryo and the formation of lateral organs. Research on the auxin-AIL/PLT pathway has recognized that in Auxin Response Factors (ARFs) act as upstream mediators of AIL/PLT genes. Additionally, AIL/PLT TFs regulate the transcription of auxin biosynthesis and transport genes in both the root and shoot, establishing an Auxin - AIL/PLT positive feedback loop. Marchantia polymorpha, a descendant of the first events of embryophyte diversification, has a single AIL/ PLT orthologue, AINTEGUMENTA (MpANT) coded in its genome. MpANT protein is very similar to that of A. thaliana AIL/PLTs. A cis-elements analysis of the 2.5 kb promoter region of MpANT revealed it has six putative Auxin Response Elements. Most importantly, we have observed that the A. thaliana plt1;plt2 double mutant phenotype is partially complemented by the overexpression of MpANT, which is evidence that the function of AIL genes in stem cell regulation and their interaction with auxin could be conserved since the last common ancestor of land plants. With this study, we aim to understand the role of MpANT and also to determine if the Auxin-AIL/PLT positive feedback could be conserved in Marchantia polymorpha.



# Development of intrinsically disordered proteins-based biosensors to monitor the effects of the environment on cell biology

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The orchestrated set of reactions that maintain cell homeostasis are modulated by the concentration, composition, and properties of its molecular components. The impact of the interactions among macromolecules on regulating cell functions is well described, but how the physicochemical properties of the cellular environment influence the different aspects of cell biology remains largely uncharacterized. The challenge of studying the effect of the environment on cell functions resides in the lack of methods to dynamically monitor physicochemical parameters *in vivo*. To better understand how the environment regulates cell biology, we built a collection of Genetically Encoded Fluorescent Biosensors. As the sensory domain, we used 200 different intrinsically disordered regions (IDRs) from various organisms in a Förster Resonance Energy Transfer (FRET) biosensor context. The biosensor library contains IDRs from 37 different organisms from all kingdoms of life with unique features. We selected regions associated with the gain of stable structure in response to physicochemical environmental changes, classical and fully characterized IDRs, Arabidopsis transcription factors, plant intrinsically disordered stress proteins and regions participating in the formation of biomolecular condensates throughout the process known as liquid-liquid phase separation (LLPS). We envision that this collection of IDR-based biosensors will constitute a valuable resource to better understand the regulation of the environment on cell function.



# *Trichoderma atroviride*-emitted volatiles improve growth of *Arabidopsis* seedlings through modulation of sucrose transport and metabolism

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Plants host a diverse microbiome and differentially react to the fungal species living as endophytes or around their roots through emission of volatiles. Here, using divided Petri plates for Arabidopsis-*T. atroviride* co-cultivation, we show that fungal volatiles increase endogenous sugar levels in shoots, roots and root exudates, which improve *Arabidopsis* root growth and branching and strengthen the symbiosis. Tissue-specific expression of three sucrose phosphate synthase-encoding genes (*AtSPS1F*, *AtSPS2F* and *AtSPS3F*), and AtSUC2 and SWEET transporters revealed that the gene expression signatures differ from those of the fungal pathogens *Fusarium oxysporum* and *Alternaria alternata* and that AtSUC2 is largely repressed either by increasing carbon availability or by perception of the fungal volatile 6-pentyl-2Hpyran-2-one. Our data point to *Trichoderma* volatiles as chemical signatures for sugar biosynthesis and exudation and unveil specific modulation of a critical, long-distance sucrose transporter in the plant.



# Design of dsRNA-based biofungicides for the control of plant pathogens *Rhizopus oryzae*, *Fusarium incarnatum*, and *Geotrichum candidum*

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Most of the important fruits and seeds highly valued in the international market are produced in the tropical humid and sub-humid regions of Mexico, where diseases caused by fungi affect the production of crops at different stages of the food chain. Extending the postharvest life of tropical fruits is fundamental to minimize economic losses. Spray-induced gene silencing (SIGS) has emerged as a modern biotechnological strategy that can be implemented for crop protection. In this work, pathogenic fungi were isolated from papaya, pineapple, banana, and melon fruits obtained in the local market. Morphological and molecular analysis through sequencing and analysis of ITS region identified *Rhizopus oryzae* (papaya, pineapple and melon), *Fusarium incarnatum* (papaya), and *Geotrichum candidum* (papaya and melon), as the predominant fungal pathogens in the fruits at the postharvest stage. DICER-LIKE (DCL) genes in these pathogens or in closely related species were identified through a search in the available databases, and regions of 250-500 bp in length with low similarity to the respective plant DCL genes were selected for amplification, confirmation by sequencing and construction of a template for the synthesis of long and short dsRNAs. The antifungal activity of these dsRNAs is under evaluation. Our results will provide useful knowledge for the use of SIGS as an alternative for extending the postharvest life of fruits produced in the tropical humid and sub-humid regions of Mexico.



# Participation of the Arabidopsis thaliana PUT2 gene, encoding a polyamine transporter, in the plant defense response against Pseudomonas syringae

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The interaction between plants and pathogens is very common in nature. For this reason, plants have developed a highly regulated and complex defense system that is activated after pathogen recognition. The regulation of the plant defense system depends on several mediators, among which are polyamines. These polycationic aliphatic amines are present in all organisms, where they play essential functions for cell viability. In plants, polyamines participate in the establishment and regulation of the defense response, through changes in their metabolism (biosynthesis, conjugation-deconjugation, and catabolism), that result in modifications of free and conjugated polyamines. However, it is unknown whether polyamine transport has an implication in biotic stress response. In the present study, the importance of Polyamine Uptake Transport 2 (AtPUT2) in the interaction between Arabidopsis thaliana and Pseudomonas syringae pv. tomato DC3000 (Pst) was analyzed. Using a reporter promoter line (promAtPUT2::GUS) and analysis of mRNA levels by qRT-PCR, it was found that AtPUT2 gene expression increased 6 hours post-inoculation (hpi) with Pst. In addition, the phenotype of a T-DNA insertional mutant line (Atput2-1) in response to Pst infection was evaluated. A diminution in bacterial titers in the Atput2-1 mutant with respect to the wild-type ecotype was found 72 hpi. Furthermore, Atput2-1 mutant line had an increase in the expression levels of the *PR-1* and *ICS* genes, two important markers of the salicylic acid pathway involved in plant response to biotic stress. These results show that AtPUT2 is an interesting candidate to further evaluate the participation of polyamine transport in plant defense.



#### Salt stress responses in Marchantia polymorpha

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Soil salinity inhibits plant growth and affects agriculture around the world. Salt stress in plants causes metabolic disorders, inhibits photosynthesis, affects ionic balance and cell permeability, and can lead to death. Plants have developed different response mechanisms to cope with saline stress. The ability of plants to respond to increase in salt concentration depends on changes in gene expression. In order to understand the physiological and molecular changes during salt stress response in *Marchantia polymorpha*, a bryophyte that colonized the landscape 470 million years ago, we characterized the physiological response to low concentration (50 mM), moderate (100 mM) and high (150 mM) NaCl. Low concentration has positive effect in growth, 100 and 150 mM impair plant growth, causes oxidative burst and delay development. Then we profiled global gene expression and identified genes differentially expressed in *M. polymorpha* when exposed to 100 mM NaCl for 2 and 24 hours. In the transcriptome analysis we identify 1,154 up regulated genes and 1,352 down regulated genes in 2 and 24 hours. We were able to identify gene pathways involved in carbohydrates, amino acids, and lipid synthesis, auxins and ROS production. We conclude that saline stress in *M. polymorpha* is redirecting its energy to maintain cellular integrity and basal functions due to osmotic stress and that high concentrations of NaCl impact resource assignment, causing a decrease in area, biomass and affecting asexual reproduction

Key words: bryophytes, ecophysiology, RNA-seq, salinity conditions, tolerance.



#### Metallothionein1A regulates rhizobial infection and nodule organogenesis in *Phaseolus vulgaris*

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Metallothioneins (MTs) constitute a heterogeneous family of ubiquitous metal ion-binding proteins. In plants, MTs participate in the regulation of cell growth and proliferation, protection against heavy metal stress, oxidative stress responses, and responses to pathogen attack. Despite their wide variety of functions, the role of MTs in symbiotic associations, specifically nodule-legume symbiosis, is poorly understood. Here, we analyzed the role of the *PvMT1A* gene in common bean (*Phaseolus vulgaris*)-*Rhizobium tropici* symbiosis using bioinformatics and reverse genetics approaches. Using *in silico* analysis, we identified six genes encoding MTs in *P. vulgaris*, which were clustered into three (MT1, MT2, and MT4) of the four classes described in plants. *PvMT* genes contain two exons and one intron, while the primary structure of *Pv*MT contains at least two characteristic cysteine residues. *PvMT1A* transcript levels were significantly higher in roots inoculated with *R. tropici* at 7 and 30 days post-inoculation (dpi) than in non-inoculated roots. Functional analysis showed that downregulating *PvMT1A* by RNA interference (RNAi) reduced the number of infection events at 7 and 10 dpi, as well as the number of nodules at 14 and 21 dpi. In addition, nodule development was negatively affected in *PvMT1A*:RNAi transgenic roots, and these nodules displayed a reduced nitrogen fixation rate at 21 dpi. These results strongly suggest that *PvMT1A* plays important roles in the infection process and nodule development in *P. vulgaris* during rhizobial symbiosis.



# *In silico* characterization of one-carbon metabolism genes during the postharvest ripening of climacteric fruits (*Persea americana and Solanum lycopersicum*)

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Folates, known as vitamin B9 or folic acid (in their synthetic form), are essential in all cells. They donate one-carbon units (1C) for amino acids, nucleic acids, and S-adenosyl methionine (SAM) biosynthesis. These cofactors are involved in several aspects of plant development, metabolism, and physiology, including embryo and root development, DNA methylation, and NADPH production. The ripening process of climacteric fruits is triggered by ethylene production, and ethylene synthesis partially depends on one folate: 5-CH<sub>2</sub>-THF. The enzyme Methionine Synthase (MS) uses 5-CH<sub>2</sub>-THF as a cofactor for Methionine synthesis, then Met donates 1C to SAM synthesis (via SAM synthase), a precursor of ethylene. Previously, we have evidenced that during postharvest ripening of climacteric fruits, the folate pool was affected by ethylene. This work aimed to in *silico* characterize 1C metabolism genes and their evolutive relationships of two climacteric fruits, avocadoes (Persea americana) and tomatoes (Solanum lycopersicum), and the effect of ripening on the expression of 1C metabolism genes (MS and SAMS) related with ethylene synthesis using databases (Ibarra-Laclette et al. 2015, Tomato Expression Atlas). Using Arabidopsis sequences as query, at least one orthologous gen was obtained from avocado and tomato fruits genomes. For each protein, the subcellular localization was predicted. In the case of MS, one possible plastidial and one cytoplasmic isoforms were predicted. Also, the MS proteins alignment showed that they were highly conserved between plants (>70%). Finally, transcriptomic data retrieved showed that MS and SAMS genes expression was significantly upregulated during postharvest ripening in both climacteric fruits.



# *Micrococcus luteus* LS570 promotes root branching in *Arabidopsis* via decreasing apical dominance of the primary root and an enhanced auxin response

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The interaction of plant roots with bacteria is influenced by chemical signaling, where auxins play a critical role. Auxins exert positive or negative influences on the plant traits responsible of root architecture configuration such as root elongation and branching and root hair formation, but how bacteria that modify the plant auxin response promote or repress growth, as well as root structure remains unknown. Here, we isolated and identified via molecular and electronic microscopy analysis a Micrococcus luteus LS570 strain as a plant growth promoter that halts primary root elongation in Arabidopsis seedlings and strongly triggers root branching and absorptive potential. The root biomass was exacerbated following root contact with bacterial streaks, and this correlated with inducible expression of auxin-related gene markers DR5:GUS and DR5:GFP. Cellular and structural analyses of root growth zones indicated that the bacterium inhibits both cell division and elongation within primary root tips, disrupting apical dominance, and as a consequence differentiation programs at the epidermis triggers the formation of longer and denser lateral roots and root hairs. Using Arabidopsis mutants defective on auxin signaling elements, our study uncovers a critical role of the auxin response factors ARF7 and ARF19, and canonical auxin receptors in mediating both the primary root and lateral root response to *M. luteus* LS570. Our report provides very basic information into how actinobacteria interact with plants and direct evidence that the bacterial genus *Micrococcus* influences the cellular and physiological plant programs ultimately responsible of biomass partitioning.



# Analysis of Pitaya (*Stenocereus thurberi*) Fruit Exocarp Transcriptome: Towards Identification of Cuticle Biosynthesis Genes

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The specie Stenocereus thurberi is a Cactaceae endemic from the Sonoran desert, adapted to survive under its prevalent environmental conditions. It produces a fleshy fruit named `pitaya'. The cuticle is the first contact between the plant and its surrounding environment. The cuticle maintains fruit integrity by avoiding water loss and it seems to control the fruit postharvest shelf life. In this work, we generated RNA-seq data from S. thurberi fruit exocarp to produce information useful for the study of the molecular mechanism of cuticle biosynthesis and its response to abiotic stress in pitaya fruit. A de novo transcriptome assembly was carried out from 243 million read pairs with a length of 150 bp and a mean Phred quality score of 29. Trinity default parameters were used to assemble a transcriptome that includes 174,449 transcripts with a N50 value of 2,110 bp, an average transcript length of 1,198.69 bp, and 85.4% of completeness. An alignment by BLAST was carried out against RefSeq, SwissProt, TFDB, iTAK, Arabidopsis, Tomato, and other fruits and cactus databases. 137,986 (79.1%) transcripts showed homology to proteins and/or transcripts from these databases (E value  $\leq 1 \times 10^{-5}$ ). TransDecoder analysis showed that 88,196 (50.56%) transcripts have a functional open reading frame (ORF). It was not found either homology or ORF for 32,718 transcripts, suggesting that they could be non-coding RNAs. This information will be helpful for future studies about the molecular mechanism of response to abiotic stress and biosynthesis of compounds of interest in S. thurberi fruit and other cactus.



# Identification of *SWEET* gene family in the *phaseolus* genome and comparative analysis of their expression profiles specific to mycorrhizal and rhizobial symbiosis

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Sugars are important molecule for the development of plants that fulfill their metabolic function. The *SWEET* (sugars will eventually be exported transporters) family, which carry sugars have been characterized in several plant species, but it's not been analyzed in legume *Phaseolus*. Plants make mycorrhizal or rhizobial symbiosis to obtain various nutrients from the soil, and these in turn give sugars to the hosts to fulfill their metabolic pathways. Herein, we performed a genome-wide analysis to identify the carrier gene *SWEET* members in *Phaseolus vulgaris* genome and examined the expression profiles of *PvSWEET* genes during mycorrhizal and rhizobial symbiosis. Phylogeny, chromosomal localization, number of transmembrane helixe (TMH), gene structure, and motifs of *PvSWEET* genes showed 7 TMH and the family was classified into four clades with 3 different types of motifs in a duplicate domain (*Mt*N3\_saliva). Further, transcriptomic analysis revealed that shared and unique *PvSWEET* genes were upregulated during arbuscular mycorrhizal and rhizobial symbiosis. Overall, the systematic analysis of the *PvSWEET* gene family provides valuable information for further studies on the biological roles of *SWEETs* in various *Phaseolus* tissues during diverse biological processes, including *Phaseolus*-mycorrhiza/rhizobia symbiosis. This work was supported by DGAPA-PAPIIT, UNAM no. IN213221 to M.-K.A, IN216321 to K.N; CONACYT 316538 to M.-K.A.



#### Ascorbic acid regulates cuticle deposition and stress responses in *Marchantia polymorpha*

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Ascorbic acid (AsA) is a multifunctional metabolite that in plants functions as a major antioxidant and as a signaling molecule that regulates growth, development, photosynthesis, hormone biosynthesis and also maintains the homeostasis of reactive oxygen species (ROS). The major biosynthetic route of AsA in plants is the D-Mannose/L-galactose pathway that involves the activity of the enzyme L-galactone-1,4-lactone dehydrogenase (GLDH) to catalyze the final reaction leading to AsA and is conserved across embryophytes. In the bryophyte Physcomitrium patens, AsA catabolism is required for the synthesis of cuticle which prevents organ fusion and desiccation. In this work, we present the characterization of CIRSPR-Cas9 induced loss-of-function mutants of Marchantia polymorpha MpGLDH. Mpgldh-1 loss-of-function mutants are affected in the formation of cuticle, they exhibit developmental defects and organ fusion. Mpgldh-1 mutants are more sensitive to salt stress and also exhibit a deferred infection when inoculated with the necrotrophic fungus Botrytis cinerea. Our results highlithg an important role of AsA in M. polymorpha for development and in the responses to abiotic and biotic stress.



#### Isolation and cellular identity of the megaspore mother cell in *Arabidopsis thaliana*

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The megaspore mother cell (MMC) is the initial female germ cell and meiotic precursor of Arabidopsis thaliana (Arabidopsis). Being tightly covered by sporophytic cells within the ovule primordium, the MMC is of difficult isolation. As a consequence, only few genes related to its differentiation and unique identity have been identified. One of the molecular pathways involved in the establishment of MMC is the RNA-dependent DNA Methylation pathway (RdDM). It has been observed that when mutating components of this pathway, more than one ectopic cell reminiscent of the MMC differentiate in the apical pole of the ovule primordium; however, the origin and identity of these ectopic cells has not been elucidated. We have established a protocol that allows the isolation of MMC populations by means of fluorescence activated cell sorting (FACS). Our results suggest that our protocol can be used to isolate sufficient germ cells from female organs to conduct whole cell-based transcriptome and methylome analysis in Arabidopsis thaliana. We also investigated the possible origin of the ectopic cells in the RdDM mutants. Using molecular markers that allow the distinction between meiotic and mitotic divisions. We evaluated early ovule development (five stages analyzed), confirming the absence of mitosis in the MMC and adjacent cells in wild type ovules. These patterns also were conserved in the ovule primordium of RdDM mutants, that suggest that the origin of the ectopic cell in the subepidermal layer of apical pole of the ovule mutants is not result of mitotic divisions of a previously differentiated MMC.



# Enhancement of Arabidopsis, tomato, and maize plant growth by biocontrol agent *Metarhizium anisopliae*

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*Metarhizium spp.* is the most used entomopathogenic fungus for integrated pest management. However, recently some Metarhizium species have been found as plant rhizosphere associates. Here the effect of three *Metarhizium anisopliae* strains (Ma-20, Ma-25 and Ma-28) to improve Arabidopsis plant growth were evaluated under *in vitro* conditions using a split system. The results showed that three Metarhizium strains significantly increased plant biomass and total chlorophyll content in Arabidopsis after 7-days post-inoculation. Also, Metarhizium strains are able to modify root architecture; the main root length was promoted by all strains without physical contact, whereas in direct contact it caused inhibition in main root growth. Under split system interactions, we identified the terpene  $\beta$ -caryophyllene as unique compound, whereas in the Arabidopsis-Ma-28 interaction *o*-cymene, *P*-cymene and  $\beta$ -caryophyllene by GC-MS. Additionally, we analyzed the interaction with tomato, Arabidopsis, and maize plants grown in pots soil, wherein the *Metarhizium anisopliae* strains significantly increased plant biomass. Our results showed that these *Metarhizium anisopliae* strains could be used for crop production, with the advantages of insect biocontrol and plant growth promotion.


## Arabidopsis plant growth is mediated by Volatile Organic Compounds emitted by Trichoderma

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Trichoderma can colonize the plant rhizosphere, providing pathogen resistance, abiotic tolerance, and enhancing crop yield. Trichoderma species are prolific producers of many bioactive metabolites. Volatile organic compounds (VOCs) emitted by Trichoderma have antifungal activity, induce resistance to plant pathogens and promote plant growth. In this study, Arabidopsis plants were exposed to mixtures of volatile organic compounds emitted by *Trichoderma atroviride* and *Trichoderma virens*, which were grown on split petri dish with PDA or MS. The VOCs released by two Trichoderma species grown on PDA under split interaction significantly increased plant biomass and lateral roots number in Arabidopsis at 3 and 5dpi. Furthermore, an analysis using the auxin *DR5:uidA* reporter line and the mutant *rhd6* (root hair-defective phenotype) in Arabidopsis revealed that VOCs released by two Trichoderma species trigger the auxin production in Arabidopsis. VOCs analysis by GC–MS revealed that when Trichoderma strains were growth in Ms or PDA culture media 50% of VOC produced were sesquiterpenes in the Arabidopsis-T. atroviride, and 87% in Arabidopsis-T. virens interactions. This study showed that blends of VOCs emitted by *Trichoderma atroviride* and *Trichoderma virens* improve Arabidopsis growth through an auxin-dependent mechanism.



# Effect of Streptomyces bacteria strains as a plant growth promoting agents

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Actinomycetes represent important microbial communities in plant rhizosphere. These produce a wide range of bioactive compounds which increase the tolerance in plants exposed to biotic and abiotic stresses. Certain species of actinomycetes have the ability to promote plant growth through the production of organic acids, phytohormones, siderophores and volatile organic compounds. In this work, four rhizospheric actinomycetes were isolated from melon and avocado fields. The isolates were characterized genetically through the amplification of the 16S rRNA, as a result they had high identity with Streptomyces species. The effect of Actinomycete strains on the growth of Arabidopsis seedlings was evaluated using an *in vitro* split system, and they showed a significant increase in fresh weight at 12dpi for 27-E and 37-E strains. For the directly contact condition, was observed that strains 30-A and 37-E promoted the highest fresh weight in Arabidopsis seedlings. The main root length was promoted by all strains without physical contact, whereas in direct contact it caused inhibition in main roots growth. Using the Arabidopsis DR5:uidA reporter line was possible to identify an accumulation of auxins in roots during a split interaction with all strains. Finally, through the Kovacs biochemical test, we identify that all strains produced indole compounds and were positive for siderophore production. Our data showed that these Streptomyces strains are good candidates to be used in commercial crops as biofertilizers.

## Identification of SOL interacting pathways, a maize protein involved in immunity

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Maize (*Zea mays*) is one of the species of grasses family with major economic importance worldwide and it is considered one of the staple foods for humans. One of the main problems facing the crop is attack by pathogens and environmental conditions that affect its development and in turn severely limit performance due a decompensation between defense and growth. The productivity of different crops has been significantly improved through the implementation of strategies that favor their development; these strategies are based on the molecular mechanisms of response to biotic and abiotic stress that plants possess. Therefore, the identification of specific regulators that control both signaling of the immune response and development of plants is a key goal to provide knowledge that helps to maximize productivity. Due to this, the investigation of the maize SOL protein has been raised, this protein has been shown to have the ability to rescue the phenotype of *Lgn-R* maize mutants which are related to the development of leaves and resistance to pathogens. Therefore, this research focuses on using the SOL protein to purify a specific antibody that allows the recognition of SOL in maize tissues by immunolocalization and also identify its possible interactors through immunoprecipitation.



## Carbon concentrating mechanisms in pods are key elements for terminal drought resistance in common bean (*Phaseolus vulgaris L*.)

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Common bean (*Phaseolus vulgaris L.*) is one of the most consumed legumes in the human diet. A major problem for this rainfed crop is the decrease in grain yield and the big losses (>80%) caused by terminal drought (TD). The aim of this research is to investigate the impact of TD on carbon distribution from leaves to pods and seeds and, its relationship and relevance to grain yield in common bean cultivars with different TD resistance. Previous reports and data obtained in our laboratory, and others, indicate that common bean cultivars considered resistant to TD are more efficient in remobilization of carbon reserves for seed filling under TD compared to TD sensitive cultivars, suggesting that carbon distribution is modulated by water deficit conditions (Polania *et al.*, Euphytica 210:17, 2016; Cuéllar-Ortiz et al., Plant Cell Environ 3:1399, 2008; Rosales-Villegas *et al.*, Plant Physiol Biochem 56:24, 2012). In this work, we present physiological and molecular data supporting the relevance of photosynthate distribution to pods and seed in TD sensitive and resistant common bean cultivars. We have also conducted TD sugar accumulation and transcriptomic experiments of source leaves, pods and seeds of these cultivars. The molecular analyses have allowed the identification of transcripts/genes responding to TD stress conditions in those organs that suffer a major impact and are critical to final yield at this stage. Altogether, these findings exhibit the relevance of carbon concentrating mechanisms in pods for terminal drought resistance in common bean.



## Genome evolution and phylogenetic relationships in *Opuntia tehuacana* (Cactaceae)

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Mexico harbors a large diversity of *Opuntia species (Cactaceae)*, which are also relevant due to their cultural and economic importance. The multiple taxonomic problems in this group of plants are related to hybridization, homoplasy and polyploidy, from which the latter allows them to survive in adverse environments and also plays an important role in speciation and evolution. The study of geographic areas with high biodiversity is of primary importance, thus we are focused in the Tehuacán-Cuicatlán Valley (Oaxaca, México), with a high diversity of Opuntia species whose phylogenetic relationships and chromosome numbers are unknown. This study aims to know the phylogenetic position of O. tehuacana and their sympatric species, and analyze the ploidy levels in five O. tehuacana localities. We performed Bayesian phylogenetic analysis using three chloroplast markers and two nuclear introns, as well as chromosome counts for three Opuntia species and flow cytometry analysis in O. tehuacana. Our phylogenetic trees shown O. tehuacana as member of Basilares clade as well as most of their sympatric species, except for O. decumbens, O. lasiacantha and O. huajuapensis which are in Nopalea clade. The ploidy level of O. tehuacana is 11x and 12x the highest reported so far for the genus. Finally, we found significative differences inside O. tehuacana genome size and their high ploidy level might be due to multiple polyploidization events occurred between individuals from the same specie as well as from other opuntias.



## *In situ* to greenhouse transplant of drought-adapted and plant-associated bacteria

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Climate change is negatively affecting crop production by increasing temperature and reducing agricultural areas. Some microbial communities from arid sites are adapted to drought and may improve plant survival to climate change. Here, we studied squash (Cucurbita pepo L.) root microbiome from historically dry and humid sites to test whether in situ diversity could be transferred to a greenhouse common garden experiment. We sequenced 16S rRNA gene amplicons from soils, rhizospheres, and root endospheres. These analyses showed that Proteobacteria was the most abundant phylum in all the samples, but Actinobacteriota prevalence was higher in arid ones. From the 1009 bacterial genera found in the root-associated samples, 199 were exclusively from arid locations. Remarkably, β-diversity analyses showed split microbiomes between arid and humid localities, demonstrating that it was possible to transplant in situ diversity to the greenhouse. Arid locations bacteria such as Cellvibrio, Ensifer, and Streptomyces were positively correlated with squash phenotype, suggesting their possible role in promoting plant growth under drought conditions. Additionally, shotgun metagenomes confirmed the in situ transfer and enrichment of previously reported genes in drought survival as protein degradation and folding, oxidative stress, and compatible solute synthesis. Finally, allowing us to describe new microbial enriched genes under drought conditions. So far, we showed that it is possible to transfer microbial communities from arid sites to other locations to recover taxa and genes with the potential to face the negative effects of drought on plants.



## Determination of heat stress-tolerant bread wheat plants field-grown in the Yaqui Valley, Mexico

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*Triticum aestivum L.* is a staple crop that provides with more proteins and calories than any other cereal. The increasing threat of climate change has had negative impact on yield of wheat in many regions of the world, and the pernicious effects are expected to be aggravated in the next century. Heat stress is an abiotic stress that limits plant growth, metabolism, and productivity worldwide. Therefore, the development of heat stress-tolerant wheat genotypes able to maintain grain yield and quality is crucial to food security and economical profits. Herein, two field experiment: control and heat stress during reproductive stage were performed with 25 CIMMYT bread wheat genotypes and a control from INIFAP at the Norman E. Borlaug research field in the Yaqui Valley, Sonora, Mexico. Based on grain yield reduction after heat-stress 10 out of 26 genotypes were labeled as heat-tolerant ( $\leq 700$  g) or heat-sensitive (> 700 g) and selected for further analysis. Genotypes 1, 3, 18, parents 22 and 23, and 24 were sensitive while 4, 6, 17 and 26 (control) were tolerant. Likewise, plant height was evaluated at both conditions. All sensitive genotypes presented a reduction > 14 cm under heat stress, except the 23, while tolerant genotypes had a reduction < 14 cm, except for genotype 4. Genotype 24 had the lowest chlorophyll concentration (µmoles per m2) and 22 the highest. Only genotype 17 presented significant reduced levels in both Fv/Fm and Eto/Rc fluorescence parameters under heat stress.



## Coexpression networks associated with auxin metabolism during transition from flower to fruit in *Vanilla planifolia Andrews* (Orchidaceae)

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The transition from flower to fruit (TFF) in angiosperms is a complex process that is coordinated through a system of co-expression networks related to auxin metabolism. However, in plants with post-pollination syndrome (PPS) such as the Orchidaceae family, the absence of ovules at the time of anthesis implies structural and functional differences that modify the general explanation about the role of auxins in the TFF of these plants. In this regard, co-expression network analysis has made possible to develop models to explain the TFF process in angiosperms. Therefore, the objective of this study was to analyze the coexpression networks of auxin metabolism during TFF in the CH-I (fruit fall-tolerant) and CH-VI (susceptible to fall) genotypes of V. planifolia. Transcriptomes related to TFF were used to build co-expression networks and functional modules, also authoritative hub genes (AHG) were identified. The results show differences in the number of functional modules in the co-expression networks of each genotype, 16 AHG associated with a developmental genetic program were identified in the VNCH-I network, while in VNCH-VI 38 AHG related to environmental interaction were recognized. In this sense, the regulation of the CH-I genotype is linked with low levels of auxins through of the hub genes VpJAR4 and VpGH3.11 (conjugation), while the CH-VI genotype presents a regulation associated with high auxin gradients through of the hub genes VpTAR1 and VpNPY (biosynthesis). It was concluded that the VNCH-VI network presents a regulatory core, related to immature stages of the ovary suggesting an incomplete fertilization.



## **Functional Analysis of Flowering Locus T During the Reproductive Transition in** *Agave tequilana*

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The reproductive transition has been widely studied in monocarpic annual species and polycarpic perennial species. Unfortunately, there are few reports for reproductive transition in monocarpic perennials such as Agave tequilana, which is a crop of interest in Mexico due to the production of tequila and mezcal. Moreover, this crop has acquired international relevance for fiber production and as a bioenergy source. Based on transcriptome analysis from leaf and shoot apical meristem tissues of A. tequilana plants from different developmental stages, a series of elements involved in the reproductive transition were identified, such as Flowering Locus T (FT), a mobile signal protein that induces the reproductive transition, also known as florigen. The manipulation of the reproductive transition represents an advantage for commercial production and basic research in Agave species. Hence, the purpose of the present study is to characterize the function of the FT homologs (AtqFTs) in A. tequilana. In order to do this, clones were constructed with the AtqFTs putatively designated as promoters or repressors and their ectopic overexpression is being studied in Arabidopsis thaliana Col-0 and ft-10. The overexpression of AtqFTs is also being characterized in A. tequilana by establishing a transformation protocol mediated by Agrobacterium tumefaciens. In addition, in situ hybridization and translational fusions of the genes to yellow fluorescent protein will be developed to determine the spatial and temporal expression. The results obtained will enable us to understand the transcriptional regulation of the reproductive transition and to improve strategies for genetical analysis in Agave species.



## Plant membrane on-a-chip platform to study COPT1 transporter function

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Plant transport mechanisms have been studied for many years; however, the study of individual proteins is a big challenge because of the non-electrogenic nature of some of them. To overcome this, we designed a plant membrane on-a-chip system that combines optical and electrical means to study these proteins. We selected copper transporter protein 1 (COPT1), which regulates copper levels in plants and is related to plant survival in copper stress conditions. First, we formed a supported lipid bilayer (SLB) using membranes from transient GFP and GFP-COPT1-transfected Arabidopsis thaliana mesophyll protoplasts. Then, the formation and fluidity of the SLB was confirmed by fluorescence recovery after photobleaching. We obtained a similar diffusion coefficient for both samples and full recovery of photobleaching, confirming a high quality SLB has formed. Next, we integrated SLBs with organic electrochemical transistors (OECTs) based on the conductive polymer PEDOT:PSS. OECTs allow for combination of high quality electrical signals and optical monitoring due to the transparency of PEDOT:PSS thin films. Electrochemical impedance spectroscopy (EIS) shows that the SLB forms a layer that blocks the passage of ions from the electrolyte to the conductive polymer, increasing the system's impedance. COPT1 SLBs were tested under different CuSO4 concentrations, 0-100 µM. For COPT1 SLB, we observe a slight decrease in the impedance in each concentration but not with the control case. When the copper was removed, the impedance was recovered to the original value. This points to the presence of copper being the cause of the impedance decrease when the COPT-1 transporter is open. In the future, our platform could be used to analyze deeply COPT1 transporter mechanism and study more plant proteins involved in transport mechanisms associated with plant survival.



## Effects of time on buffelgrass rhizosphere microbiome

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Buffelgrass (*Pennisetum ciliare*) is an invasive desert shrub out-competing native species. One exclusion mechanism used by buffelgrass is allelopathy, which is based on the synthesis and release of allelochemical compounds by one plant that influences another plant's growth. Buffelgrass produces and releases phenolic acids into the rhizosphere, where they interact with microorganisms. However, little is known about the associations between buffelgrass and soil microorganisms. In this study, the 16S rRNA gene Amplicon Sequence Variants (ASV) were used to describe the buffelgrass roots microbiome under the autotoxic effects of its allelochemicals in a short time scale. We tested the hypothesis that microbiome composition would change according to allelochemical treatment. For this, buffelgrass roots were exposed to root exudates or aqueous leachates from shoots. A total of 2,164 ASVs were identified. Actinobacteria was found as the dominant phylum, just as is commonly found in desert and arid zones. Contrary to what was expected, the presence of allelochemicals did not affect the composition of the buffelgrass' rhizosphere microbiome. However, another trend was found, microbiome convergence across time with a merging trend. This tendency could be a consequence of thriving in a phenolic-rich environment, which probably changes as the plant grows and develops. Finally, we identified a buffelgrass' core root microbiome comprising bacteria previously reported as antimicrobial or vitamins producers, thus allowing them to act as pivots to shape the rest of the microbial community in the roots of buffelgrass.

# Role of flotillin in root hair growth and during the interaction *Phaseolus vulgaris*-rhizobia

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The symbiotic relationship between bacteria of genus *Rhizobium* and the plant of common bean (*Phaseolus vulgaris*), is one of the most common successful plant-microbe interactions. The bacteria are internalized in the root through specialized cells such as the root hair, to do so, they induce the formation of a structure called infection thread and subsequently penetrate the root cortex. It was shown that membrane microdomains are important for membrane shaping, trafficking and signal transduction. Flotillins are lipid microdomains components in both vegetal and animal cells and in plants were shown to play important role in plantmicrobe interaction. In this study, we identified one flotillin gene in *P. vulgaris* genome and conducted transcriptional profiling in common bean roots to determine the specific expression patterns and subcellular localization of flotillin during nodulation. Our results reveal that flotillin is expressed in the root and nodule meristem development. Subcellular localization indicates that flotillin have a role in vesicular trafficking and root hair polar growth.



## The plant beneficial rhizobacterium *Achromobacter sp.* 5B1 influences root development through auxin signaling and redistribution

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Roots provide physical and nutritional support to plant organs that are above ground and play critical roles for adaptation via intricate movements and growth patterns. Through screening the effects of bacterial isolates from roots of halophyte Mesquite (Prosopis sp.) on Arabidopsis thaliana, we identified Achromobacter sp. 5B1 as a probiotic bacterium that influences plant functional traits. Detailed genetic and architectural analyses in Arabidopsis grown in vitro and in soil, cell division measurements, auxin transport and response gene expression and brefeldin A treatments demonstrated that root colonization with Achromobacter sp. 5B1 changes the growth and branching patterns of roots, which were related to auxin perception and redistribution. Expression analysis of auxin transport and signaling revealed a redistribution of auxin within the primary root tip of wild-type seedlings by Achromobacter sp. 5B1 that is disrupted by brefeldin A and correlates with repression of auxin transporters PIN1 and PIN7 in root provasculature, and PIN2 in the epidermis and cortex of the root tip, whereas expression of PIN3 was enhanced in the columella. In seedlings harboring AUX1, EIR1, AXR1, ARF7ARF19, TIR1AFB2AFB3 loss-of- function mutations, or in a dominant (gain-of-function) mutant of SLR1, the bacterium caused primary roots to form supercoils that are devoid of lateral roots. The changes in growth and root architecture elicited by the bacterium helped Arabidopsis seedlings to resist salt stress better. Thus, Achromobacter sp. 5B1 fine tunes both root movements and the auxin response, which may be important for plant growth and environmental adaptation.

Keywords: Arabidopsis, auxin signaling, development, root biology.



# **O**-Glycosylation modulates the localization of pollen class I formins during tip growth

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Pollen tube elongation is a tightly regulated process where targeted secretion of new cell wall material and cytoskeleton dynamics coordinate to maintain an apical domain of growth. The molecular players linking extra and intracellular dynamics to enable proper pollen tube growth remains elusive. Polarized growth in pollen tubes depends on the organization of the actin cytoskeleton in functionally distinct subarrays. In Arabidopsis, two members of the class I formin family, AtFH3 and AtFH5, participate in F-actin dynamics by nucleating cortical actin in the shank and the tip, respectively. Both AtFH3 and AtFH5 are transmembrane proteins with extracellular domains (ECDs) akin to hydroxyproline (hyp)-rich cell wall glycoproteins: AtFH3 possess putative Arabinogalactan protein-like glycosylation motifs (hyp-Oarabinogalactan glycans), whereas AtFH5, possess putative Extensin-like motifs (hyp-O-arabinose). We demonstrate that the ECD of both AtFH3 and AtFH5 is necessary for their plasma membrane localization. We provide evidence suggesting that AtFH5 is immobilized in the apical region of the pollen tube via hyp-Oarabinosylation and endocytosis. Furthermore, mutant pollen tubes lacking hyp-O-arabinose display F-actin cytoskeleton disorganization, possibly due to AtFH5 mislocalization. Finally, biochemical approaches revealed that the ECD of AtFH3, but not AtFH5, is post translationally modified (PTM) by the addition of hyp-O-arabinogalactan glycans and such PTMs greatly influence their lateral mobility in the membrane. Our findings suggest that the presence of distinct glycans in the ECDs of pollen class I formins modulate their interaction with the extracellular matrix, spatially restricting them to specific domains in the plasma membrane to perform their intracellular functions.



## **Evolution of the putative RETINOBLASTOMA-RELATED LxCxE-mediated interaction landscape across Viridiplantae**

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Key steps in evolution are often singularities. One of such was the colonization of land by a single charophyte algae lineage some 450 million years ago. This evolutionary success has been linked to multiple key innovations such as 3D growth, alternation of generations, as well as innovations inherent to the birth of major plant lineages, such as the origins of vascular tissues, seeds and flowers Multicellularity, which evolved multiple times in the Viridiplantae lineage, coupled with precise spatiotemporal control of proliferation and differentiation were instrumental for the evolution of the aforementioned traits. RETINOBLASTOMA-RELATED (RBR), the plant homolog of the metazoan Retinoblastoma protein (pRB), is a highly conserved core cell cycle regulator that has been implicated in the evolution of multicellularity in green algae as well as plant multicellularity-related processes such as proliferation, differentiation, stem cell regulation and asymmetric cell division . RBR participates in many of these processes through context-specific proteinprotein interactions (PPI) with proteins containing the Leu-x-Cys-x-Glu (LxCxE) short-linear motif (SLiM), however, how RBR-LxCxE interactions have changed throughout major innovations in the Viridiplantae kingdom is a question that remains unexplored. Here, taking advantage of recently published genomes of representative species of major Viridiplantae lineages, we employ an in silico evo-devo approach to analyze how potential RBR-LxCxE interactions have changed in major Viridiplantae lineages. Our data suggests that potential RBR-LxCxE interactions with chromatin modifiers/remodelers, DNA replication and repair machinery are highly conserved throughout the Viridiplantae, while LxCxE interactions with DNA-binding transcription factors likely accompanied the water-to-land transition.





## The novel phytochrome interacting proteins ERF55 and ERF58 are repressors of light-induced seed germination in *Arabidopsis thaliana*

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The moment at which a seed germinates is controlled by interactions between environmental and developmental cues. Three such cues are, light, abscisic acid (ABA) and gibberellin (GA); however, the molecular nature of interactions between them remain largely obscure. Here we show that light-dependent seed germination is enhanced in mutants deficient in the AP2/ERF transcription factors ERF55 and ERF58. Light-activated phytochromes bind ERF55/ERF58 and displace them from promoters of *PIF1* and *SOM*, genes that encode germination-repressing transcriptional regulators. The same mechanism controls the expression of genes that encode enzymes which regulate the cellular concentrations of GA and ABA. Such light-dependent ERF inactivation stimulates germination by reducing the expression of *PIF1* and *SOM*, and differentially regulating the expression of ABA and GA metabolic genes to decrease levels of ABA and possibly increase levels of GA. Interestingly, *ERF55* and *ERF58* are themselves under transcriptional control of ABA and GA suggesting that, under permissive light regimes, they form a self-reinforcing signalling loop which drives germination.

Keywords: Light signalling; phytochromes; abscisic acid (ABA); gibberellic acid (GA); seed germination; AP2/ERF transcription factors



## **DEFECTIVE IN INDUCED RESISTANCE 1 play** a key role in the progress of the infection thread and the organogenesis of nodules in *Phaseolus vulgaris*

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*Phaseolus vulgaris* is known to establish a symbiotic relationship with *Rhizobium tropici*, forming new organs called nodules where the bacteria fix atmospheric nitrogen. Beneficial soil microorganisms such as rhizobacteria can induce systemic defense responses by optimizing the symbiosis and conferring resistance to various plant. *DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1)* encodes a small protein implicated in the activation of the systemic defense response in *Arabidopsis thaliana*. *DIR1* belongs to the family of non-specific lipid protein transporters (LPTs), the members of which are involved in membrane remodeling, cell expansion, plant growth, stress responses and the establishment of symbiosis. In the *P. vulgaris* genome we identified four genes homologous to *AtDIR1*. Herein, we analyzed and compared the *PvDIR1* phylogeny with others legumes and with *A. thaliana*, and determined its role in nodulation. Phylogenetic analysis shows that *DIR1* from legumes clustered into two separate clades of *A. thaliana*. On other hand, the silencing of *PvDIR1* by RNAi interferes with the advancement of the infection thread formed by the rhizobia to colonize the cortical cells of the nodule, driving a reduction in the number of nodules, as well as changes in the differentiation of symbiosomes. In addition, *PvDIR1*:RNAi nodules show a smaller size and a multinodular phenotype with aberrant vascular tissue development. Our data suggest that *PvDIR1* acts as a positive regulator of nodulation and prevents the occurrence of more than one infection event at the same location.



#### Role of the m<sup>6</sup>A-reader protein ECT8 in the response to stress conditions in *Arabidopsis thaliana*

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Methylation of adenosine at the N6 position (m6A is the most common internal modification found in eukaryotic mRNA. It is a conserved post-trancriptional regulation mechanism that affects the fate and function of the modified mRNA, thus regulating fundamental biological processes. Addition of m6A is a reversible and dynamic process, where the protein complexes responsible for its addition, removal and recognition have been described. Most of the "reader" proteins recognize m6A through a highly conserved domain known as YTH, found only in eukaryotes. In Arabidopsis, 13 proteins with this domain have been identified. Among these, previous work in our group showed accumulation of the transcript of the AtYTH06 gene (*ECT8*) under different conditions associated with low water availability or upon abscisic acid (ABA) addition. Furthermore, in germination tests in the presence of ABA we observed a partial insensitivity to this hormone when we used two different T-DNA insertional mutant lines for this gene (*ect8-1* and *ect8-2*), indicating a possible defect in its perception and/or signaling.

Therefore, we suggest an important role for ECT8 in the response to situations of water deficit. However, the biological functions, the molecular partners, and the biochemical properties of the ECT8 protein remain unknown. Thus, it is of our interest to evaluate the molecular role and biological function of ECT8 under conditions of water limitation, identifying the interacting proteins and mRNAs during germination in the presence of ABA, and thus understand their contribution to the stress response.



## Small pieces of a big picture: Upstream and downstream regulation of microRNA zma-miR528

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MicroRNAs (miRNAs) are essential regulators involved in nearly all aspects of plant growth and development. Throughout the miRNA lifetime stages, different factors determine its accumulation and the mode of action over its mRNAs targets. Over the last years, miR528, a monocot-specific miRNA, has been described in several plant species with multifaceted roles during development and stress response. We have investigated important aspects of zma-miR528 expression and target regulation in the Mexican maize landrace Tuxpeño (VS535). zma-miR528 accumulation was characterized during early germination, seedling establishment, and crucial stages of somatic embryogenesis. Likewise, we experimentally validated targets, previously predicted only by computational methods, and examined their level correspondence with the miRNA accumulation. Several targets showed a clear inverse correlation in total RNA, indicating that miRNA-mediated cleavage occurred. However, zma-miR528, distributed in heavy polysomal fractions, displayed negative correspondence with particularly one target, suggesting it also regulates some targets by translational repression. Also, Accumulation of both, precursor and mature miRNA corresponding to zmMIR528a gene, increased with treatments such as nitrogen luxury and exogenous auxin application during early germination and seedling establishment. Such increments resulted from Pol II-depended transcriptional events, as exposure to a-Amanitin reverted the positive effect of both treatments on *zmMIR528a* gene transcription. Finally, functional analysis of the *zmMIR528a* promoter region identified binding sites for known transcription factors involved in auxin signaling pathway and nitrate perception network supporting the observed increases of miR528 during certain developmental stages and treatments. Altogether, our findings contribute to elucidate the miR528 regulome assembly, an amazing miRNA with multiple roles and potential applications.



#### Anti-pancreactic lipase of edible and medicinal plants

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One of the options to combat obesity is finding plants that have a strong activity to reduce the digestion of lipids from the diets. The main of this study is evaluating 37 ethanolic extracts of plants, some edible, medicinal or belonging to a family that have the inhibitory activity of pancreatic lipase, to found a new anti-obesity agent. The degree of inhibition of pancreatic lipase in vitro was compared using orlistat as a control. Results revealed that 9 plants had a low percentage of inhibition of lipase activity <41%, 9 extracts with a moderate percentage of inhibition of 41-50%, 8 extracts in the high inhibition range 51-60% and 11 extracts with the highest percentage of lipase inhibition  $\geq 61\%$ . Ethanolic extract of *Hibiscus rosa-sinensis* of dry leaf, displayed the highest inhibitory activity of pancreatic lipase 71.90 % at 400 µg/mL, very similar to orlistat (70%). When performing the enzymatic kinetics of H. rosa-sinensis, it was found that it has uncompetitive inhibition, that is, the extract can inhibit both the enzyme and the substrate enzyme complex, unlike orlistat, which presents a competitive inhibition (70%).



## Understanding the tripartite interaction between a plant (*Zea mays*), a fungal pathogen (*Fusarium verticillioides*) and a beneficial bacterial endosymbiont (*Bacillus cereus*)

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We have developed a tripartite model system to study the interaction involving maize (Zm), the fungal pathogen of maize *Fusarium verticillioides* (*Fv*) and the maize rhizospheric bacterium *Bacillus cereus* (*Bc*) reported as an effective biological control agent of Fv in the field. A preliminary transcriptomic analysis to study the direct confrontation Fv vs. Bc showed that when Bc encounters Fv it uses different molecular weapons directed to control Fv growth or conidia germination. Some of these mechanisms include production of siderophores, antibiotics, biofilm and induces a set of fungal cell wall lytic enzymes. Plant chitinases are part of a surveillance mechanism which produces small oligomers of the fungal cell wall and elicit plant defense responses to avoid fungal invasion. Fv produces chitinase-modifying proteins (Fv-cmp) that target plant chitinases, cutting and inactivating them. We demonstrated that bacterial chitinases are resilient to *Fv*-cmp, and we are working to understand if bacterial chitinases may also act recovering the plant defense mechanisms lost by the action of Fv-cmp. We propose a model were Bc acts directly on Fv to control fungal growth inside the plant (they both are endosymbionts), but Bc may also restore plant defense working together with the plant to stop the fungal invasion. We are currently conducting an RNA-seq analysis. A total of 5302, 6475, and 3332 differentially expressed genes were identified in maize roots in response to  $Bc_{\rm c}$ . Fv, and Bc+Fv, respectively, which will be used for functional category enrichment and gene co-expression network analyses.



## Atomic structures of respiratory complex III<sub>2</sub>, complex IV, and supercomplex III<sub>2</sub>+IV from vascular plants

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Mitochondrial complex III (CIII<sub>2</sub>) and complex IV (CIV), which can associate into a higher-order supercomplex (SC III<sub>2</sub>+IV), play key roles in respiration. However, structures of these plant complexes remained unknown. We present atomic models of CIII<sub>2</sub>, CIV, and SC III<sub>2</sub>+IV from *Vigna radiata* determined by single-particle cryoEM. The structures reveal plant-specific differences in the MPP domain of CIII<sub>2</sub> and define the subunit composition of CIV. Conformational heterogeneity analysis of CIII<sub>2</sub> revealed long-range, coordinated movements across the complex, as well as the motion of CIII<sub>2</sub>'s iron-sulfur head domain. The CIV structure suggests that, in plants, proton translocation does not occur via the H channel. The supercomplex interface differs significantly from that in yeast and bacteria in its interacting subunits, angle of approach and limited interactions in the mitochondrial matrix. These structures challenge long-standing assumptions about the plant complexes and generate new mechanistic hypotheses.



## Obtention of betalains from *Beta vulgaris* out of an *in vitro* culture

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Betalains are natural pigments with an elevated potential of pigmentation and multiple color ranges found in different plant species, such as beetroots (*Beta vulgaris*); they are considered as an alternative for usage of the colorant Red 40, which is highly allergenic, in cosmetics and foods. Complete beetroots are needed for betalains'extraction, provoking waste in roots destined for human consumption; it is considered as an alternative in vitro culture of callus and cells of *Beta vulgaris* for the pigments' obtention. Beetroot sprouts and bulbs were cultivated from seeds in hormone free Murashige-Skoog (MS) solid culture medium, tissues were obtained for callus and cultivated in four solid MS culture mediums with growth hormones for five months. Hormones used in this project were Bencylaminopurine (BA), 2,4-Dichlorophenoxyacetic Acid (2,4-D), Picloram and Naphthaleneacetic Acid (NAA); BA was used independently (1 mg/l) and combined with the rest of the hormones (0.5 mg/l). Callus cells were disaggregated and cultivated in liquid MS culture medium with the same hormones for two months meanwhile both callus and cells were submitted to growth kinetics; betalains from cells, callus and a mature root were extracted and quantified by colorimetry. BA and the combination of BA-NAA were the best for callus and cell culture as well as presenting total pigments' concentration ranges from 0.1mg/l to 3.5 mg/l from both callus and cells. The obtention of betalains from *Beta vulgaris* out of an in vitro culture is possible depending on growth hormone combinations.



#### Phenotyping Agave spp under abiotic stress

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Using a PlantScreen System (PSI) with hyperspectral/fluorescence cameras for imaging chlorophyll fluorescence kinetics and evaluating photosynthesis parameters as Fv/Fm, NPQ or QY, the physiological and metabolic responses of *Agave tequilana* and *A. angustifolia* (two ecotypes) facing hydric or nutritional/high-lighting stress were monitored. Standard morphological/biochemical parameters such as size, leaf turgor, stomatal status, and chlorophyll, anthocyanin and proline levels, were also registered. The relative expression of a set of photosynthetic and stress-related genes, including *lhcb*, *rbcS*, *pepc*, *dhn*, *leap*, *apx*, *cat* and *sod*, was also compared by qRT-PCR. Our data show that the evaluated agave species keep the photosynthetic capacity even under extreme and prolongated drought, with full recovery after rehydration, even though some protection systems typically involved in control of oxidative stress are turned off. Agglutination of chloroplasts as an effect of drought was registered in *A. angustifolia* and chlorophagy was observed under nutritional depletion. Our data suggest that the agave drought tolerance involves constitutive expression of specific members of gene families to avoid stress effects. Subcellular reorganization seems also implied. The agave performance as homoiochlorophyllous or poikilochlorophyllous plants will be disscused.



## Tissular and Subcellular Localization of a Protein Induced by Water Deficit in *Arabidopsis Thaliana*: The case of AtLEA4-5 Protein

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Late Embryogenesis Abundant (LEA) proteins accumulate during the last stages of seed development and in vegetative tissues in plants under water deficit. Previous work uncovered the relevance of group 4 LEA proteins in plant tolerance to water deficit. In *Arabidopsis thaliana* this group is formed by AtLEA4-1, AtLEA4-2 and AtLEA4-5, and mutant plants in any of these members are more susceptible to water deficit; also, *in vitro* assays demonstrated that AtLEA4-2 y AtLEA4-5 proteins could protect the activity of reporter enzymes when they were subjected to partial dehydration or freezing/defrosting cycles. This evidence indicate that these proteins could be exerting a protective function in the plant against to water deficit, nevertheless, little is known about if their function in the plant is limited to certain tissues and cellular compartments. In this project, we address the localization of AtLEA4-5 protein, since is one of the most studied members of group 4. Using fusions to GFP, so far, we know that AtLEA4-5 protein is localized in all the tissues of imbibed seeds and apparently this localization is maintained during the first days after germination. In seedlings expose to salt, this protein localize in vascular tissues from roots. At subcellular level this protein localizes in nucleus and cytoplasm, but it remains to be determined if localization in cytoplasm corresponds to a certain(s) organelle(s). The information obtained from this analysis could indicate us where this protein carries out their function(s) in the plant and suggest us in which biological/molecular processes is involved.



## **Biological traps to recruit plant** growth promoting bacteria

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Microorganisms found at the roots can be positive, negative or neutral in the development and growth of plants. Our goal was to design and implement a system to identify communities of plant growth promoting bacteria (PGPB) in *Solanum lycopersicum* (tomato). We established a PGPB screening system for microbial communities, using tomato in a common garden experiment under hydroponic conditions. We used hydroponic tomato cultures inoculated with multiple soil sources, then plant phenotyping as a reporter for the root interacting microbiomes. Multiple plant phenotypic variables were evaluated: amount of chlorophyll, length, weight dry, weight wet, stem diameter and leaf surface. We also tested multiple sterile inoculants and nutrient controls to discriminate PGPB positive phenotypes. We selected the plants with PGPB positive phenotypes to describe the communities both by 16S rRNA gene amplicons and shotgun metagenomics to describe their genes. Our results showed an enrichment of the genera *Luteolibacter*, *Rhodobacter* and *Sphingobium* in the PGPB communities. Likewise, we found some previously reported PGPB bacteria such as the genera *Flavobacterium*, *Rhodobacter* and *Sphingobium*. On the other hand, the abundance of the genera *Acinetobacter* and *Hydrogenophaga* correlated positively with dry weight and stem length. Finally, the system developed in this work uses plant roots for selecting whole microbial communities and their interactions without culture-based constraints from small soil inoculants.



## *Azospirillum brasilense* Sp245 lipopolysaccharides induce target of rapamycin signaling and growth in *Arabidopsis thaliana*

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The Target of Rapamycin (TOR) protein kinase plays a pivotal role in metabolism and gene expression, which enables cell proliferation, growth and development. Lipopolysaccharides (LPS) are a class of complex glycolipids present in the cell surface of Gram-negative bacteria and mediate plant-bacteria interactions. In this study, we examined whether LPS from *Azospirillum brasilense* Sp245 affect *Arabidopsis thaliana* growth via a mechanism involving TOR. *A. thaliana* plants were treated with LPS and plant growth and development were analyzed in mature plants. Morphological and molecular changes as well as TOR expression and activity were analyzed in root tissues. LPS increased total fresh weight, root length and TOR::GUS expression in the root meristem. Phosphorylation of S6k protein, a downstream target of TOR, increased following LPS treatment, which correlated with increased or decreased expression of CycB1;1::GUS protein upon treatment with LPS or TOR inhibitor AZD-8055, respectively. Long term LPS treatment further increased the rosette size as well as the number of stems and siliques per plant, indicating an overall phytostimulant effect for these signaling molecules. Taken together, the results suggest that A. brasilense LPS play probiotic roles in plants influencing TOR-mediated processes.



## **Communicating Plant Science using Comics, LEGO bricks, and beyond**

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There is a huge void between our work as Plant scientists and what happens in the field or society. Thomas Kuhn emphasizes science not shared is like it does not exist. We must put effective science communication in front of making citation numbers and one of the best ways to achieve this is by recognizing fieldwork and every lifestyle by ourselves since we also must know how to communicate in every context. Science communication is an important part of whatever we want to change, in this way, I propose to incorporate friendly and pop-culture tools to illustrate theoretical and practical knowledge. If everyone loves superheroes then we should create a superhero who can be heard by everyone; if K-12 scholars like LEGO bricks, then we should use them to show how a sequencer works; if farmers are interested in movies, we should create cinematography-like videos and so. I encourage academia to focus on sharing before publishing, being always integrative and inclusive. Science is for everyone, not for journals.



## ROS generation is decreased during Host-Induced Gene Silencing of *Phytophthora capsici* GIP1 (Glucanase Inhibitor Protein 1) in *Nicotiana benthamiana*

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The high production of reactive oxygen species (ROS) is one of the initial defense mechanisms induced when oomycete pathogens enter a host plant. *Phytophthora capsici* is a late blight disease agent in many Solanaceae and Cucurbitaceae plants, able to secrete a Glucanase Inhibitory Protein (GIP1) which inhibits endoglucanase activity involved in plant defense response against infection. In this way, we performed diamino-benzidine staining to elucidate in situ hydrogen peroxide accumulation after host-induced gene silencing of *P. capsici* GIP1. We observed that transient expression of *P. capsici* dsRNA-GIP1 gene shows reduced ROS production and accumulation and posterior cell death in *Nicotiana benthamiana* in comparison to an empty vector plus infiltration with *P. capsici* zoospores. Also, we found that overexpression of PcGIP1 induces high ROS generation in the absence of *P. capsici* infection. This behavior could be explained by compromised pathogen development when  $\beta$ -1,6-Glucanases are no longer available, and the infection suddenly ceases. Our results suggest that *P. capsici* GIP1 plays an essential role during infection by interfering with ROS homeostasis.



## Differential expression of Arabidopsis Glycine Rich Domain Protein (*AtGRDP2*) gene under abiotic stress and hormone application

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Abiotic stresses are responsible for ~50% of crops losses worldwide. Genes encoding glycine-rich proteins are accumulated in response to abiotic stress. AtGRDP2 gene encodes a glycine rich domain protein that is auxin-responsive gene and it was implicated in salt stress response. Herein, we characterize AtGRDP2 transcriptional regulation under several abiotic stresses, such as cold and salinity, and exogenous hormone application such as abscisic acid (ABA) and indole acetic acid (IAA). This was performed trough transcriptional fusion of AtGRDP2 promoter and GUS gene reporter (pAtGRDP2::GUS). Results showed an increase in GUS signal in seedlings, in particular in roots under abiotic stress, specially accumulated in the root tip and the elongation zone. It is worth to mention that cold stress strongly induces AtGRDP2 promoter in comparison to salt stress. Similar results were observed in exogenous hormone application, ABA triggered the highest AtGRDP2 expression. In addition, we analyzed in the pAtGRDP2::GUS lines the effects of three auxin repressors, N-1-naphthylphthalamic acid, L-kynurenine and Phenoxyphenyl boronic acid, the application showed an accumulation of GUS signal particularly on the root tips. Additionally, six abiotic response genes were selected from the Atgrdp2 mutant and 35S::AtGRDP2 over-expression microarray under salinity by bioinformatics analysis. These six genes were upregulated in the 35S::AtGRDP2 overexpression line and downregulated in the Atgrdp2 mutant. Our results suggest that AtGRDP2 gene respond to abiotic stress and hormones, furthermore the over-expression of AtGRDP2 gene enhance the expression of several abiotic response genes.



## <sup>14</sup>C-Partitioning and biomass allocation in common bean (*Phaseolus vulgaris L.*) under different moisture levels during pod filling

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Plants modify their carbon allocation as a response to low water availability. The objective of this study was to evaluate, using a 14CO2 pulse-chase analysis, the effect of moisture restriction on biomass production in common bean plants var. OTI. The plants were maintained with irrigation until the beginning of pods filling; then, three groups were formed, kept at 100, 75 or 50 % field capacity (FC). After 10 days, the plants were 14CO2-labeled during 4 h. The plants were harvested at 1, 3 and 7 days after applying the label. Ripe fruits imported more than 50 % of the total 14C. Particularly, in pericarps at mid-pod filling stage (16-20 days after anthesis; DAA) the label presented greater changes. The fructose concentration doubled that of the glucose and decreased with the age of the fruit, that of sucrose increased in pericarps at mid-pod filling and late pod-filling stages (21-30 DAA) in relation to those at early pod filling stage (10-15 DAA). In pericarps of early pod filling, sucrose concentration was low, as well as the starch content that decreased by half in the 50 % FC condition. The latter coincided with the highest amylolytic activity as evaluated in native gels. These findings open new opportunities to research the carbon allocation mechanism under moisture restriction.



## Effect of heat stress on biomass production in bread wheat genotypes in the Yaqui Valley, Mexico

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Among the several abiotic stress factors that the agricultural system has to confront, heat stress has the mayor impact in crops yield. With the increase in annual temperatures the new average climate in places where wheat crops where located has increased as well, causing physiological damage and crop failure. This work examines the performance of four genotypes of bread wheat under heat stress. Two field experiments were carried out: a control and a heat stress during reproductive stage; characterized by daily maximum temperatures of 33°C and 38°C. Four repetitions of each genotype were sown, tillers and spikes were collected and their length and weight were measured; afterwards, the spikes were shelled and the biomass was evaluated. The results showed that heat stress reduces the time to reach the anthesis and indicate that the heading stage is affected, causing a decrease in the length and the weight of the tiller. The biomass reminded mostly unaffected under heat stress field experiments, with a weight change of 7% and 1.5%. This suggest that the wheat genotypes differ in their ability to respond to heat stress, with some of them being able to better maintain their biomass under heat stress, which could be useful as genetic stock to develop wheat tolerant varieties in breeding programs.



# **OpsDHN2** an acidic dehydrin SK3 isolated from **Opuntia** strepthacanta

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In the Cactaceae family, the genus Opuntia represents an important economic source that is distributed mainly in the arid and semi-arid regions of Mexico. Prickly pear (*Opuntia* spp.) is used as forage, fruits and green food. These succulent plants have a carbon fixation metabolism called Crassulacean Acid Metabolism as well as an efficient system of water use. In our work group, we previously reported the isolation of *OpsDHN1* dehydrin from an *Opuntia streptacantha* cDNA library. *OpsDHN1*, paralogous of OpsDHN2, belongs to a large family of disordered and highly hydrophilic proteins known as Late Embryogenesis Abundant (LEA) proteins. In this study, we isolated and characterized the *OpsDHN2* gene of *O. streptacantha* and carried out a phylogenetic analysis that revealed that OpsDHN2 is paralogous to OpsDHN1 and orthologous to Arabidopsis ERD14. Expression analysis demosntrated that *OpsDHN2* mRNA was accummulated in response to abiotic stress, such as such as extreme temperatures and salinity. Also, we analyzed the cellular localization of the *GFP::OpsDHN2* fusion in *Nicotiana benthamiana* epidermal cells and found that *OpsDHN2* localizes in nucleus and cytosol as similar results observed previously in OpsDHN1.

# Characterization and pharmacological evaluation of *Callistemon citrinus* leaf herbosomes

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Is a technology developed by pharmaceutical and nutraceutical manufacturers to complex standardized plant extracts or water-soluble phyto-constituents with phospholipids to produce lipid-compatible vesicular structures, named herbosomas or phytosomes. *Callistemon citrinus* leaf extract (CCLE) has shown its potential effect as a possible anti-obesogenic agent. The biological activity of C. citrinus has been mainly attributed to phenolic compounds and terpenoids previously reported. The standardization and pharmacological characterization of *C. citrinus* leaf herbosomes (CCLH) was evaluated. The pharmacological evaluation of CCLH was dosed at 200 mg/kg. CCLE and soybean phospholipids (1:1 v/v ratio) were taken to load the *C. citrinus* extract into the standardized phytosome. In addition, drug entrapment efficacy and solubility profile studies of *C. citrinus* herbosomes were carried out.

Standardization of CCLH: The prepared herbosomes showed vesicular structures under the light microscope with a size ranging from 50 to 100 µm with great similarity in terms of the size of the vesicular particles. Regarding solubility, it was found that the solubility of CCLH was much higher than that of CCLE. Finally, it was found that the encapsulation efficiency of the herbosomes was 80.49%, represented by the concentration of unbound CCLE (200 mg/kg). With these results, the optimization of the herbosome preparation technique was obtained in terms of conditions such as reaction time and temperature. In addition, the CCLH also developed into vesicles (herbo vesicles) that were further characterized by the shape, size, and trapping efficiency of the vesicles.

Key words: Anti-obesogenic, Phytosomes, Callistemon citrinus.



## Changes induced by lead in root system architecture of Arabidopsis seedlings are mediated by PDR2-LPR1/2 phosphate dependent way

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In soil, in addition to water and nutrients, there are toxic elements such as heavy metals (HMs). Lead (Pb) is one of the most hazardous pollutants. We found that the Arabidopsis root response to Pb presents a stimulatory effect at 400  $\mu$ M, whereas 600  $\mu$ M or higher concentrations are inhibitory. We found that the repression of primary root growth modifies root system architecture (RSA) similarly to phosphate (Pi) starvation stress, increasing lateral root density (LRD). Thus, it was hypothesized that both the metal and nutritional stress might be linked. Expression of *AtPT2* a low Pi response inducible gene, is down-regulated under high Pb supplements, indicating that this metal regulates physiological responses in root system. Mutants unable to response to Pi starvation, which show root architectures comparable to plants growing in non-stressed physiological conditions (*stop1*, *lpr1/2* and *lpi3*) were grown under + Pb and Pi starvation. We found two different responses on +Pb supplemented media: i) *stop1* showed a PRL short and ii) *lpi3* and *lpr1/2* were insensitive to Pb, as if they were growing on medium without Pb. Our results show that activity of *AtPT2* is down-regulated by Pb and that changes in RSA by Pb are genetically regulated by the pathways that mediate the Pi starvation response including PDR2-LPR1/2 and LPI3 genetic elements.

## Analysis of *Amaranthus hypochondriacus* seed development DNA methylation patterns by WGBS

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Amaranth is a highly important crop, provides cereals, leafy vegetables and a better diet than the predominant staple crops making it a viable micronutrient supplier. Molecular information regarding amaranth embryogenesis is very limited, genomic data suggest that one of the molecular mechanisms that modulate this process is DNA methylation (DNAm). In plants, DNAm occurs in CG, CHG and CHH contexts. Furthermore, recent studies reveal an emerging relationship between phytohormones (plant hormone signaling) and epigenetic regulation. To determine the DNAm dynamics on A. hypochondriacus seed development, whole genome bisulfite sequencing (WGBS) was performed. A total of 18 plants were grown in greenhouse conditions. Germinal tissue (ovule), early and late embryo (after pollinated ovule) were recollected and processed to extract DNA and generate WGBS reads. Global DNAm levels were obtained, ovule: 28.6%, early embryo: 29.1% and late embryo: 29.4% of which the methylation levels per context for ovule were: 79.3%, 48.7% and 16.05%; early embryo: 79.55%, 49.15% and 16.7%; late embryo: 78.45%, 50% and 17.1% in the contexts CG, CHG and CHH, respectively. Results showed that in all three stages a large number of cytosines in CG and CHG contexts were methylated, in contrast to CHH context where a smaller fraction of cytosines were methylated. Further statistical analysis confirmed that DNAm levels per tissue of each context were independent to each other (value was not significant p>0.05). The differences in the ovule and seed methylation profiles during amaranth embryogenesis could give indication of the molecular dynamics involved in this process.


## Population genomics of *Quercus macdougallii* (Fagaceae), an endemic oak of the Sierra Juárez, Oaxaca, Mexico

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Mexico is considered the country with the largest number of species of the *Quercus* genus worldwide. It houses many endemic species, such as *Quercus macdougallii*, which is distributed in the Sierra Juárez de Oaxaca and is classified as a vulnerable (IUCN) and a threatened species (NOM-059). In this study, we analyzed the genomic variation of the species considering eight sampling sites located across the north and south of its known distribution (approx. 28 km). We identified 8,186 single nucleotide polymorphisms (SNPs) and found moderate genetic diversity (Ho and He  $\sim 0.2$ ) as well as low genetic differentiation and inbreeding levels (FST = 0.0204, FIS = 0.0101). By using isolation by distance analysis, a genetic variation gradient between the North and South zones was identified. Although the species remains as a single population, a genetic group with incipient structuring was identified in the southern zone. The analysis of SNPs outliers did not show evidence of locally adapted genotypes however the presence of private alleles associated with particular areas was observed. These results allow us to complement ongoing conservation and monitoring strategies of the species, particularly in the southern zone, with the distribution of individuals with a high genetic variation and where we estimated that in the coming decades, climate change will negatively affect this species.



## **Plastid chromatin**

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Although the organization of the nuclear chromatin and its role in transcriptional regulation are well defined mechanisms, little is known about the structural organization of the plastid nucleoid and its consequences in the plastid gene regulation. In this work, using several high throughput-sequencing techniques, we found that the plastid nucleoid as a complex and specific organization, which is primarily caused by protein binding, transcription, and the association with membranes. Moreover, these features are variable and dynamically modulated among different regions in the plastid genome. Given those evidences, we propose that the plastid nucleoid structure is complex, stable and functionally relevant.



# *Phaseolus vulgaris* tetraspanin 8 plays a key role during mutualistic interactions

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The root-nodule and arbuscular mycorrhiza symbiosis are the most important mutualistic interactions in plants for nitrogen and phosphorus uptake under conditions of limited nutrient availability. These symbioses required many responses and mechanisms for their development, such as the release of signal molecules, calcium oscillations, reactive oxygen species (ROS) production, cell reorganization and vesicular traffickingmediated symbiont accommodation. In this work we have addressed the exploration of a new family of protein called "tetraspanin" which have emerged as a new membrane component able to organize as tetraspanin enriched microdomain or web in the plasma membrane. These proteins have been broadly studied in human cells, nematodes, fungi and flies, however, in plants is very limited. Tetraspanins are integral membrane proteins with two extracellular loops containing several highly conserved cysteine residues. These loops allow their association to microdomain forming clusters in different levels of complexity. Tetraspanins also contributes to exosome formation, which are extracellular vesicles derived from the multivesicular body that carry DNA, mRNA, microRNA, proteins and lipids. Thus, the key role for exosomes is the intercellular and interkingdom communication. We analyzed the transcriptional activity of the PvTET8 promoter during interaction with arbuscular mycorrhizae and determined the phenotypes associated with *PvTET8* silencing or overexpression during nodulation or under mycorrhizal association. Our results demonstrate that PvTET8 transcription is modulated during mycorrhizal and rhizobial association. Furthermore, PvTET8 silencing and overexpression affected nodule size and numbers, as well as nitrogen fixation and arbuscule formation during mycorrhizal association. Therefore, we propose that tetraspanin are new players regulating the mutualistic interaction in addition to its role in pathogenic responses.



# Functional characterization of a LORELEI-like gene during the symbiotic interaction

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Reactive oxygen species (ROS) in plant cells play an important role in several physiological processes, for example: in plant development, hormonal signaling, polar growth, biotic and abiotic interactions etc. It is well known that ROS, has a dual role during stress and development processes, which means that the concentration, dynamics, and subcellular distribution could have antagonistic responses depending on the biotic stimuli perceived in a similar way to what has been described with intracellular calcium. In plant cells, the biogenesis of ROS has been widely linked to NADPH oxidase activity acting downstream the signaling cascade. For instance, FERONIA (a receptor-like kinase) has emerged as an important regulator of the NADPH oxidase, which regulates the localized production of ROS at the apical pollen tube and root hairs. Both FER and NADPH oxidases are localized in the apex of the root hair, where lipid rafts domains have been described. These membrane microdomains are sterols enriched regions and contain some proteins modified with a glycosyl-phosphatidyl-inositol (GPI). In Arabidopsis thaliana, a mutation in llg1-2 a LORELEI-like protein (LGPI) which has a GPI motif result in a phenotype very similar to fer4 and affects the ability of FER to localize in the plasma membrane and affect the ROS generation, therefore, FER is sequestered in the endoplasmic reticulum. Since FER requires LGPI protein for correct targeting to the plasma membrane, we have analyzed the role of a LGPI in *Phaseolus vulgaris* during mutualistic interaction. In beans there are 2 LGPI proteins, one of them is expressed exclusively in floral tissue and the other in vegetative tissues. We selected this LGPI because the promotor is active in infected root hairs, and mature root nodules, in a similar way to the transcript accumulation. This suggested an important role in the nodulation process. In order to analyze the phenotypic defect, we generated silenced and over-expressed hairy roots in composite plants. Our results showed an important defect both in the infection process and in the development of the root nodules. Since LGPI proteins have only been described in the pathogen interaction, it results interesting that this function can be expanded to rhizobia-host plant interaction during mutualistic interactions.



## Submergence stress affects the expression of the Evening Complex genes and produces altered transcriptomic outputs of the diurnal cycle

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Submergence causes multimillion crop losses worldwide. The transcriptomes of model and crop plants have been studied in different submergence conditions. However, there is not a transcriptome that characterizes it in a diurnal cycle. Many responses to submergence are diurnally regulated, such as starch degradation, growth, or hormone synthesis. Here, we studied the diurnal transcriptome under submergence and control conditions of two ecotypes with contrasting flooding tolerance of the monocot model Brachypodium distachyon. We applied submergence stress to 15-day-old plants under a diurnal cycle (16h light/8h dark) and collected samples at ZT0 (dawn), ZT8 (midday), ZT16 (dusk), ZT20 (midnight), and again at ZT0 (dawn). All collects were triplicates and RNA from leaves was sequenced to obtain RPKM, log2, gene clusters and GO. These analyses indicated that the circadian clock genes showed an altered expression by the stress, both by intensity and phase change. Notably, the PRR gene family of the Evening Complex was up regulated and phase-shifted by 8h. The Evening Complex genes are master checkpoints that control a backwards inhibition cascade that produces the characteristic expression oscillation of the circadian clock. Although this disruption was detected in both the tolerant and sensitive ecotypes, the transcriptomic output was different. The tolerant ecotype diurnally up regulated genes of polyamine synthesis, phenylalanine metabolism, and gluconeogenesis. On the other hand, the sensitive ecotype diurnally up regulated genes of ABA biosynthesis, JA perception, and cellulose synthesis. These results can be used to increase tolerance of plants to submergence based on the diurnal gene oscillation.



#### Sulforaphane extraction from Brassica Oleracea Var. Italica

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Brassica Oleracea Var. Italic from Europe possesses a bioactive compound called sulforaphane, which acts against gastritis, gastric ulcer and stomach cancer. This compound reduces tumor size by acting as an activator of the transcription factor Nrf2 which provides a preventive effect against stomach cancer. Sulforaphane has no adverse effects; however, an extensive study is needed to determine the maximum doses to be administered. This research postulates Sulforaphane as a possible candidate in the development of new antimicrobial agents Helicobacter Pylori infection and its use in other lines or research. An extraction will be carried out using dehydration and Soxhlet extraction methods to obtain Sulforaphane from Brassica Oleracea, followed by pre-clinical tests. As this is an extraction proposal, three alternatives will be considered: Bioactive Principle, Extract, Vegetable Mode.

Also consider the data obtained from the survey carried out, which shows the most important statistical data, with the aim of finding out the average percentage of people in the population aged between 16 and 54 years (applied in the State of Mexico, Puebla and Mexico City) who suffer from gastritis, and how much they know about the benefits of Brassica Oleracea. The inflorescence is the main source of Sulforaphane in broccoli, as well as the stem, which presents significant levels of this bioactive compound.



## WUCHEL-RELATED HOMEOBOX 9/STIMPY (WOX9/STIP) is important for ovule development and female germline progression in Arabidopsis thaliana

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*Wuchel-related homeobox (WOX)* genes encode for a family of transcription factors, sharing important roles in a wide range of processes during plant development. In *Arabidopsis thaliana WOX9/STIP* gene is reported to be necessary for the correct patterning of the embryo and for the shoot apical meristem maintenance. We have investigated the role of *WOX9/STIP* in ovule development by the analysis of *loss-of-function* and *gain-of-function* mutant alleles. Our results showed that *WOX9/STIP* is required for the correct patterning of the outer integument and the anatropy of the ovule. In fact, knockout mutant of *WOX9/STIP* is characterized by severe defects in outer integument development, hence determining a radialized ovule phenotype. In addition, alteration of *WOX9/STIP* expression in the ovule affects the correct differentiation and progression of the female germline.

Our results unravel an important role of *WOX9/STIP* during ovule development and female germline progression, contributing to better dissect and reconstruct the regulatory networks determining ovule development.

# Antiobesogenic effect of *Callistemon citrinus* in rats fed a high-calorie diet rich in fat and sucrose

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The excessive consumption of diets with a high caloric intake and the decrease in physical activity, among other factors, together rapidly develop overweight and obesity in the human population worldwide. During these conditions the excessive accumulation of adipose tissue is favored, and increase in the generation of reactive oxygen species and decrease of the antioxidant system, producing oxidative stress Obesity is associated with numerous diseases hyperlipidemia, diabetes mellitus, hypertension and cardiovascular diseases. *Callistemon citrinus* is a plant that produces compounds with a high pharmacological potential and antioxidant activity. In the present study, the effect antiobesogenic of *C. citrinus* in rats fed with a high fatsucrose (HFSD) was determined. Fifteen female Wistar albino rats were used which were randomly divided into three experimental groups (n=5). Group I (normal diet), Group II, HFSD and Group III HFSD plus daily oral *CC* extract at doses of (250 mg/kg) for 15 weeks. The morphometric and biochemical parameters for the HFSD-only group were significantly increased compared to the control group (\*) (P $\leq$ 0.05). While Group III showed significant suppression of weight gain. The morphometric parameters were strongly correlated with the body weight. *C.citrinus* extract decreased the parameters compared to the HFSD group, acting similarly to the control group. These results suggest beneficial antiobesogenic activity of *C. citrinus* leaf extract.



## Regulation of gene expression under extreme temperatures in *Arabidopsis thaliana:* The eukaryotic initiation factors eIF4E family-mediated responses

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Under global warming, extreme temperatures become a very important issue for agricultural productivity and food safety. Plants elicit highly regulated responses, at several levels, to deal with stressful stimulus and survive. One important step of this process is the control of gene expression to activate molecular weapons leading to optimum acclimation. In our laboratory we are interested in understanding plant translation regulation under high and low temperatures exerted by eukaryotic initiation factors 4E family members. We found that the absence of either canonical isoform, eIF4E or eIFiso4E, resulted in susceptibility phenotype of 5 weeks-old *Arabidopsis thaliana* plants under freezing stress and altered selective stress-related mRNAs translation. On the other hand, the absence of eIF4E or eIFiso4E did not affected early seedling development under heat stress. Interestingly, the non-canonical isoform 4EHP displayed a novel, apparently dual role under extreme temperature. The *4ehp* null mutant was significantly affected in cold acclimation and freezing tolerance, in combination with altered transcriptional induction of cold-responsive genes, nevertheless exhibited improved response to heat stress. 4EHP orthologues have been reported as non-canonical since they are not directly involved in translation, but rather participate in other aspects of RNA metabolism. Our current aim is to understand how regulatory networks mediated by non-canonical 4EHP isoform work under stress.



## Characterization of a *Phaseolus vulgaris THESEUS1* gene that encodes for a receptor of the CrRLK family during mutualistic interactions

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Several plants promote the associations with different beneficial microorganisms to capture nutrients in exchange for carbon sources generated by the plant. These implies an early reorganization of the root hair cells. These cells respond to nodulation factors by inducing changes such as: ionic, alkalinization of the cytoplasm, fluctuations in intracellular Ca2+, rearrangement of the cytoskeleton, gene expression, etc. During mycorrhization, the activation of genes involved in signaling, transcriptional regulation, synthesis of cell wall components and lipid metabolism has also been described. The plant uses multiple receptors to sense the cell wall integrity (CWI), this includes Receptor Like Kinases such as CrRLK. The CrRLK is a subfamily of RLKs characterized by presenting two malectin domains at their extracellular end, involved in binding to carbohydrates present in the cell wall or small peptides called RALFs (Rapid Alkanization Factors); and an intracellular kinase domain to transduce the changes in cell wall, as well as to regulate the production of reactive oxygen species. THESEUS1 (THE1) is a transmembrane receptor member of the CrRLKs subfamily, reported as a sensor of CWI. In order to better understand the role of the THESEUS1 receptor during symbiotic relationships, we focus on Phaseolus vulgaris as model plant. We determined the promoter activity of the THESEUS1 in the early stages of nodulation using a transcriptional fusion with GFP-GUS (*pPvTHE::GFP-GUS*) and studying the activity of  $\beta$ -glucuronidase at different times of the process of nodulation. THESEUS1 promoter activity was observed in the root hairs, as well as in coiled root hairs in plants inoculated with Rhizobium tropici. This activity was also observed in nodule primordia, mainly in the cells of the cortex and the epidermis, implying that this receptor acts from the earliest stages of the interaction.



# *In vitro* assessment of dsRNAs as biofungicides against pathogens of pineapple, orange, and cacao

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Mexico is the world's leading exporter of tropical fruits. However, tropical crops are affected by a diversity of fungal pathogens that impact at different stages of the productive chain. Based on the evidence of cross-kingdom RNA interference between plants and fungi, new strategies for the control of fungal pathogens have been developed that involve the use of dsRNAs as biofungicides. In this work, we aimed to design dsRNA-based biofungicides for the protection of tropical crops such as pineapple, orange, and cacao. For this, pathogenic fungi were isolated directly from pineapple and cacao plantations in the area of Loma Bonita and Valle Nacional, Oaxaca, respectively, and oranges in the postharvest stage in local markets. Microscopic and molecular analysis via sequencing of the ITS region identified the pathogens as *Phytophthora nicotianae* and *Fusarium oxysporum* (pineapple), *Phytophthora tropicalis* and *Lasiodiplodia theobromae* (cacao), and *Penicillium digitatum* (orange). The sequences of Dicer-like (DCL) genes in these pathogens were searched in the databases, and regions of 250-500 pb were selected for the synthesis of dsRNAs. *In vitro* assays showed that a combination of DCL1-DCL2- derived short dsRNAs inhibited spore germination by up to 80% in *Penicillium digitatum* compared to controls. *In vitro* assays with pathogens from pineapple and cacao are in course. These results will be useful for further exploration of spray-induced gene silencing (SIGS) as a biotechnological strategy for protection of tropical crops.



## Amino acid signaling in the regulation of *Arabidopsis thaliana* root growth

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Amino acids have been critical molecules in the origin and evolution of organisms, acting as structural units of proteins, precursors of secondary metabolites, and signal molecules to mediate cell communication and regulate the expression of genes. In plants, these organic molecules also serve as nitrogen sources, as precursors of phytohormones, and as signals that activate ion channels to induce defense systems and regulate stomatal movement. In this work, we carried out a detailed analysis of *Arabidopsis thaliana* root growth responses to the 20 L-amino acids. 15 of the evaluated amino acids decreased the primary root growth, of which L-glutamate (L-Glu), L-Leucine (L-Leu), L-Lysine (L-Lys), and L-tryptophan (L-Trp) were the most bioactive. The localized application of the four amino acids on the root system indicated that the root apex is responsible for perceiving them. These four biomolecules affected cell division and growth processes altering the expression of auxin response genes and the level of PIN transporters. Furthermore, an *Arabidopsis* mutant defective in the gene encoding the MAP kinase MPK6 was resistant to the effect of L-Glu, L-Leu, L-Lys, and L-Trp. With the results obtained, we conclude that despite their physical-chemical properties and influence on the auxin pathway, the four amino acids converge in MPK6 to alter the primary root growth.



## **MEDIATOR16** orchestrates local and systemic responses to phosphate scarcity in Arabidopsis roots

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Phosphate (Pi) is a critical macronutrient for the biochemical and molecular functions of cells. Under Pi limitation, plants manifest adaptative strategies to increase Pi scavenging. However, how low Pi sensing links to the transcriptional machinery remains unknown. The role of the MEDIATOR (MED) transcriptional co-activator, through its MED16 subunit in Arabidopsis root system architecture remodeling in response to Pi limitation was assessed. Its critical function acting over SENSITIVE TO PROTON RHIZOTOXICITY1 (STOP1)-ALUMINUM ACTIVATED MALATE TRANSPORT1 (ALMT1) signaling module was tested through a combination of genetic, biochemical, and genome-wide transcriptomic approaches. Root system configuration in response to phosphate scarcity involved MED16 functioning, which modulates the expression of a large set of low-Pi-induced genes that respond to local and systemic signals in the Arabidopsis root tip, including those directly activated by STOP1. BiFC analysis suggests that MED16 is required for the transcriptional activation of STOP1 targets including the membrane permease ALMT1, to increase malate exudation in response to low Pi. Our results unveil the function of a critical transcriptional component MED16, in the root adaptive responses to a scarce plant macronutrient, which helps understanding how plant cells orchestrate root morphogenesis to gene expression with the STOP1-ALMT1 module.



## Contribution of microRNAs miR482, miR2118 and miR1510 to the regulation of early stages of nodulation between *Phaseolus vulgaris and Rhizobium tropici*

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Legumes establish a symbiotic relationship with specific soil bacteria called rhizobia, resulting in the formation of specialized root organelles called nodules, where the rhizobia synthetize nitrogen compounds useable by the plant. The success of this interaction depends on the recognition of the specific partner and the exclusion of possible pathogenic bacteria. Communication between the symbionts is necessary for the molecular and physiological changes that allow the initial stages of nodulation. Recent studies have demonstrated that recognition of molecular patterns that belong to rhizobia and other bacteria present in the rhizosphere, is in many cases necessary for successful symbiotic nitrogen fixation. As part of its immune system, plants have a diverse family of proteins that function as cytoplasmic receptors to recognize strainspecific effectors. These proteins contain two domains: a nucleotide binding site and leucine rich repeats (NBS-LRRs). Their regulation by miRNAs, a group of small RNAs involved in post-transcriptional gene regulation, has gained relevance. Three families of miRNAs have emerged as important regulators of NBS-LRRs: miR482, miR2118 and miR1510. Nevertheless, their participation during the establishment of symbiosis has not been studied. Thus, we are characterizing the accumulation of these miRNAs and confirming selected mRNA targets during the early stages of nodulation between Rhizobium tropici and Phaseolus vulgaris. We found that the accumulation of the three miRNAs tend to decrease over the first hours of interaction. In addition, using the hairy roots methodology to reduce the levels of miR482 and miR2118, we observed a compensation between them, however the reason is not entirely clear. This work was supported by a grant from DGAPA-PAPIIT (IN-202918) to JLR.



## Investigating the effects of retrotransposon activity during female gametophyte development in Arabidopsis.

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Retrotransposons are the most prevalent class of transposable elements (TEs) in the genome of *Arabidopsis thaliana* (Arabidopsis). Although their retrotransposition can be a source of genome rearrangements and modifications of gene expression, the consequence of their activity in the haploid female gametophyte remains unknown. EVADE (EVD) is an endogenous single copy retrotransposon of the ATCOPIA family capable of escaping transcriptional gene silencing (TGS) in Arabidopsis. To better understand the consequences of retrotransposon activity in the female gametophyte, we are studying the endogenous activity of EVD in the developing ovule, defining its *in situ* pattern of expression, and directly activating its transcription through CRISPR-SUNTAG under both constitutive and specific promoters. RT-PCR and whole-mount *in situ* hybridization showed that EVD is transcriptionally repressed in most cases, in agreement with restricted localization of endogenous antisense transcripts. Transgenic *Col*-0 plants expressing CRISPR-SUNTAG under the pUBIQUITIN10 promoter express the reporter GFP gene in nuclei at premeiotic and postmeiotic stages of female gametogenesis. We are currently investigating the eventual phenotypic consequences of EVD activity in the female haploid phase of the life cycle. Our current results suggest that either TGS or PTGS in the female gametophyte could prevent retrotransposition prior to double fertilization and seed development.



## Suppressor of RNA silencing encoded by Pepper huasteco yellow vein virus

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RNA silencing is an important gene regulation mechanism conserved in eukaryotic organisms; in plants, it plays a key role in antiviral defense. The silencing process can act at posttranscriptional gene silencing level (PTGS) that directs viral and cellular mRNA degradation, and a transcriptional gene silencing level (TGS) by RNA-directed DNA methylation (RdDM) and viral chromatin modifications, to restrict viral replication, transcription and proliferation. As a counterdefense, many plant viruses encode suppressors of RNA silencing.

Pepper huasteco yellow vein virus (PHYVV) is a bipartite begomovirus that infects solanaceous crops such as pepper, tomato and tobacco in México. To test if PHYVV encoded genes are TGS suppressors, we inoculated silenced N. benthamiana 16c-TGS with either PHYVV or recombinant PVX vectors expressing PHYVV encoded genes. PTGS assays were performed in N. benthamiana 16c plants (expressing constitutively GFP) by agroinfiltration-mediated transient expression, using co-infiltration of 35S-GFP construct with either pBINX expressing PHYVV encoded proteins under control of 35S promoter, or pBINX empty vector as control. GFP fluorescence was monitored (in inoculated leaves or in new emerged systemic leaves for PTGS and TGS assays, respectively) under the UV light.

We have found that PHYVV infection induces TGS suppression in 16c-TGS plants allowing the expression of GFP and the expression of REn (PVX::REn) is also able to suppress TGS. On the other hand, the expression of Rep, TrAP, CP, MP and NSP do not suppress TGS. Furthermore, the expression of Rep (35S:Rep) suppressed PTGS.



## Viruses infecting trees and herbs that produce edible fleshy fruits with a prominent value in the global market: an evolutionary perspective

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Trees and herbs that produce fruits represent the most valuable agricultural food commodities in the world. However, the yield of these crops is not fully achieved due to biotic factors such as bacteria, fungi and viruses. Viruses are capable of causing alterations in plant growth and development, thereby impacting significantly the yield of their hosts. In this work, we first compiled the world's most comprehensive list of known edible fruits that fits our definition. Then, plant viruses infecting those trees and herbs that produce fruits with a commercial importance in the global market were identified. The identified plant viruses belong to 30 families and according to the nature of their genomes, most of them contain single-stranded RNA genomes. On the other hand, we show the overall picture of host range for some virus families following an evolutionary approach. Further, the current knowledge about plant-virus interactions, focusing on the main disorders they cause, as well as yield losses, is summarized. Also, since accurate diagnosis methods are of pivotal importance for viral diseases control, the current and emerging technologies for the detection of these plant pathogens are described. Finally, the most promising strategies employed to control viral diseases in the field are presented, focusing on solutions that are long lasting.



## Selection and metagenomic examination of a rhizospheric community capable of promoting plant growth on mine tailing substrate

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We have previously described a plant community able to colonize mine tailings that are deposits of mine residues and are a potential source of contamination when exposed to erosion. These plants could be used to buffer erosion in a bioremediation strategy known as phytostabilization. Since both substrate characteristics and local adaptation failed to explain the establishment of this community, we predicted that rhizospheric communities should positively contribute to the plant growth in the mine tailings. We screened the culturable and metagenomic diversity of mine tailing rhizospheric bacteria using 16S rRNA gene amplicons and metagenomic shotgun sequencing. Then we built a synthetic community (SC) of culturable bacteria from the mine tailings. The SC was subject to a mesocosm experiment with plant-derived nutrient sources, testing for community dynamics and tolerance to heavy metals. The result of this procedure was named the 'final synthetic community' (FSC). We found genes involved in heavy metal homeostasis, iron scavenging, and stress responses in the predicted protein profiles of both environmental and cultivated communities. The FSC was dominated by a single lineage that was represented by a metagenome-assembled genome that contained genes related to plant symbiosis. We performed a greenhouse experiment in which the FSC was inoculated to mine tailing colonizing plants. The FSC inoculated plants showed higher relative plant growth rates in sterile substrates. These results indicate that the FSC could be used to enhance in situ phytostabilization.



## Increased trehalose synthesis in wheat plants benefit photosynthesis and growth under drought stress

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Trehalose is a widely studied molecule because its role on abiotic stress tolerance in many organisms, such as yeast, bacteria, and resurrection plants. The most common pathway for trehalose synthesis found in nature is the trehalose-phosphate synthase (TPS)-trehalose phosphate phosphates (TPP) pathway. Plenty studies have shown that the overexpression of TPS and/or TPP genes from microorganisms for increasing trehalose synthesis in different crop species resulted in an improved tolerance to abiotic stress. Here we studied how drought stress affect photosynthesis and development in transgenic wheat plants overexpressing a bifunctional TPS-TPP enzyme from yeast. Transgenic and non-transformed (NT) wheat was subjected to drought stress (DS) by maintaining soil humidity at 30% for five days, and their recovery was tested after 5 days of re-watering (RW). Relative water content (RWC) in the leaf of both NT and transgenic plants was reduced by drought and it recovered after RW. Gas interchange and chlorophyll fluorescence parameters were analyzed using a LI-6400/XT equipment. Stomatal conductance was reduced in all wheat lines during DS. Assimilation of CO2 and performance of PSII was more efficient in transgenic lines under DS than in NT wheat. Photosynthetic parameters recovered after RW in both transgenic and NT plants. Aerial biomass was reduced by DS in a greater extent in NT plants than in transgenic wheat lines, 5d after RW as well as in their final developmental stage. These results indicate that trehalose synthesis supports a better photosynthetic performance and protects plant growth during and after DS.

#### **Comparative transcriptomics in flower development** of Disocactus species (Hylocereeae, Cactaceae)

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Cacti have stunning and beautiful flowers which made this family popular. Cacti flowers are diverse in size, organ number, color and forms between the genera and species in the family. Some structures in cacti flowers such as tepal and the pericarpel are subject of debate because their homology is not clear, that is, we do not know the genetic signatures of these organs. Here we present the results of sequencing, *denovo* assembly, and analysis of flower development transcriptome for two *Disocactus* species, namely, *D. speciosus* and *D. eichlamii*. These species were chosen as they develop flowers with differences in size and color, which also have contrastants pericarpel. We generated two well-assembled transcriptomes, with ~90 % completeness and ~60,000 proteins annotated. Around 15,600 transcripts have ortholog sequences to *S. undatus*, which is closely related to *Disocactus*, and it is currently the best reference genome assembled so far for Cactaceae. Our transcriptome allowed the identification of putative orthologous genes to the ABC model of flower development; genes that might be involved in flower color, such as those in the betalain and carotenoid synthesis pathways; and genes related to cell proliferation and cell wall expansion. They were also identified leaf genes which could be related with the pericarple development.



## Characterization of the role of two MBF1 transcription factors during fruit ripening in *Solanum lycopersicum*

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Until now five members of the Multiprotein bridging factor 1 (MBF1) family have been identified in tomato: MBF1a, MBF1b, MBF1c, MBF1d and MBF1/ER24. The transcription cofactors MBF1 are directly related to the stress response in plants. MBF1a is ubiquitously expressed in the tomato plant but its function is not characterized. MBF1/ER24 is expressed during ripening, expressing mostly in Breaker + 10 fruits. To gain information about the MBF1a and MBF1/ER24 protein in tomato specific antibodies against these proteins will be produced and affinity purified. Immunolocalization will be carried out in tissues at different fruit developmental stages. Additionally, Co-immunoprecipitation (IP) experiments and BiFC assays will be carried out in order to explore the interaction pathways in which these proteins function, and to know whether they interact to each other. This information will help to understand the role of these transcription cofactors in tomato fruit.



# *Arabidopsis* transcriptional response due to exposure to cold and freezing stress

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Low temperature can induce stress in plants, leading affectations in which the severity depends on whether cold stress or freezing, and exposure time to this condition. In order to have an overview of the impact of low temperature stress on plants, we performed a transcriptomic analysis. For this, 14-day-old *Arabidopsis thaliana* plants were exposed to 0°C, 4°C and 10°C for 24 h, and changes in gene expression were analyzed by RNAseq analysis. It was found that, within the genes with greater induction, certain shared processes were favored in the three temperatures analyzed. Some of these biological processes are cold acclimation, RNA secondary structure unwinding, rRNA processing, *de novo* protein folding, ribosome assembly, among others. To deepen the study of the transcriptomic profile, the first 50 genes induced at 0°C, 4°C and 10°C were selected. We found that 16 genes are shared at the three temperatures, 0°C and 4°C share 11 genes and the conditions with the highest affinity are 4°C and 10°C with 13 genes in common. While between 0°C and 10°C, only 6 are shared. Whitin the 16 shared genes in the three conditions, we have the following: AT1G08410, which codes for a GTPase involved in ribosome biogenesis, another is the *LON1* gene, which is related to the selective degradation of damaged polypeptides, and two genes related with saline stress, *Sec23/Sec24* and P5CS2, encoding a transporter located in COPII vesicle coat and delta 1-pyrroline-5-carboxylate synthetase, respectively.



## *In silico* analysis and gene expression of transcription factors related to cuticle biosynthesis during the ripening of soursop fruits (*Annona muricata*)

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The soursop (Annona muricata) is a climacteric fruit with a great appeal for consumption due its pleasant flavor and aroma in addition to its high nutritional and pharmacological value. Mexico is one of the main global soursop producers, producing in 2019 more than 30 thousand tons with a value of 12 million USD. Nevertheless, this fruit presents a short postharvest life due to its high respiration rate and ethylene production. It has been reported that the structure and composition of fruit cuticle plays an important role in the quality and postharvest life of fruits and the cuticle biosynthesis is regulated at various levels, including transcriptional level, where has been proposed a putative gene regulatory network to modulate the cuticle components biosynthesis. However, this network is not completely elucidated and is even less studied in non-model fruits like the soursop. Due to this, the goal of this work is to identify and characterize transcription factors (TF) related to cuticle biosynthesis in soursop fruits. For this, the soursop transcriptome was compared with iTAK and PlanTFDB databases through a homology search using BLAST. This allowed us to identify sequences encoding TF of various families including WRKY, MYB, HD-Zip, among others. Additionally, we performed an analysis using InterPro to identify functional domains that are characteristic of TF to know whether the soursop transcriptome includes transcripts that encode for TF not yet reported on databases. The identification of the TF will allow us to reconstruct the gene regulatory network of cuticle biosynthesis in soursop fruits.





# Chemical and physiological perturbation as a dissecting tool of Arabidopsis circadian clock

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Plant circadian clock is tightly intertwined with physiological and metabolic processes. With the aim to study metabolic and circadian clock interactions, we used a chemical approach to perturb metabolism through a series of compounds including chemicals that alter redox cellular status, metal ions and hormones while monitoring circadian oscillations. Molecules that alter chloroplast related functions affected circadian rhythms. Some chemicals as vitamin C and paraquat altered circadian period in a light dependent manner, whereas antibiotics as rifampicin only had an effect under darkness. We found that sucrose, which is a recurrent constituent of MS media in experiments with seedlings, lengthened or prolonged circadian period with a dependence of the circadian clock mutant background. Salicylic (SA) acid improved the robustness of the circadian clock, however its effect on period was dependent of both light quality and genotype. Furthermore, the effect of SA was dependent on the presence of sucrose in the media. Our results suggest that SA may act as a zeithnemer of the circadian clock through phytochrome B and that sucrose could function as a derived oxidative stress signal, thus acting as a signal hub between metabolism and the circadian clock.



## Bioinformatics analysis of the xyloglucan endotransglucosylase/hydrolase (XTH) gene family in *Solanum lycopersicum* and expression profile during the arbuscular mycorrhizal symbiosis

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Xyloglucan endotransglucosylase/hydrolase (XTHs) are a family of xyloglucan modifying enzymes that are mainly responsible for the cleavage and rearrangement of xyloglucan backbones, which results in changes of cell wall extensibility during plant growth. Solanum lycopersicum is one of the most important crops around the world due to its increasing commercial production, as well as its use as a model for genetic and physiological studies. Although the XTH gene family has been characterized in several plants species, this is not the case for S. lycopersicum. In the present study, XHT gene family was analyzed using bioinformatics approaches. Furthermore, we analyzed the expression profiles of XTH genes in leaves of S. lycopersicum during the arbuscular mycorrhizal symbiosis by qRT-PCR. We identified 37 SIXTH genes through genome wide screening of the S. lycopersicum genome based on the PF00722 and PF06955 domains. Phylogenetic analysis showed two distinct subfamilies: Group I/II and Group III. This was supported by gene structure and conserved motif analysis, as well as by the identification of the conserved catalytic domain in XTH proteins. Evolutionary relationship of XTH from S. lycopersicum, Nicotiana tabacum, Solanun tuberosum, Petunia axi and Arabidopsis thaliana were also evaluated. Expression profiles based on S. lycopersicum genome database showed that *SIXTH* members have differential expression p atterns in several t issues. Finally, the r elative expression by q R T-PCR revealed that *SIXTH* genes display different expression patterns in *S*. lycopersicum leaves colonized by Rhizophagus irregularis in comparison to non-colonized leaves. All these results provide a comprehensive and systematic understanding of XTH gene family for further studies in S. lycopersicum during different growth cycles, stress, and interaction with microorganisms. This work was funded by CONACYT (CB A1 S 31400) and SIP- IPN (20211500).



## *Medicago truncatula* miR2199 coordinates environmental responses

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Plant microRNAs participate in multiple processes including development, stress responses and their own biogenesis. Their activity is exerted through cleavage of target mRNA or by translation inhibition. Several plant microRNAs regulate transcripts encoding transcription factors, thus amplifying the miRNA effects through regulation of downstream genes.

We previously identified miR2199 in *Medicago truncatula* and other Fabaceae. In *M. truncatula* four bHLH mRNAs (encoding for basic helix-loop-helix transcription factors) are targeted by Mt-miR2199. One of them, named TSAR1 is a key transcriptional activator of saponin biosynthesis, a pathway previously shown to enhance symbiotic nodulation.

In this work we determined that Mt-miR2199 increases its abundance in roots and leaves while TSAR1 mRNA abundance is reduced upon water deficit. We are employing genome editing, hairy-root transient transformation and mutant lines to determine the molecular and phenotypical consequences of affecting Mt-miR2199 and TSAR1 levels, and in turn identify downstream gene networks regulated by this module involved in water deficit responses and during root development.

Our aim is to understand how TSAR1 silencing mediated by miR2199 is involved in the plant response to water deficit and root development. TSAR1 role in saponin biosynthesis is known to promote plant-bacteria symbiosis, which represents an expensive process for the plant in terms of carbon usage, while at the same time this element becomes one of the first affected nutritional fluxes under a water deficit condition. How these processes are coordinated could be addressed by studying the gene expression changes caused by miR2199 and TSAR1 regulation.



## Host-Induced Gene Silencing of *Phytophthora capsici GIP1* (Glucanase Inhibitor protein 1) decreases infection in *Nicotiana benthamiana*

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*Phytophthora capsici* is an oomycete transmitted via soil and late blight disease agent in many solanaceae and cucurbitaceae plants all around the world triggering big economic crops damage, by that reason it is very important to understand its pathogenicity and ways to combat it. To establish infection, *P. capsici* must evade or suppress the host defense. And the first barrier is the plant cell wall. In plants, the major components of the cell wall are cellulose and hemicellulose con  $\beta$ -linked, and in oomycetes is composed of 1-3 and 1-6  $\beta$ -glucans. Plants synthetize a variety of proteins that inhibit the enzymes that degrading cell wall secreted by phytopathogens; and during pathogenesis, many pathogens also secrete endo- $\beta$ -1, -4-glucanases. *P. capsici* is able to secrete a Glucanase Inhibitory Protein (GIP1) that specifically bind and inhibit the activity of plant extracellular endo- $\beta$ -1,3-glucanases, thereby suppressing the degradation of glucans in the oomycete cell wall and /or the release of defense-eliciting oligosaccharides by host. In this study, we developed a strategy of host-induced gene silencing (HIGS) to silenced *GIP1* gene, and we observed that transient expression *P. capsici* dsRNA-GIP1 gene shows reduced symptoms in *Nicotiana benthamiana*, in comparison with the pHellsgate 12 empty vector and posterior infection with *P. capsici* zoospores. To our knowledge this is the first-time that the host-induced gene silencing in GIP1 gene has been analyzed and we confirmed that the HIGS method could be an effective strategy to confer resistance against the oomycete *P. capsici*.



## Isolation and identification of fungal pathogens in tropical fruits and design of synthetic dsRNAs for postharvest protection via a SIGS strategy

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Plant diseases are responsible for up to 30% of losses in crop production in the world, many of which are attributed to fungal pathogens. In recent years, the need to produce enough food to feed an increasing population has remarked the urgency to develop new strategies for crop protection. A novel approach known as SIGS (spray-induced gene silencing) has emerged that is based on the spraying of dsRNAs to inhibit the development and growth of fungi through the silencing of key genes in the pathogens. To propose a SIGS strategy for the protection of tropical fruits in the postharvest stage, we isolated pathogenic fungi in mango and pineapple fruits from the local markets. Morphological and molecular identification by sequencing of the ITS region was performed in the isolates. After sequence and phylogenetic analysis, two of them were identified as Lasiodiplodia pseudotheobromae in mango, and Aspergillus tubingensis in pineapple fruits. Then, DICER-LIKE (DCL) genes were selected as molecular targets for silencing due to their importance in the biogenesis of sRNAs that regulate several molecular processes including pathogenesis in fungi. A search in the databases revealed the existence of two DCL sequences for each pathogen. Regions of 250bp between the DEAD-LIKE HELICASE and HELICASE C-terminal domains were selected from the DCL sequences, amplified by PCR from genomic DNA, fused in a single construct, and used as template for dsRNA synthesis. Ongoing bioassays will reveal the potential of SIGS as a strategy for extending the postharvest life of tropical fruits.



## The PvRALF1/PvRALF6- PvFER1 module differentially regulates the optimal number of nodules in common bean roots depending on the nitrogen status of the soil

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The cysteine-rich peptide RALF and FERONIA, which is a CrRLK1L, are part of a unique ligand-receptor complex in plants, which in the recent years has gained relevance due to its versatility of functions, ranging from development in plants, to biotic and abiotic responses. Genomic and bioinformatic analysis of the RALF family and the CrRLK1L subfamily in different plant species, indicates that both groups are highly conserved, and appeared almost simultaneously with the emergence of the terrestrial plants. In this study, the promoter activity and the expression of the PvFER1, PvRALF1, and PvRALF6 genes were evaluated in common bean roots inoculated and not inoculated with rhizobia. The results obtained showed that these genes are expressed in roots and nodules at various stages of their organogenesis. Reverse genetic analysis of *PvFER1*, *PvRALF1*, and *PvRALF6* revealed a negative role in determining the number of nodules under symbiotic conditions, while a positive role in determining the number of nodules under inhibitory symbiotic conditions in the presence of nitrate in the soil. Data from qPCR analysis show a correlation between the effect on the total number of nodules and changes in the expression of genes involved in the autoregulation of nodulation (AON) pathway and in the regulation of nodulation mediated by nitrate (NRN). These data strongly suggest that the PvRALF1/PvRALF6-PvFER1 complex participates in determining the optimal number of nodules in common beans by regulating the expression of genes involved in AON and NRN, depending on the nitrate content in the soil.



#### Root growth adaptation under water deficit

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Growth and development of the root system require the coordinated regulation of developmental programs and environmental signals; however, the knowledge about its interconnection is scarce. The balance between cell division, regulated cellular expansion, and differentiation in the root apical meristem directs primary root growth in Arabidopsis. Cellular expansion requires cell wall controlled relaxation, which ensures cell integrity during the expansion process. In field conditions, the root faces different kinds of stress, including osmotic stress. Mutations in the Arabidopsis Tetratrico-peptide Thioredoxin-Like 1 (TTL1) cause hypersensitivity to osmotic stress evidenced by root tip swelling, making it an attractive model to explore how root growth is regulated under osmotic stress conditions. We found that osmotic stress decelerates root growth by reducing first cell elongation in the elongation zone and second the number of cortical cells in the proximal meristem. Using atomic force microscopy, we measure the stiffness of epidermal cell walls in the root elongation zone of *ttl1* mutants, and we found that the mean apparent elastic modulus was 448% higher for live Col-0 cell walls than for *ttl1* (88.1  $\pm$  2.8 vs. 16.08  $\pm$  6.9 kPa) in plants grown in control conditions. Furthermore, cell walls of epidermal cells in the elongation zone increase their stiffness 87% and 84% for Col-0 and *ttl1*, respectively, in response to seven days of osmotic stress. These findings suggest that TTL1 may play a role in controlling cell expansion orientation during root growth, necessary for osmotic stress adaptation.



## **Over-expression of a ripening related ramnogalacturonan lyase ( Solyc11g011300) bring improvement in fruit tomato shelf life**

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The enzyme rhamnogalacturonan lyase Solyc11g011300 (RGL) is responsible for the breakdown of the polymer rhamnogalacturonan-l, a component of pectin. Pectin degradation is associated with firmness decreases during tomato fruit ripening. In this work, the effect of the overexpression of the Solyc11g011300 gene in tomato plants cv Ohio 8245 was assessed . We collected fruit during the ripening process, including 39, 42, 49 and 56 days after anthesis (DDA). We evaluated the transcriptional changes of genes involved in biosynthesis, signaling and ethylene response, ethylene hormone production, chemical quality tests and physiological tests in tomato fruits. Our result from two transgenic line(L1 and H2 lines) presented a delay of one day to reach the breaker stage relative to isogenic line (Ohio 8285), causing a delay in the appearance of climacteric peak and, therefore, in the ripening phenomena. Interestingly, no significant changes were observed in the amount of soluble solids, titratable acidity and pH, but there were differences in softening. The firmness of the H2 transgenic line was higher at 39 and 56 DAA. Transcript quantification indicate a putative alteration on ethylene pathway because both transgenics lines shown ACS2 (Solyc01g095080) y ACS4 (Solyc05g050010) maintained the same behavior from 42 to 56 DAA but not in 39 DAA. The genes E4 (Solyc03g111720) was observed in transgenic line H2 close to breaker and repressed in 56 DAA. An opposite response was found in L1 transgenic line. E8 (Solyc09g089580) gene was found active at 49 DAA. With our result we suggest that the over expression of Solyc11g011300 gene increases fruit shelf life by altering the ethylene effect.



## **RNA-Seq analysis of** *Ditylenchus dipsaci-Allium sativum* interaction

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*Ditylenchus dipsaci*, is a plant parasite considered within the top 10 of the most important parasites for agriculture<sup>1</sup>. It is considered the most important phytopathogenic nematode of garlic<sup>2</sup>, which is a crop of economic importance at regional, national, and world levels. Garlic has a wide variety of compounds that it uses as a defense system<sup>3</sup>, however, this is not enough to control the nematode infection processes<sup>4</sup>. Our aim was to analyze the *D. dipsaci*-garlic interaction to elucidate which are the mechanisms, both of defense of the host and attack of the nematode. For this, an RNA-Seq transcriptome analysis was performed. Slices of garlic of 2 cm<sup>2</sup> x 0.3 mm, with and without nematode inoculation, were prepared to evaluate different time points, 0, 24, and 72 h. Inoculation was carried out with a suspension of nematodes containing an approximate amount of 10 thousand nematodes, mostly at the juvenile state. We proceeded to macerate with N<sub>liq</sub>, and RNA was extracted to carry out a massive 2x100 bp pair-end sequencing, using the Illumina platform. The results of the differential expression analysis show an induction of different transcripts related to defense in garlic, among them those related to Pathogenesis-Related (PR) proteins and defense at the first stages of infection. The expression of most of these transcripts is reduced as nematode exposure time increases. The information generated helps us to understand the processes that are taking place during the interaction of *D.dipsaci* and *A. sativum* which can be further studied to find better control strategies.



## Characterization of a motif that leads the secretion of a thioredoxin type h and probably to any protein that contains it

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NaTrxh is a protein recently characterized as an essential protein in the gametophytic S-RNase selfincompatibility system. An important feature of NaTrxh is that it possesses the information to lead its secretion, which is different to the classical signal peptide. A motif called N $\beta$ , comprising from Ala-17 to Pro-27, has been identified as the responsable of NaTrxh extracellular localization. A BLAST was performed identifying the N $\beta$  and N $\beta$ -like sequences within different proteins of all biological domains. A total of 383 protein sequences were obtained, from which 266 belong to animals, 23 to fungi, 16 to plants, and 77 to Archae and Bacteria. When the sequences were analyzed by function and its relation to the localization of the N $\beta$  motif in the primary structure, 83.2% of these proteins that contain the NB towards the first half of the N-terminal are secreted or involved in a membrane-associated function. Additionally, to define the minimal unctional size of the motif, we generated different deletions on the NB and fused them to GFP in order to transiently express these fusions in onion epidermal cells. The results will be discussed.



## An Arabidopsis transformation platform of sensitive and tolerant ecotypes to test biotechnological strategies aimed to improve submergence tolerance in plants

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Submergence is an important plant production constraint, but a few cultivars of superior tolerance are available. Arabidopsis is well established as a model plant and can be used to translate lab results to field applications. Additionally, Arabidopsis has been used extensively for the discovery of mechanisms acting on submergence stress. Here, we characterized reported Arabidopsis ecotypes with contrasting tolerance and determined the best Arabidopsis-Agrobacterium combination to avoid recalcitrance. We selected the ecotypes from previously reported results and replicated the experiments. Next, we applied floral dip transformation to all ecotypes with three different Agrobacterium strains (EHA105, GV3101 and GV2260) to characterize the best combination in terms of transformation efficiency. The results indicated that the tolerance was Cvi-0 < Ita-0 < Bay-0 < Col-0 < Kin-0 < Lp2-6 < C24. Ita-0 and C24 were highly recalcitrant but still produced recombinants (<0.1%). The Agrobacterium strain that could consistently transform all ecotypes was GV3101. Finally, to use this potential biotechnological platform, we constructed plasmids to express plant hemoglobin (NO scavenger) and bZIPs (sucrose homoeostatic regulators) with the miRNA156 recognition site in the 3' UTR to gradually increase the transgene expression with age. The characterization of transformants under submergence conditions is an ongoing experiment. We conclude that having the availability of ecotypes with robust performance (either sensitive or tolerant) under submergence stress conditions that are genetically transformable will be an important tool to quickly test biotechnological strategies to increase tolerance.



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

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Plant aquaporins are a large family of proteins solutes transporters (water, sugar and NH4) that play an important role in several physiological processes in living organisms. On the other hand, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels and transport have been related with plant growth, development, biotic, and abiotic stress responses. It has been proposed that aquaporins can also transport H<sub>2</sub>O<sub>2</sub>, regulating the subcellular distribution and thus signal strength. However, little is known about this process. It's well known that reactive oxygen species (ROS) are highly involved in polar growth, but also during the mutualistic interactions such as the rhizobia-legume or mycorrhizal association. ROS generated in the apoplast by NADPH oxidases and SOD activity, such as H<sub>2</sub>O<sub>2</sub>, 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<sub>2</sub>O<sub>2</sub> in root hairs during the polar growth and 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 H2O2 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. Our new results describing the subcellular localization, nodulation and nitrogen fixation phenotype, will be presented and discussed.