SMB

XV National Congress of Plant Biochemistry and Molecular Biology & VIII Symposium Mexico / USA

CONFERENCE PROGRAM

XCARET, Quintana Roo, Mexico
October 21 – 25, 2013

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Sociedad Mexicana de Bioquímica A.C.

Promover la investigación y la educación en el área Bioquímica en México

XV National Congress of

Plant Biochemistry and Molecular Biology

&

8th Symposium Mexico-USA

Xcaret, Quintana Roo, Mexico, October 21st – 25th, 2013

Scientific Committee

Academic Program

Sarah Hake

Manuel Martínez Estévez

Donald Ort

José Juan Zúñiga Aguilar

With collaboration of:

Miguel Lara Flores

Abstracts

Alejandra Covarrubias Robles

Zvetanka Dimitrova Dinkova

Luis Eguiarte Fruns

Gregorio Godoy Hernández

Georgina Hernández Delgado

Jorge Molina Torres

Oscar Moreno Valenzuela

Quintín Rascón

Mario Rocha Sosa †

Rogelio Rodríguez Sotres

María de la Paz Sánchez Jiménez

June Simpson Williamson

Posters

USA and Mexican researchers and students

Funding Institutions and Sponsors

Bienvenida

Estimados participantes,

El Comité Organizador tiene el placer de recibirlos en el **XV Congreso Nacional de Bioquímica y Biología Molecular de Plantas,** que se llevará a cabo en Xcaret, Quintana Roo, del 21 al 25 de octubre de 2013. Como ha sucedido desde el Congreso de 1995 en Cococyoc, Morelos, esta reunión científica se desarrollará en paralelo con el **Simposio México-Estados Unidos**, que en esta oportunidad la octava edición.

La celebración de la décimo quinta reunión organizada por esta rama de la Sociedad Mexicana de Bioquímica, nos motiva a rememorar la decisión tomada por los socios numerarios durante la reunión de 1993 en la Ciudad de Morelia, para transformar la entonces Reunión de Bioquímica Vegetal en el ahora Congreso de Bioquímica y Biología Molecular de Plantas. La diversidad de áreas de estudio representadas actualmente en este congreso, entre las que se encuentran temas básicos y aplicados de la biología vegetal, demuestran el carácter visionario de aquella decisión. La evaluación de los trabajos presentados en los congresos celebrados a lo largo de estos 18 años demuestra que las contribuciones al conocimiento universal de los grupos de investigación, distribuidos ahora a lo largo del territorio nacional, son cada vez más significativas.

Un elemento importante de esta nueva etapa lo ha constituido el **Simposio Mexico – Estados Unidos**, especialmente para los jóvenes estudiantes, quienes han aprovechado este foro para interactuar con investigadores estadunidenses, quienes durante estas reuniones han compartido sus experiencias de manera generosa.

En su versión décimo quinta, este congreso contará con siete plenarias con la participación de investigadores nacionales y extranjeros, quienes abordarán diversas temáticas de la biología vegetal. En las sesiones concurrentes, hemos pretendido favorecer la participación de los estudiantes, cuyas presentaciones en formato oral fueron seleccionadas por comités científicos con base en criterios académicos. Finalmente, contaremos con dos sesiones de exposición de carteles, en la que se ejecutará de manera interactiva la esencia de esta reunión, la discusión objetiva de los resultados experimentales. Un comité científico denominará los tres mejores carteles, los cuales recibirán un premio como primer lugar.

Esperamos saludarles personalmente en Xcaret, lugar que constituye un buen ejemplo de los esfuerzos para miniminar el impacto de las actividades humanas en el entorno natural.

El Comité Organizador,

MÉXICO:
Manuel Martínez Estévez, CICY
Jose Juan Zúñiga Aguilar, CICY, ITSR

USA:
Sarah Hake, University of California, Berkeley
Donald Ort, University of Illinois

Welcome

Dear participants

The Organizing Committee is pleased to receive you in the **XV National Congress of Plant Biochemistry and Molecular Biology**, which will be carried out in Xcaret, Quintana Roo, from 21 to 25 October 2013. As it has occurred since the 1995 Congress in Cococyoc, Morelos, this scientific meeting will be held in parallel with the **Symposium Mexico –Unites States**, which on this occasion will see its eighth edition.

The celebration of the XV meeting organized by this branch of the Mexican Society of Biochemistry, motivate us to evoke the decision taken by the SMB members, during the 1993 meeting in Morelia, to transform the former Meeting of Plant Biochemistry into the current Congress of Plant Biochemistry and Molecular Biology. The areas of study currently represented in this Congress, which includes a diversity of basic and applied topics in Plant Biology, demonstrates the visionary character of that decision. The evaluation of the works presented in these 18 years clearly shows that the contributions to the universal knowledge of the research groups, which are distributed throughout the national territory, are increasingly significant.

A very important component of this new period has been the **Symposium Mexico - United States**, especially for young students, who have used this forum to interact with researchers from the United States, who have shared their experiences in a generous way during these meetings.

In his XV version, this congress will have seven Plenary Sessions with the participation of national and foreign researchers, who will address various topics of plant biology. In concurrent sessions, we pretended to favor the participation of students, whose presentations in oral format were selected by scientific committees on the basis of academic criteria. Finally, we will have two sessions of posters discussion, in which we expect that the essence of this meeting will be developed in an interactive manner, the objective discussion of the experimental results. A scientific committee will design the three best posters, which will receive each a prize as a unique first place.

We hope meeting you personally at Xcaret, an iconic place that is a good example of the efforts to minimize the negative impact of human activities on the natural environment.

The Organizing Commitee,

MEXICO:
Manuel Martínez Estévez, CICY
Jose Juan Zúñiga Aguilar, CICY, ITSR

USA:
Sarah Hake, University of California, Berkeley
Donald Ort, University of Illinois

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Programme of Events

Monday, October 21st, 2013

10:00 — 19:00	**Registration**
17:30 — 18:00	**Inauguration**
18:00 — 19:00	**Inaugural Conference** Rosario Muñoz Clares *Enzymological Aproaches to Understand Plant Responses to the Environment* Facultad de Química, UNAM
19:00 — 19:15	**Dr. Mario Rocha, In Memoriam**
20:00 — 22:00	**Welcoming Cocktail**

Tuesday, October 22nd, 2013

Plenary Session I

Plant Microbe and Insect Interactions /Plant Response to the Environment

Chair: Federico Sánchez

8:00 – 8:25	Jose Dinneny Hydropatterning: how local moisture controls branching in roots Stanford University
8:25 – 8:50	Luis Cárdenas Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth Instituto de Biotecnología, UNAM
8:50 – 9:15	Donald Ort Food, Fuel and Photosynthesis University of Illinois
9:15 – 9:40	José López Bucio Chemical signaling in plant growth promotion by rhizobacteria Universidad Michoacana de San Nicolás de Hidalgo
9:40 – 10:05	Jennifer D. Lewis The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a University of California, Berkeley
10:05 – 10:30	Rebecca Bart The *Xanthomonas Cassava* bacterial blight pathogen employs TAL effectors to induce a pectate lyase and sugar transporter during host colonization University of California, Berkeley
10:30 – 11:00	Coffe Break

Plenary Session II

Secondary Metabolism/ Plant Nutrition

Chair: Leon Kochian

11:00 — 11:25	Tony Kutchan A transcriptomic/metabolomic approach to biochemical pathways in non-model systems Donald Danforth Plant Science Center
11:25 — 11:50	Jorge Molina Torres *Thevetia thevetiodides* seed cardenolides Centro de Investigación y de Estudios Avanzados, Irapuato
11:50 — 12:15	Joe Chappell Nothing like we imagined – uncovering the terpenome of liverworts University of Kentucky
12:15 — 12:40	Leon Kochian Molecular and biochemical strategies for cereal crop adaptation to acid soils Cornell University
12:40 — 13:05	Renata Rivera Madrid Bixin synthesis and carotenoid gene expression in *Bixa orellana* L. Centro de Investigacion Científica de Yucatán
13:05 — 13:30	Aida Martínez Hernández Testing the role of well-known molecular mechanisms involved in metal uptake, translocation and homeostasis in Agave Colegio de Posgraduados, Campus Campeche
13:30 — 13:50	Congress Photo
13:50 — 15:00	Lunch

Concurrent Session IA: Plant Microbe and Insect Interactions I

Chair: José Juan Zúñiga Aguilar

Room Xcaret 1

15:00 – 15:15	Randy Ortiz Castro *Pseudomonas putida* and *Pseudomonas fluorescens* regulate Arabidopsis root architecture through an auxin mediated pathway and produce bioactive cyclodipeptides Universidad Michoacana de San Nicolás de Hidalgo
15:15 – 15:30	Juan Elías Olivares Grajales Analysis of nodulin 41 expression in early stages of symbiosis and cellular localization in transgenic root nodules Instituto de Biotecnología, UNAM
15:30 – 15:45	Loreto Holuigue A salicylic acid-induced lectin-like protein plays a positive role in the effector-triggered immunity response in *Arabidopsis thaliana* to *Pseudomonas syringae* AVR-RPM1. Instituto de Biotecnología, UNAM
15:45 – 16:00	Lucila Méndez-Morán The *Arabidopsis thaliana* peroxidase expression during *Ustilago maydis* infection Centro Universitario de Ciencias Biológicas y Agropecuarias
16:00 – 16:15	Gabriela Chávez-Calvillo Interactions between two unrelated RNA viruses and their host: a case of classic synergism and contrasting viral antagonism Instituto Potosino de Investigación Científica y Tecnológica
16:15 – 16:45	Coffee Break

Concurrent Session IB: Plant Microbe and Insect Interactions II

Chair: Damaris Godínez (Mario Rocha †)

Room Xcaret 1

16:45 – 17:00 Edmundo Lozoya
Response of the promoter of a phytoalexin biosynthetic gene from pepper to virus, insects and parasitic plants
Centro de Investigación y de Estudios Avanzados, Irapuato

17:00 – 17:15 Ruth Sarahi Pérez-Alfaro
Characterization of histone H3 family from *Capsicum annuum* and differential expression in response to Pepper Golden Mosaic Virus (PepGMV) infection
Centro de Investigación y de Estudios Avanzados, Irapuato

17:15 – 17:30 Yolanda Ortega-Ortega
Phosphoproteomic analysis of *Phaseolus vulgaris* roots during the early stages of the rhizobia-legume symbiosis
Instituto de Biotecnología, UNAM

17:30 – 17:45 Claudia Ramíez Valdespino
Characterization of genes encoding potential effector of *Trichoderma* spp. differentially expressed in interaction with *Arabidopsis thaliana*
Universidad de Guanajuato

17:45 – 18:00 Manoj-Kumar Arthikala
Overexpression of a *Phaseolus vulgaris* NADPH oxidase gene increases symbiosome number, bacteroid size and nitrogen fixation in nodules and impairs mycorrhizal colonization
Instituto de Biotecnología, UNAM

18:00 – 20:00 **POSTER SESSION I: Odd numbers** **Room Xcaret 6**

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Plant Systematics and Biodiversity	107–113
Crop Improvement/Crop Evolution	114–147

Concurrent Session IIA: Response to the Environment/Pant Nutrition I

Chair: Jorge M. Santamaría Fernández

Room Xcaret 2

15:00 – 15:15	Julio A. Massange Sánchez Overexpression of a novel ethylene response factor gene AhERF of *Amaranthus hypochondriacus* as a strategy to confer dual resistance to water stress and bacterial infection in transgenic *Arabidopsis plants* Laboratorio Nacional de Genómica para la Biodiversidad
15:15 – 15:30	Jorge M. Santamaría Fernández Characterization of the entire family of HSF in Carica papaya and expression analysis of six of those genes, in response to heat stress and during recovery Centro de Investigacion Científica de Yucatán
15:30 – 15:45	Damaris Godinez-Vidal Mutations in AtFBS genes alter the response to abiotic stress in *Arabidopsis thaliana* Instituto de Biotecnología, UNAM
15:45 – 16:00	Aída Araceli Rodríguez Hernández AtGRDP1 gene encoding a glycine-rich domain protein, a new component of the ABA signaling pathway? Instituto Potosino de Investigación Científica y Tecnológica
16:00 – 16:15	Abigael López Córdova *AtLEA-1* and *AtLEA-2* are involved in stomata patterning and water stress tolerance in *Arabidopsis thaliana*
16:15 – 16:45	Coffee Break

Concurrent Session IIB: Response to the Environment/Plant Nutrition II

Chair: Alejandra Covarrubias

Room Xcaret 2

16:45 — 17:10 Gladys Cassab
The relationship of drought tolerance to the hydrotropic response of maize and Arabidopsis roots
Instituto de Biotecnología, UNAM

17:10 — 17:25 Cesar Luis Cuevas Velázquez
Changes in the environmental conditions induce structural order in intrinsically unstructured stress proteins from plants
Instituto de Biotecnología, UNAM

17:25 — 17:40 Francisca Morayna Gutiérrez-Luna
Intracellular localization of the inorganic soluble pyrophosphatase isoforms 5 and 6 in *Arabidopsis thaliana*
Facultad de Química, UNAM

17:40 — 17:55 Alejandra Chamorro Flores
Involvement of ABA in salt stress tolerance in the moss *Bryum billarderi*
Centro de Investigación en Biotecnoogía Aplicada, IPN

17:55 — 18:10 Emanuel Bojórquez-Quintal
Proline accumulation and ion flux in the roots of two varieties of habanero pepper (*C. chinense* Jacq.) with different tolerance to NaCl
Centro de Investigación Científica de Yucatán

18:00 — 20:00 **POSTER SESSION I: Odd numbers** **Room Xcaret 6**

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Thursday, October 24th, 2013

Plenary Session III

Developmental Patterning

Chair: Sarah Hake

8:00 – 8:25	Dave Jackson New players in CLAVATA signaling control shoot meristem size and yield in maize Cold Spring Harbor Laboratory
8:25 – 8:50	Jean Philippe Vielle-Calzada On the (epi)genetic control of apomixis: learning from sexual experience Laboratorio Nacional de Genómica para la Biodiversidad
8:50 – 9:15	Miguel Lara Flores Target of rapamycin is required for root growth and nodule development in *Phaseolus vulgaris* Escuela Nacional de Estudios Superiores Unidad León, UNAM
9:15 – 9:40	Sarah Hake Wab1 encodes a TCP transcription factor and regulates LG1 expression University of California, Berkeley
9:40 – 10:05	Patricia León Novel signals regulating chloroplast biogenesis and leaf development Instituto de Biotecnología, UNAM
10:05 – 10:30	Joseph Dubrovski The RAM determinacy versus indeterminacy: developmental programs and their regulation Instituto de Biotecnología, UNAM
10:30 – 11:00	Coffe Break

Thursday, October 24th, 2013

Plenary Session IV

Epigenetic/Genetic Regulation of Plant Processes

Chair: Enrique Castaño de la Serna

11:00 – 11:25	Doris Wagner Chromatin remodeling ATPases at the interface of environment, development and the genome University of Pennsylvania
11:25 – 11:50	Enrique Castaño de la Serna Phosphatidylinositol-4, 5-bisphosphate in the nucleus and its involvement on nuclear myosin 1 function Centro de Investigación Científica de Yucatán
11:50 – 12:15	Jay Hollick Non-Mendelian inheritance of epigenetic variation in maize The Ohio State University
12:15 – 12:40	Stefan de Folter Factors guiding gynoecium development Laboratorio Nacional de Genómica para la Biodiversidad
12:40 – 13:05	Carroll Vance The impact of phosphate deficiency on carbon metabolism and sucrose University of Minnesota
13:05 – 13:30	José Luis Reyes Water deficit responses regulated by microRNA in *Phasoelus vulgaris* Instituto de Biotecnología, UNAM
13:30 – 15:00	Lunch

Concurrent Session IIIA: Developmental Patterning I

Chair: María de la Paz Sánchez Jiménez

Room Xcaret 1

15:00 – 15:15	Nayelli Marsch-Martínez The role of the phytohormone cytokinin in the design of the plant gynoecium Centro de Investigación y de Estudios Avanzados, Irapuato
15:15 – 15:30	Berenice García-Ponce New MADS-box genes in the floral transition network Facultad de Ciencias, UNAM
15:30 – 15:45	Edith Muñoz-Parra Melatonin regulates Arabidopsis root system architecture likely acting independently of auxin signaling Universidad Michoacana de San Nicolás de Hidalgo
15:45 – 16:00	Ángel Arturo Guevara García *Arabidopsis thaliana* MPK6 mutation drives three distinct classes of seed phenotypes, which correlate with alterations in cellular processes that affect root architecture Instituto de Biotecnología, UNAM
16:00 – 16:15	Alma Fabiola Hernández-Bernal Regulation of ABA-INSENSITIVE (ABI) 4 transcription factor in *Arabidopsis thaliana* Instituto de Biotecnología, UNAM
16:15 – 16:45	Coffee Break

Concurrent Session IIIB: Developmental Patterning II

Chair: Felipe Cruz García

Room Xcaret 1

16:45 — 17:00	Javier Andrés Juárez Díaz BiFC shows that the S-determinants from *Papaver rhoeas* directly interact *in vivo* in an S-specific manner University of Birmingham
17:00 — 17:15	Lilia Angélica Bernal Gracida The proteases and proteinase inhibitors game in pollen rejection in *Nicotiana alata* Facultad de Química, UNAM
17:15 — 17:30	Silvia Karina Godínez Palma Complexes of cyclins D with CDKs during maize germination: activity and regulation Facultad de Química, UNAM
17:30 — 17:45	Aarón Giovanni Munguía-Rodríguez Study of the involvement of jasmonic acid on epidermal cell differentiation processes in *Arabidopsis thaliana* Universidad Michoacana de San Nicolás de Hidalgo
17:45 — 18:00	Svetlana Shishkova RNA-seq assisted insight into molecular mechanisms of determinate root growth in Cactaceae Instituto de Biotecnología, UNAM

18:00 — 20:00	**POSTER SESSION I: Even numbers**	**Room Xcaret 6**
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Concurrent Session IVA: Epigenetic/Genetic Regulation of Plant Processes

Chair: Tzvetanka Dimitrova Dinkova

Room Xcaret 2

15:00 – 15:15	Tzvetanka Dimitrova Dinkova Regulation by small RNAs during somatic embryogenesis in maize (*Zea mays* L.) Facultad de Química, UNAM
15:15 – 15:30	Luis Joel Figueroa Yáñez Functional and phylogenetic analysis of a CBF/DREB gene in *Carica papaya* var. Maradol Centro de Investigación Científica de Yucatán
15:30 – 15:45	Keren Martínez Aguilar Transgenerational epigenetic modifications as a result of priming in common bean (Phaseolus vulgaris L.) Centro de Investigación y de Estudios Avanzados, Irapuato
15:45 – 16:00	Ulises Rodriguez Corona Interaction between fibrillarin and phosphatidylinositol 4,5-bisphosphate in the nucleolus of *Arabidopsis thaliana*. Centro de Investigación Científica de Yucatán
16:00 – 16:15	David Díaz Ramírez amiRNA-based gene silencing of the gene families WIP and ERF B1 Centro de Investigación y de Estudios Avanzados, Irapuato
16:15 – 16:45	Coffee Break

Concurrent Session IVB: Secondary Metabolism

Chair: Felipe Vázquez Flota

Room Xcaret 2

16:45 — 17:00	Beatriz King-Díaz Piperine, photosynthetic electron transport and vegetal growth inhibitor Facultad de Química, UNAM
17:00 — 17:15	Fray Martin Baas-Espinola Possible relationship between primary and secondary metabolisms in placental tissue of *Capsicum chinense* Jacq. Centro de Investigación Científica de Yucatán
17:15 — 17:30	Ernesto García-Pineda Avocado roots treated with salicylic acid produce phenol-2,4-bis (1,1-dimethylethyl), a compound with antifungal activity Universidad Michoacana de San Nicolás de Hidalgo
17:30 — 17:45	Patricia Ríos Chávez Developmental regulation of valine decarboxylase in *Acmella radicans* Centro de Investigación y de Estudios Avanzados, Irapuato
17:45 — 18:00	Magda Lisette Arce Rodríguez Virus-induced silencing of a putative capsaicin synthase (AT3) gene affects the expression of genes related to the capsaicinoid biosynthetic pathway in chili pepper fruits (*Capsicum annuum* L.) Centro de Investigación y de Estudios Avanzados, Irapuato

18:00 — 20:00	**POSTER SESSION I: Even numbers**	**Room Xcaret 6**
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	CropImprovement/Crop Evolution	114—147

Plenary Session V

Plant Molecular Systematics and Biodiversity

Chair: Luis Eguiarte Fruns

8:00 – 8:25	Luis Eguiarte Fruns The evolution of biodiversity in plants: Classic questions - new approaches and paradigms, with special reference to studies of Mexican diversity Instituto de Ecología, UNAM
8:25 – 8:50	Susana Magallón A metacalibrated relaxed molecular clock analysis of flowering plants Instituto de Biología, UNAM
8:50 – 9:15	Angélica Cibrián Jaramillo Beyond natural selection in phylogenomics: uncovering in genes with functional importance Laboratorio Nacional de Genómica para la Biodiversidad
9:15 – 9:40	Juan Pablo Jaramillo Correa Predicting climate maladaptation in forest trees: perspectives for Mexican conifers Instituto de Ecología, UNAM
9:40 – 10:05	Michael Clegg Tracing Population History with Haplotype Data University of California, Irvine
10:05 – 10:30	Andrew Doust Evolution and domestication in grasses Cold Spring Harbor Laboratory
10:30 – 11:00	Coffe Break

Friday, October 25th, 2013

Plenary Session VI

Crop Improvemenet/ Crop Evolution

Chair: June Simpson

11:00 — 11:25	Patrick Brown Genetic architecture of flowering time in sorghum University of California, Davis
11:25 — 11:50	June Simpson Strategies for conservation and sustainable use of Mexican maize landraces Centro de Investigación y de Estudios Avanzados Irapuato
11:50 — 12:15	Frank Dohleman Title Pending Monsanto Company
12:15 — 12:40	Verónica Lira-Ruán Exploring the *Physcomitrella patens* genome for the two main enzymatic nitric oxide-producing mechanisms: nitrate reductase and nitric oxide synthase Universidad Autónoma del Estado de México
12:40 — 13:05	Aida Odette Avendaño Vázquez Functional diversity of plant-soil relations in maize and wild relatives Centro de Investigación y de Estudios Avanzados Irapuato
13:05 — 13:30	Quintín Rascón Cruz Traditional and genetically improvement of sugar cane Universidad Autónoma de Chihuahua
13:30 — 15:00	Lunch

Concurrent Session VA: Plant Molecular Systematics and Biodiversity

Chair: Renata Rivera Madrid

Room Xcaret 1

15:00 — 15:15	José Pablo Lara-Ávila Assessment of genetic diversity in Mexican strains of phytopathogen *Clavibacter michiganensis* subsp. michiganensis Universidad Autónoma de San Luis Potosí
15:15 — 15:30	Germán Fernando Gutiérrez Opaco2 mutant gene and phylogenetic relationships of quality protein maize Unidad Profesional Interdisciplinaria de Biotecnología-IPN
15:30 — 15:45	Carlos Alberto Puch-Hau Isolation and characterization of a superfamily of candidate disease-resistance genes of the nucleotide binding site (NBS) type from *Cocos nucifera* L. Centro de Investigacion Científica de Yucatán
15:45 — 16:00	José Antonio Corona Gómez Ecological genomics of the interaction cyanobacteria-cycads in Mexico Laboratorio Nacional de Genómica para la Biodiversidad
16:00 — 16:15	Rodolfo Pech Hoil Molecular genetic analysis of the mating system of annatto plants (*Bixa orellana* L.) cultivated under different agricultural conditions in the state of Yucatán Centro de Investigacion Científica de Yucatán
16:15 — 16:45	Coffee Break

Concurrent Session VIB: Crop Improvement - Crop Evolution

Chair: June Simpson

Room Xcaret 2

15:00 – 15:15	Gustavo J. Acevedo-Hernández ISTR markers in the study of genetic variability in cultures of *S. edule* Universidad de Guadalajara
15:15 – 15:30	González-Segovia, Eric Gerardo Identification of presence absence variation in the landrace Palomero Toluqueño Laboratorio Nacional de Genómica para la Biodiversidad
15:30 – 15:45	J.C. Raya Pérez Characterization of a maize Celaya landrace mutant Midrib brown Instituto Tecnológico de Roque
15:45 – 16:00	Jorge Herrera Díaz Biomarker discovery using bottom up analysis: Differential protein accumulation in barley seeds from five Mexican varieties grown under field conditions Facultad de Química, UNAM
16:00 – 16:15	June Simpson Variation in environmental conditions leads to an "identity crisis" during bulbil formation in *A. tequilana* Centro de Investigación y de Estudios Avanzados, Irapuato
16:15 – 16:45	**Coffee Brake**

Friday, October 25th, 2013

Plenary Session VII

Closing Conferences

Plant Proliferation and Differentiation

Chair: Jorge M. Vázquez Ramos

16:50 — 17:15	**Vernonica E. Franklin-Tong** Integrating the signalling networks that trigger programmed cell death in self-incompatible Papaver pollen University of Birmingham
17:15 — 17:40	**Maria de la Paz Sánchez Jiménez** Chromatin dynamics during plant cell proliferation Instituto de Ecología, UNAM
17:40 — 18:05	**Jorge M. Vázquez Ramos** Ciclinas de Gl en maiz. Ciclinas D y la germinación Facultad de Química, UNAM
18:05 — 18:30	**Best Posters Award**
18:30 — 18:45	**Closing Ceremony**

20:00 — 22:00 **Farewell Party**

Plenary and Concurrent Sessions Abstracts

Enzymological approaches to understand plant responses to the environment

Rosario A. Muñoz-Clares**.**

Departamento de Bioquímica, Facultad de Química. UNAM. clares@unam.mx

Plants use a variety of strategies to respond to the demands of their environment, some of them involving the evolution of novel metabolic pathways from old ones. This is the case of the route of synthesis of the osmoprotectant glycine betaine (GB), or of the photosynthetic C4 cycle. GB is formed from betaine aldehyde (BAL) in an oxidative reaction catalyzed by betaine aldehyde dehydrogenases (BADHs), which belong to the ALDH10 family. Plant ALDH10 enzymes oxidize several aminoaldehydes but only some of them are able to use BAL as substrate. This difference in BAL specificity among the ALDH10s was puzzling given the high structural similarity between BAL and the other aminoaldehydes, and between the ALDH10 proteins. By means of x-ray crystallography, docking, site-directed mutagenesis, and kinetic studies of the enzyme from spinach (*So*BADH) we found that the size of a single amino acid residue is critical for accepting or rejecting BAL as substrate. We also found a perfect correlation between the ability of the plant to accumulate GB and the presence of the appropriate ALDH10 isoenzyme, and that the BADH activity evolved after gene duplication several times during plant evolution. By studying the four *So*BADH variants that could be the evolutionary intermediates, we conclude that the acquisition of the new BADH function occurred without detriment of either the oxidation of other aminoaldehydes or protein stability. Regarding the C4 cycle, we have studied the C4-phosphoenolpyruvate carboxylase isozymes from maize (*Zm*PEPC-C4) and amaranth (*Ah*PEPC-C4), as representative of monocot and dicot plants. Again, we found that a single residue is responsible for the lack of sensitivity of the dicot PEPC-C4 isozymes to the activator glycine, which is the most relevant activator of *Zm*PEPC-C4 under near physiological conditions.

Supported by PAPIIT-UNAM (IN204708 and IN216911) and CONACYT (167122 and 101986) grants.

Hydropatterning: how local moisture controls branching in roots

José Dinneny

Satnford University

Plant development provides a context for the perception of and response to changes in the environment and is also a product of interactions between genes and the environment. Our lab is focused on understanding how developmental parameters provide a regulatory context for controlling the response to water-associated stimuli. Current work is aimed at understanding how plants sense the local availability of water surrounding the root. We have discovered that the primary root positions root branches towards environments with high water content. We have termed this process hydropatterning and have found that similar responses occur in all plant species tested. Our investigation of hydrotropism has revealed the spatial scale with which plants perceive heterogeneity in their environment and implicates polar auxin transport as an important mechanism to control branch angles. I will also present new work on the GLO-Roots system. GLO-Roots (Growth and Luminescence Observatory for Roots) is a system based on rhizotrons that allows us to study root system growth and gene regulation in a soil environment. Using luminescence-based reporter systems we are able to uncover root structures from plants grown under physiologically relevant conditions. GLO-Roots will provide many advantages over tissue-culture based imaging systems and will open up new areas of root research that require a soil environment to study.

Dynamic of reactive oxygen species in root hair cells and pollen tubes are essential for polar growth.

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In plant cells reactive oxygen species (ROS) accumulation have been involved in several processes such as: development, hypersensitive response, hormonal perception, gravitropism and stress response. In guard cells from *Vicia faba* regulates the opening of stomata and more recently in root hair cells from *Arabidopsis* ROS can be observed at the tip region by using fluorescent dyes. This ROS accumulation plays a key role in root hair tip growth and suggested to play a similar role in pollen tubes. Herein we report a new molecular probe to depict the ROS dynamic in living hair cell and pollen tube during apical growth. Hyper is a new generated GFP fused to the OxyR domain that result in a hydrogen peroxide specific probe. This molecular probe was expressed in root hair cells and pollen tubes (1). By using high resolution microscopy we depicted for the first time an apical H_2O_2 gradient at the tip dome where the polar growth occur, furthermore we were able to visualize dynamic ROS oscillations, which are couple to growth changes. In pollen tubes we also found a particular ROS distribution, with clear oscillations couple to growth fluctuations. In both tip growing cells, the apical regions are the site where the more dynamic ROS changes were observed.

1. Hernandez-Barrera, A., Quinto, C., Johnson, E. A., Wu, H. M., Cheung, A. Y., & Cardenas, L. (2013) *Methods Enzymol* **527,** 275-290.

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Food, Fuel and Photosynthesis

Donald Ort

University of Illinois

Feeding the world's current population already requires 15% of the total net primary productivity of the globe's land area and that will need to increase to 25% in order to meet the projected increase in agricultural demand this century. This near doubling of food production will have to be accomplished on globally declining acreage and during a time in which there will be ever increasing demand on cultivated lands for the production of bioenergy crops, while in the face of a changing global environment that has already resulted in decreasing global yield of some of the world's most important food crops. The yield potential of crops is determined by their efficiency of capturing available light energy (ε_i), the efficiency of converting intercepted light into biomass (ε_c), and the proportion of biomass partitioned into grain (**η**). The remarkable yield gains of the Green Revolution in the middle of the 20th century resulted from plant breeders bringing **η** and ε_i for major crops close to their theoretical maxima, leaving improved photosynthetic efficiency as the only yield determinant with sufficient capacity to double crop productivity. Opportunities to improve photosynthetic efficiency exist in readapting photosynthesis to the rapid changes in atmospheric composition and temperature, in redesigning photosynthesis for agricultural production and in applying synthetic biology to bypass evolutionary limitations and inefficiencies in photosynthesis.

Chemical signaling in plant growth promotion by rhizobacteria

José López Bucio

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Gram-negative bacteria produce small molecules to interact with plants. The *Pseudomonas* genus includes many species of plant growth-promoting rhizobacteria (PGPR), but the molecular mechanisms by which these beneficial organisms enhance plant growth and health remain to be clarified. In this study, we performed experiments co-cultivating *Arabidopsis thaliana* seedlings with either *Pseudomonas putida* or *Pseudomonas fluorescens* in order to determine the growth and development responses to these bacteria. Both *P. putida* and *P. fluorescens* stimulated lateral root and root hair formation and increased plant biomass, which correlated with induction of auxin responsive gene expression in roots. Genetic analyses suggest that growth promotion by the bacteria involves auxin signaling as *tir1, tir1afb2afb3, arf7-1, arf19-1 and arf7arf19* auxin-related mutants show altered lateral root response to inoculation and because *P. putida* and *P. fluorescens* normalize root hair development in the *rhd6* mutant. It was found that the bacteria produce the cyclodipeptides cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Tyr) and cyclo(L-Pro-L-Tyr), which were able to induce auxin-responsive gene expression when supplied to the culture media of seedlings. These findings indicate that DKP production by *P. putida* and *P. fluorescens* modulates auxin signaling and likely participates in plant growth promotion.

The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a

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The plant pathogen *Pseudomonas syringae* causes disease in more than 100 plant species using the type III secretion system to secrete and translocate effector proteins into the plant. Many of these effector proteins are believed to function primarily in the suppression of host defense signaling. However recognition of these effector proteins by resistance (R) proteins induces a defense response. The YopJ/HopZ family of effector proteins is evolutionary diverse and found in both animal and plant pathogens. We previously demonstrated that HopZ1a elicits effector-triggered immunity, when it is recognized in *Arabidopsis* by the ZAR1 R protein. However, recognition of HopZ1a does not require any known defense-related proteins. To identify additional genes involved in innate immunity to HopZ1a, we designed a forward genetics screen based on a loss of HopZ1a recognition. We identified several alleles of the *hopz-effector-triggered-immunity-deficient* (*zed1*) mutant. *zed1* is impaired in ZAR1-mediated defense responses but is not affected in the recognition of other unrelated T3SEs or in basal immunity. ZED1 is a previously uncharacterized pseudokinase that is modified by HopZ1a. This work reveals novel genes involved in innate immunity, and additional immune signaling pathways in *Arabidopsis*.

The *Xanthomonas* Cassava Bacterial Blight Pathogen Employs TAL Effectors to Induce a Pectate Lyase and Sugar Transporter During Host Colonization

Rebecca Bart

University of California, Berkeley

We focus on the staple food crop cassava and its pathogen, *Xanthomonas axonopodis* pv. *manihotis* (*Xam*). Bacterial pathogens within the genus *Xanthomonas* deliver type three effectors (T3Es) into the cells of their plant hosts to promote virulence. We use genomic approaches to understand the conservation of T3Es on a pathogen population level. Highly conserved effectors represent ideal targets for disease resistance. In addition, we characterize a specific class of T3Es, the transcription activator-like (TAL) effector family and report a major role for bacterial population growth and symptom development for $TAL14_{Xam668}$ and $TAL20_{Xam668}$, respectively. We identify pathogen-induced transcriptional changes using RNA-seq and identify cassava target genes for each TAL effector. $TAL20_{Xam668}$ specifically induces *MeSWEET10a*, a member of the sugar transporter family of susceptibility genes previously characterized in rice. $TAL14_{Xam668}$ induces a pectate lyase, a novel class of TAL-targeted susceptibility genes. We show that induction of these genes is a highly conserved virulence strategy employed by *Xam* during infection of cassava and propose strategies for durable engineered resistance.

A transcriptomic/metabolomic approach to biochemical pathways in non-model systems

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A large number of plant species are proven to have medicinal or health-protective value. In fact, approximately 25% of contemporary pharmaceuticals are either directly obtained from, or are structurally based upon, natural products. Many potent plant-derived pharmaceuticals have chemically complex structures and contain multiple stereocenters that make commercially feasible syntheses unattainable. Most of these drugs are produced in non-model plant systems and the knowledge of the biochemical pathways that lead to even the most effective plant-derived pharmaceuticals contains gaps at best. In cases where the supply of a plant-derived pharmaceutical is limited and commercially feasible chemical production is precluded by structural complexities, a biotechnological approach is necessary for production of sufficient quantities for patients in need of the drug for treatment. The goal of our research is to elucidate the biochemical pathways that lead to selected potent plant-derived pharmaceuticals and to use this knowledge to develop alternative production systems and novel homologs. We seek to develop methodologies with which to bioinformatically interrogate medicinal plant deep transcriptome datasets to yield candidate biosynthesis genes and then to use biochemistry to link these genes to biochemical pathways that lead to plant-derived pharmaceuticals. A comparison of the expression profiles of genes in deep transcriptome datasets can be compared across species and to the accumulation pattern of selected medicinal metabolites to yield genes that are expected directly involved in the formation of the target drug. Results will be presented from efforts to date to produce deep transcriptome datasets and directed metabolite profiles by LC-MS/MS.

Thevetia thevetiodides seed cardenolides

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Cardenolides, also known as cardiac glycosides, are steroid with hydroxyl (OH) in position 14b. In these tetracycles, the rings C and D are *cis* in contrast with most steroids. In 17β position is linked to a five-membered unsaturated lactone (cardenolides) or six members (bufadienolides). In the 3β position deoxy or O-methyl, sugars are present, that is unusual but characteristic of cardenolides. Cardiac glycosides are present in several plant families; eg. Scrophulariaceae, Ranunculaceaae, Asclepiadaceae, Apocynaceae, and Liliaceae. The cardenolides are the most common derivatives. In tropical America, from a variety of species containing cardenolides highlights the presence of the genus *Thevetia* (Apocynaceae). From extensive trade is known *Thevetia peruviana* synonymous of *Thevetia neriifolia* (Yellow oleander) distributed from northern South America to Florida, the temperate North America. Thevetine, a cardenolide present in this species, found frequent use in Europe, as it is considered particularly useful in cases of intolerance to Digitalis. The toxicity of this plant has been frequently exaggerated. Mesoamerica, despite having a wealth of flora and traditional herbal knowledge, has few species studies on the national pharmacopoeias, paying little attention to the more than 3000 species used traditional herbal medicine in Mexico. *Thevethia thevetioides* (HBK) K. Schum, endemic to Mexico, is distributed in the central and southern states, including Guanajuato, Queretaro, Hidalgo, Michoacan, Mexico, Morelos, Puebla and Guerrero. Nowadays, this attractive tree is rare in the wild at the Bajio, but cultivation is not uncommon in many villages in the region. It is recognized its use in folk medicine against various diseases (Rzedowski and Rzedowski, 1998). This species, although having biotechnological, pharmacological and medicinal value, has not been studied in its phytochemical bioactive components.

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Nothing like we imagined – uncovering the terpenome of liverworts

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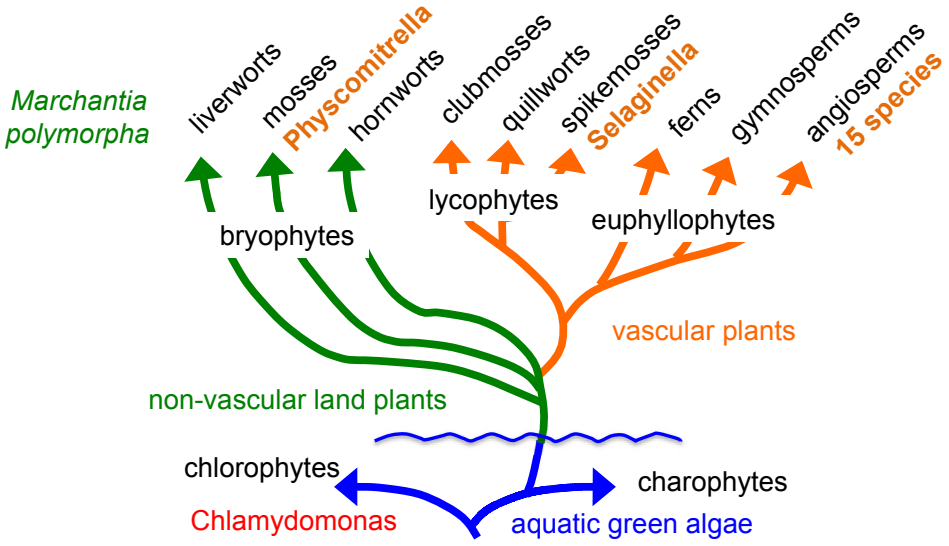
Plants produce a wealth of terpenes that serve physiological and ecological roles, and the basic biosynthetic pathways for many classes of terpenes have been elucidated. These studies have logically led to sophisticated structure-function studies of terpene synthase enzyme families using a variety of molecular genetic and biochemical approaches. Interestingly, these latter studies hold promise for uncovering the biogenic origins of the structural complexity found within each class or family of terpene compounds. An alternative and complimentary approach to uncovering the reaction mechanism(s) specificity for chemical diversity might be evident in evolutionary comparisons between terpene synthases associated with lower plants, like bryophytes, versus higher plants. But this notion is not well founded. The chemical complexity of terpenes in lower plants equals or exceeds that in evolutionary advanced angiosperms and gymnosperms, and attempts to isolate terpene synthase gene homologs from lower plants have largely failed. Using new computational search algorithms, we now report the discovery of evolutionarily distinct terpene synthases that portent new routes to chemical diversification within the terpenome.



Banks et al. 2011 Science 332:960

Molecular and Biochemical Strategies for Cereal Crop Adaptation to Acid Soils

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Aluminum (Al) toxicity is a major limiting factor both for food and bioenergy crops on acid soils that comprise up to 50% of the world's potentially arable lands. A large proportion of the acid soils occur in developing countries in the tropics and subtropics where food and energy security are the most tenuous. Also, there is a significant area of acid soils in the Southeastern U.S., which may be a useful region for the production of bioenergy sorghum. Because of the agronomic importance of crop Al toxicity, identifying the molecular determinants for Al tolerance has attracted significant interest from a number of laboratories around the world. We are now poised, based on recent discoveries by our labs and others, to develop the molecular and genetic resources required to address a worldwide agronomic problem that is only exceeded by drought stress with regards to abiotic limitations to bioenergy and food crop production. In this talk, the isolation of the major sorghum Al tolerance gene, *SbMATE*, via high-resolution mapping has opened up new avenues for improving cereal acid soil tolerance. The role of this gene in controlling the wide range of Al tolerance in sorghum via regulation of SbMATE function and expression will be described. The combination of genetics, genomics and protein biochemistry has shown us that other molecular determinants reside in the sorghum genome that help regulate both *SbMATE* expression and SbMATE protein function, resulting in greater levels of Al tolerance. This research is allowing us to assemble a molecular toolbox that is being used to translate these discoveries into more Al tolerant sorghum lines for production on acid soils both in Brazil and in developing countries in sub-Saharan Africa.

Bixin synthesis and carotenoid gene expression in *Bixa orellana* L.

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Annatto (*Bixa orellana*) is a tropical shrub from the intertropical regions of the Americas. *B. orellana* is rich in carotenoids, principally the pigment bixin. Synthesis of this pigment has become the focus of studies by a number of research groups. Knowledge of the limiting steps in carotenoid biosynthesis, as well as factors relating to its storage and its relation with other biosynthetic pathways are significant areas under study. The aim of this study was to analyse the expression of the key genes (based on complementary DNA) involved in carotenoid and bixin synthesis, such as phytoene desaturase (*pds*), lycopene beta-cyclase (*βlcy*) and lycopene epsilon-cyclase (*εlcy*), during the different stages of development of the plant organs which present greatest bixin accumulation, such as the leaves, buds, flowers and seeds in different stages of maturation taken from four *B. orellana* varieties (P65, P13, N4, N20) with contrasting characteristics. Expression of these genes was analysed by the real-time RT-PCR technique. The results of this research suggest that plants with pink flowers (P65 and N4) have greater expression of the *pds* gene and plants with white flowers have greater expression of the *βlcy* gene. The level of *pds* and *βlcy* gene expression increased during the seed maturation stage, but decreased in mature stages. Plant N4 exhibited the greatest bixin accumulation. Maximum bixin accumulation occurred in the early stages of seed maturation, and was reduced by almost 50% in mature stages. The level of *pds* gene expression showed a 53% correlation with bixin accumulation, whilst the correlation for *βlcy* gene expression was just 22%. Regulatory mechanisms for carotene production and accumulation were observed to be specific to each tissue and, in some cases, specific to each plant. This explains why regulatory events found in the specific organ of a plant often cannot be confirmed in others.

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Testing the role of well-known molecular mechanisms involved in metal uptake, translocation and homeostasis in *Agave*.

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Agave is a large monocot genus of succulent plants capable of growing in stressful environments and in different types of soils. Using transcriptomic data from sequenced cDNAs, we previously found that several tissues of *A. tequilana* accumulate high levels of genes putatively related to tolerance to biotic and abiotic stress. Several physiological assays have shown that these genes are constitutively expressed in *Agave*, including a gene family of metallothioneins, proteins capable of binding divalent cations and known to confer tolerance to toxic metals. Three different Agave species were challenged with high or low metal concentrations using hydroponic conditions. We found that agaves possess extremely high tolerance to metal ions such as Cu, Zn, or Cd, such that they can grow under high metal concentrations and accumulate high quantities of these metals in aerial tissues. In other hand, these plants are capable of growing in the absence of iron without any symptoms of low iron status. We also tested some biochemical responses and the regulation of agave homologs of some of the most important genes involved in metal ion homeostasis in plants, including several ion transporters such as NRAMPs, ZIPs and OPTs, along with metallothioneins and other genes involved in stress tolerance. The possible role of well-known mechanisms of uptake, translocation to aerial tissues, and homeostasis of metal ions in *Agave* plants, which display an overall physiology extremely different from other well-studied plant species, will be discussed.

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Pseudomonas putida and *Pseudomonas fluorescens* regulate *Arabidopsis* root architecture through an auxin mediated pathway and produce bioactive cyclodipeptides

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Gram-negative bacteria produce small molecules to interact with plants. The *Pseudomonas* genus includes many species of plant growth-promoting rhizobacteria (PGPR), but the molecular mechanisms by which these beneficial organisms enhance plant growth and health remain to be clarified. In this study, we performed experiments co-cultivating *Arabidopsis thaliana* seedlings with either *Pseudomonas putida* or *Pseudomonas fluorescens* in order to determine the growth and development responses to these bacteria. Both *P. putida* and *P. fluorescens* stimulated lateral root and root hair formation and increased plant biomass, which correlated with induction of auxin responsive gene expression in roots. Genetic analyses suggest that growth promotion by the bacteria involves auxin signaling as *tir1, tir1afb2afb3, arf7-1, arf19-1 and arf7arf19* auxin-related mutants show altered lateral root response to inoculation and because *P. putida* and *P. fluorescens* normalize root hair development in the *rhd6* mutant. It was found that the bacteria produce the cyclodipeptides cyclo(L-Pro-L-Val), cyclo(L-Pro-L-Tyr) and cyclo(L-Pro-L-Tyr), which were able to induce auxin-responsive gene expression when supplied to the culture media of seedlings. These findings indicate that DKP production by *P. putida* and *P. fluorescens* modulates auxin signaling and likely participates in plant growth promotion.

Analysis of nodulin 41 expression in early stages of symbiosis and cellular localization in transgenic root nodules

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Nodulin 41 (PvNod41) is an aspartyl peptidase from *Phaseolous vulgaris.* It is expressed in mature root nodules and is considered a late nodulin. By western blot assays, we detected this protein in root nodules from 12 to 30 days post infection (dpi) with *Rhizobium tropici*, but not in 10 dpi root nodules, nodule-stripped roots, or uninoculated roots. However, *PvNod41* transcripts were also detected by RT-qPCR in 10 dpi root nodules and at a much lower level in nodule-stripped roots (Olivares *et al. BMC Plant Biology* 2011, 11:134). In order to determine more accurately the expression pattern of the *PvNod41* gene, a 1074 bp fragment of its promoter region was cloned and the eGFP-GUS fusion protein was put under its control. In common bean transgenic roots inoculated with *R. tropici*, we detected GFP and GUS expression in root tips and nodule primordia. This finding prompted us to see if inoculation with *R. tropici* is necessary for the promoter activation in root tips. In this case, we detected GFP expression only in 3 dpi root tips and not in 3 days uninoculated root tips. Additionally, *PvNod41* transcript accumulation levels were determined by RT-qPCR in wild type root tips. *PvNod41* transcripts were detected in 3 dpi root tips but not in 3 days uninoculated root tips. In spite of these results, we have been unable to detect the PvNod41 protein by western blot in root tips. This could indicate that *PvNod41* is under a strong post-transcriptional regulation in this tissue. By the other hand, we are interested in determining the subcellular localization of PvNod41. For this purpose, we compared, by fluorescence microscopy, the expression of PvNod41-eGFP under the 35S promoter and also under its own promoter in transgenic root nodules. These data will be presented and discussed.

This work was funded by CONACYT 177744

A salicylic acid-induced lectin-like protein plays a positive role in the effector-triggered immunity response in *Arabidopsis thaliana* to *Pseudomonas syringae* AVR-RPM1.

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Salicylic acid (SA) is one of the key hormones that orchestrate the pathogen induced immune response in plants. Here, we report the identification and functional characterization of a SA-induced legume lectin-like protein 1 (SAI-LLP1), which is coded by a gene that belongs to the group of early SA-activated *Arabidopsis* genes. We studied the role of *SAI-LLP1* gene in the defense response of *Arabidopsis* against *Pseudomonas* strains. SAI-LLP1 expression is induced upon inoculation with avirulent strains of *Pseudomonas syringae pv tomato* (*Pst*), via a SA-dependent mechanism. Constitutive expression of *SAI-LLP1* restrains proliferation of *Pst* Avr-Rpm1 and triggers more cell death in inoculated leaves. Using confocal microscopy and biochemical assays, we found evidence indicating that SAI-LLP1 is a glycoprotein located primarily at the apoplastic side of the plasma membrane. Results obtained in this work indicate that SAI-LLP1 is involved in resistance to *Pst* Avr-Rpm1, playing a positive role in the effector-triggered immunity triggered by *Pst* Avr-Rpm1 in *Arabidopsis*.

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The *Arabidopsis thaliana* peroxidase expression during *Ustilago maydis* infection

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Ustilago maydis Dc (Cda) is a pathogenic fungus that infects only maize (*Zea mays* L.). However, there is conclusive evidence that *U. maydis* infects other monocotyledons and dicotyledons plants under experimental axenic conditions (Leon-Ramírez *et al.,* 2004). The inoculation with mixtures of sexually compatible or single *U. maydis* haploid strains produced similar symptoms in *Arabidopsis thaliana* plantlets, the signs of disease include the increased anthocyanin formation, development of chlorosis, increased formation of secondary roots, induction of malformations in the leaves and petioles, induction of tissue necrosis, and stunting (Mendez-Moran *et al.,* 2005). From previous results of gene expression analyzed with microarrays, some representative genes related with the antioxidant defense system were obtained; principally the peroxidases involved in plant/fungal response. We analyzed the expression of peroxidases genes in *A. thaliana* plants. Total RNA was isolated from *A. thaliana* at different times post inoculation with *U. maydis* haploid cells. The gene expression was analyzed with sqRT-PCR. The peroxidases genes showed differential expression in at least one of the sampling times, and noticeably up-regulated and down-regulated expression in the infected plants were obtained. The oxidative response was represented with highly induced expression of disease resistance proteins and pathogenesis-related protein. Now we are related the *A. thaliana* preoxidases expression with *POX12* peroxidase gene from maize. It is concluded that the *A. thaliana*-*U. maydis* pathosystem offers new alternatives to study plant-fungal interactions. The project was supported by grants from CONACYT-CB 2007-00079530 and the Universidad de Guadalajara, Mexico.

1. León-Ramirez *et al.* New Phytologyst: 2004, 164:337-346.
2. Mendez-Moran *et al*. Phytopathology: 2005, 95(5):480-488.

Interactions between two unrelated RNA viruses and their host: a case of classic synergism and contrasting viral antagonism.

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Synergism in plants is the classic example of potex-potyvirus interaction and has been reported in different hosts. Antagonism has been described only between phylogenetic related viruses. Our studies reveal that two nonrelated viruses: *Papaya ringspot virus* (PRSV), a potyvirus; and *Papaya mosaic virus* (PapMV), a potexvirus produce a contrasting phenotype on its natural host, *Carica papaya.* The outcome of the disease depends on the order of arrival and infection time to their host. This determines the development of symptoms: a synergistic (detrimental) or an antagonistic (beneficial) response. When the host was simultaneously inoculated with both viruses, a synergistic phenotype was observed concomitant with a 1.5-fold increase PapMV accumulation, as compared to its single infection, however its translation rate remains unaffected. When PRSV is firstly inoculated, PapMV is able to 5-fold increase their transcripts but its translation decreases and the phenotype is also synergistic. Our polysome profiling suggests that, PRSV VPg hijacks some initiation translation factors that prevent the association of genomic PapMV with the cellular machinery and the massive PapMV RNA viral accumulation is producing the detrimental phenotype. Unexpectedly, when PapMV is the primary infecting virus, the PRSV phenotype and its coat protein cannot longer be detected and the potyviral genome accumulation decreases until 0.1-fold. We estimated that PapMV moves faster and produces more transcripts than PRSV, but PRSV is more efficient on its translation and produces more protein per RNA molecule. Also, when PapMV arrives first, PRSV is incapable of produce transcripts and consequently protein. This attenuation over PRSV is probably not mediated by iRNA silencing, because there is no viral sequence similarity between these viruses to turn on the Post-translational Gene Silencing (PTGS) machinery, instead, a prolonged production of Systemic Acquired Resistance (SAR) was detected by the induction of the Pathogenesis-related protein 1 (PR1) gene.

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Response of the promoter of a phytoalexin biosynthetic gene from pepper to virus, insects and parasitic plants

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The promoter of the *PEAS*1 gene of pepper (*Capsicum annuum*) controls the expression of the 5-*epi*-aristolochene synthase enzyme, involved in the biosynthesis of the bicyclic sesquiterpene phytoalexin capsidiol. This 1450 bp promoter responds to various biotic stimuli like arachidonic acid, cellulase and other pathogen-associated molecular patterns (PAMP's). Here we analyzed the response of this promoter to TEV (Tobacco Etch Virus), whitefly (*Bemisia tabaci*) and dodder (*Cuscuta* sp). The analysis was done by GUS staining in tobacco transgenic plants (*Nicotiana tabacum* var. *Xhanti*) containing the GUS reporter gene under control of the PEAS1 gene promoter. Our results showed that GUS gene expression was induced in the glands of the trichomes of leaves and stems after TEV virus infection, and during oviposition of eggs after whitefly infestation but the interaction with dodder didn't induce the expression of GUS gene.

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Characterization of histone H3 family from *Capsicum annuum* and differential expression in response to *Pepper golden mosaic virus* (PepGMV) infection.

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Geminiviruses are DNA viruses that infect economically important plants. The genomes of these viruses interact with the host's histones to organize a minichromosome, structure responsible for regulating viral replication and transcription. A previous study of the transcriptome of pepper (*Capsicum annuum*) suggested a preferential expression of histone H3 (and/or variants) during infection with *Pepper golden mosaic virus* (PepGMV). First, we performed an analysis of the genome of *C. annuum* and found 14 genes highly related to histone H3 (H3 family, canonical H3 and variants). Secondly, we quantified by real-time PCR the expression of the canonical H3 and two variants, H3.3 and H3.X. No clear differential expression was observed in either case. Then, we measured the transcription of these 3 genes in leaves from different stages that are present in a plant 24 days after inoculation. In this experiment we observed that in mature leaves, the expression of histone H3 and H3.X was eight times higher in infected plants than that observed in not infected control plants. Possible implications of this differential expression will be discussed.

Phosphoproteomic analysis of *Phaseolus vulgaris* roots during the early stages of the rhizobia-legume symbiosis

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Legumes possess the unique ability to form symbiosis with a family of gram-negative soil bacteria known as rhizobia to acquire fixed nitrogen. This interaction is preceded by a molecular dialog between the host and bacterium that takes place within the rhizosphere. Rhizobia secrete lipochitooligosaccharides, known as Nod Factors (NF), which triggers molecular and physiological changes within the root. Specific protein phosphorylation events are known to be critical for the initiation of rhizobial infection and during nodulation (1, 2). Plant mutants defective in nodulation led to the identification of key protein kinases essential for both processes (3). Herein, the phosphoproteome of *Phaseolus vulgaris* roots treated with *Rhizobium etli* NF at 10, 30 and 60 min, was analyzed. To this end, phosphoproteins from total protein extracts obtained from *P. vulgaris* root*s* treated with NF were purified by IMAC$Fe^{+}3$ affinity column. The phosphoproteins were then isolated by two-dimensional gel electrophoresis and identified by mass spectrometry. Thirty-three phosphoproteins were obtained using this approach. Among these, twenty-one were found to be upregulated (>1.3 fold) in response to NF, including actin, actin depolymerizing factor-2, pathogenesis-related protein 1, chalcone isomerase, ascorbate peroxidase, peroxidase, superoxide dismutase and chaperones among others. To do a semi--quantitative analysis of the phosphorilation of each protein, a western-blot assays using anti-serine, anti-threonine and anti-tyrosine antibodies is in progress.

1. Madsen, et al., 2003, *Nature* 425, 637; 2. Libault, et al., 2010, *Plant J.* 63, 86; 3. Radutoui, et al., 2003, *Nature* 425, 585.

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Characterization of genes encoding potential effector of *Trichoderma* spp. differentially expressed in interaction with *Arabidopsis thaliana*

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Among species that commonly inhabit the soil are *Trichoderma* species; the genus includes filamentous fungi used as biocontrol agents. Some species has the ability to activate both induced systemic resistance (ISR) and systemic acquired resistance (SAR) in plants, probably mediated by Microbe-associated molecular patterns. Some of them are effector-like proteins, which have the capacity to modify host-cell structure and function. These alterations either facilitate infection or trigger defense responses. Little is known about the function of these proteins in the establishment of beneficial interactions, mainly in mycorrhizal. In *Trichoderma spp.* nine molecules with effector characteristics has been proposed, but only one has been characterized as effector-like protein: SM1, which is implicated in the establishment of plant-fungus interactions, activating SAR and ISR mechanisms in cotton plants. Nowadays, our work group is searching for novel effector-like proteins in *Trichoderma* species interacting with the plant *A. thaliana*. By using bioinformatics tools, we have selected 21 genes that encode for possible effector-like proteins. We have found at least 4 genes up regulated, and at least 1 gene down regulated when the fungus is co-cultivated with the plant. Additionally, we are analyzing their expression in fungal cultures added with root exudates. We will confirm these results by Q-PCR in order to generate null *Trichoderma* mutants on these effector-like proteins and evaluate their participation during the *Trichoderma-Arabidopsis* beneficial interaction.

Overexpression of a *Phaseolus vulgaris* NADPH oxidase gene increases symbiosome number, bacteroid size and nitrogen fixation in nodules and impairs mycorrhizal colonization

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RBOHs (Respiratory Burst Oxidase Homologs) are plant membrane proteins that catalyze oxygen reduction to produce superoxide, a form of reactive oxygen species (ROS). ROS generation by RBOHs activity is essential in diverse plant-signalling processes; their role in symbiotic associations is poorly understood. This prompted us to explore the role of RBOHs in the *Phaseolus vulgaris-Rhizobium* and -AM symbiosis. Herein, the role of *RbohB* during the symbiotic interaction between *P. vulgaris* and *Rhizobium tropici*, and *P. vulgaris* and *Rhizophagus irregularis* was assessed by over-expression using a hairy root system. The results obtained indicate that hairy roots overexpresing *PvRbohB* transcripts increased levels of superoxide accumulation, infection threads (ITs), nodule biomass and nitrogenase activity significantly. Ultrastructure of these nodules show packed symbiosomes, enhanced bacteroid number and size per symbiosome. Expression levels of *CAT*, early nodulins, *SS1* and *GOGAT* transcripts were also elevated in nodules. On the other hand, when mycorrhized with *R. irregularis*, *PvRbohB*-OE roots, displayed a 'reduced mycorrhizal colonization phenotype'. Thus, we concluded that *PvRbohB*-OE augmented nodule efficiency by fixing more nitrogen and regulates a brief delay in nodule senescence but hampered mycorrhizal colonization in *P. vulgaris*.

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Overexpression of a novel ethylene response factor gene *AhERF* of *Amaranthus hypochondriacus* as a strategy to confer dual resistance to water stress and bacterial infection in transgenic *Arabidopsis* plants

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Amaranthus hypochondriacus is a C4 dicot plant used by Mesoamerican farmers, noted by its ability to tolerate stressful conditions and produce highly nutritious seeds. Recently, in an attempt to understand the stress response of amaranth, our research group performed the transcriptomic analysis of grain amaranth (*A. hypochondriacus*). Approximately 1900 genes increased their expression in response to at least one of four stress treatments tested (water stress, salinity, bacterial infection and insect herbivory). The analysis of the function of multistress genes is essential for the understanding of the molecular mechanisms underlying physiological tolerance to several types of stress and consequently it is a biotechnological strategy to generate better crops through targeted genetic manipulation. The ERF proteins (**E**thylene **R**esponse **F**actors) are plant-specific transcription factors that play essential roles in stress responses. However, almost no information regarding stress-related ERF genes is available in amaranth. Expression analysis by qRT-PCR revealed that *AhERF* was strongly induced by water stress and bacterial infection (10- and 8-fold higher, respectively), followed by methyl jasmonate treatment, insect herbivory and mechanical damage. Sequence analysis showed that *AhERF* is a novel transcription factor that has an open reading frame of 1,022 bp and encodes a nuclear protein of 254 amino acids. We cloned the full length gene and right now, we are developing transgenic *Arabidopsis* plant that constitutively express *AhERF*. We hypothesize that the overexpression of novel transcription factors from amaranth will alleviate water stress and bacterial infection in transgenic *Arabidopsis* plants.

Characterization of the entire family of HSF in *Carica papaya* and expression analysis of 6 of those genes, in response to heat stress and during recovery

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Heat shock factors (HSF), are transcription factors that have been associated to plant response to environmental changes, particularly to heat stress. We characterized *in silico*, the entire family of 18 HSF genes in *Carica papaya.* Primers were then designed for six CpHSF genes, representing the three groups within the family. Using RT-PCR, changes in the expression of the 6 genes were evaluated when plants were exposed to temperatures as high as 50 ^{o}C, for as long as 4 h. In addition, we evaluated changes in the expression of those genes during a recovery period, when plants were returned to standard temperatures of 25 ^{o}C. Two of those genes were particularly responsive to those temperature changes and they might be associated to the high heat tolerance shown by this tropical species. It is important to emphasize that CpHsfB1 increased their expression during the heat stress itself, while CpHsfA1 increased their expression only during the recovery period, what might be associated with the triggering of repair mechanisms. It is possible that the over-expression of those heat-responsive genes in this and other species, might result in increased tolerance to heat, what is particularly relevant to minimize possible negative effects associated to climate change and global warming.

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Mutations in *AtFBS* genes alter the response to abiotic stress in *Arabidopsis thaliana*

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AtFBS1-4 genes encode proteins with an F box. Such proteins are essential components of ubiquitin ligases called SCF. Previous studies with three of these genes show that only *AtFBS1* and *AtFBS3* transcripts increase their levels in response to different biotic and abiotic stresses. To understand the role of AtFBS proteins in stress responses in plants, the analysis of mutants in the corresponding genes was carried out; for this purpose, mutants in each of these genes have been obtained from previously existing collections and double, triple and quadruple mutants have been generated. The single and double mutants have no observable phenotype either in normal or in plants subjected to some type of stress. So far three triples mutants and the quadruple mutant were obtained. The triple mutant 3M1 (*atfbs2, atfbs3, atfbs4*) and the quadruple 4MU (*atfbs1, atfbs2, atfbs3, atfbs4*) show low germination rate, a reduction in the length of roots and poor growth under osmotic or saline stress, or the treatment with abscisic acid. Under drought stress both mutants had a higher percentage of dehydration with respect to the wild-type Col1.

AtGRDP1 gene encoding a glycine-rich domain protein, a new component of the ABA signaling pathway?

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The sessile life style of plants has led to the development of mechanisms by which to increase their tolerance of these through both physical adaptations and interactive molecular and cellular changes that begin after the onset of stress. The first step in switching on such molecular responses is to perceive the stress as it occurs and to relay information about it through a signal transduction pathway. The plant hormone abscisic acid (ABA) plays a key role in a variety of developmental processes and adaptive stress responses to environmental stimuli in plants. Nearly 10% of the protein coding-genes in Arabidopsis are likely to be regulated by ABA. In this study, we show interesting data about a novel gene encoding a glycine-rich domain protein, called AtGRDP1. The *Atgrdp1*-null mutant line showed an increased sensitivity to salt and osmotic stress in germination and cotyledon development, whereas 35S::*AtGRDP1* over-expressing lines resulted in increased tolerance to abiotic stress. Interestingly, 35S::*AtGRDP1* over-expressing lines showed resistance to ABA, resembling a well-known ABI phenotype, whereas the disruption of *AtGRDP1* gene resulted in ABA hypersensitivity, mimicking the *ABI3*-overexpression phenotype. Furthermore, we analysed the *ABI3* and *ABI5* genes, which are central regulators in ABA signalling, in *Atgrdp1*-null mutant and 35S::*AtGRDP1* over-expressing lines. Under ABA treatments, *Atgrdp1*-null mutant seedlings showed higher *ABI3* and *ABI5* transcript levels, whereas in 35S::*AtGRDP1* over-expressing line, the *ABI3* and *ABI5* transcripts were repressed. Analysis of *WRKY2* expression levels in 3*5S::AtGRDP1* over-expressing line further indicated that ABA-induced *WRKY2* accumulation correlates with the expression patterns of *ABI3* and *ABI5* genes. These results suggest that *AtGRDP1* gene plays a regulatory role in ABA signalling and tolerance to abiotic stress.

AtLEA-1 and *AtLEA-2* are involved in stomata patterning and water stress tolerance in *Arabidopsis thaliana*

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Water is an essential element for growth and development of plants, but in recent years, the droughts have become longer and more extreme Worldwide, causing irreversible damage to agriculture. That is why it has been paid special attention to LEA (Late embryogenesis Abundant) proteins since it has been found that are associated with abiotic stress tolerance, the accumulation of these proteins occurs during seed maturation. In a screening for LEA protein in *Arabidopsis* genome we identified two LEA type genes, *AtLEA-1* and *AtLEA-2*. In order to analyze the function of these, we worked with T-DNA lines (*AtLEA-salk1* and *AtLEA-salk2*), which have the insertion in the regulatory region of each of the genes. Tests were conducted to analyze the relation with water stress tolerance. We found that insertional lines are more sensitive to water stress. Furthermore, phenotypic analyzes were done and interestingly the stomatal density is also affected. We analyzed the function of *AtLEA-1* and *AtLEA-2* genes and its role in development, by crosses with stomata development markers. To elucidate the expression pattern of *AtLEA-1* and *AtLEA-2* genes we are generating a construction containing the fusion of the putative promoter region with the GUS reporter gene. Additional results will be show during the congress.

The relationship of drought tolerance to the hydrotropic response of maize and Arabidopsis roots

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While water shortage remains the single-most important factor influencing world agriculture, there are very few studies on how plants grow in response to water potential, i.e., hydrotropism. Terrestrial plant roots dwell in the soil, and their ability to grow and explore underground requires many sensors for stimuli like gravity, humidity gradients, light gradients, mechanical stimulations, temperature, oxygen, etc. To date, extremely limited information is available on the components of such sensors; however all of these stimuli are sensed in the root cap. Directional growth of roots is controlled by gravity, which is fixed in direction and intensity. However, other environmental factors, such as water potential gradients, which fluctuate in time, space, direction, and intensity, can act as a signal for modifying the direction of root growth accordingly. Hydrotropism may help roots to obtain water from the soil and at the same time may participate in the establishment of the root system. Current genetic analysis of hydrotropism in *Arabidopsis* has offered some players, mainly *AHR1, AHR2*, *NHR1, MIZ1*, and *MIZ2*, which apparently control how root caps sense and respond hydrotropically. We will discuss the mechanism(s) by which these genes and those that regulated phototropism coordinate the root hydrotropic response. We hypothesized that some aspects of water stress avoidance have evolved by natural selection of root tropic responses, most probably hydrotropism and phototropism. For testing this hypothesis, we are also using crop plants such as maize to examine root hydrotropic response and their growth responses to drought in the field conditions in a research program for encouraging agricultural diversity and sustainability in Mexico. We think that it should be a priority of plant scientists to use their creativity for the implementation of sustainable agriculture since the global agricultural sector will need 19% more water by 2050 to meet a 70% increase in demand for food (Hoekstra and Mekonnen, 2012).

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Changes in the environmental conditions induce structural order in intrinsically unstructured stress proteins from plant

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Late Embryogenesis Abundant (LEA) proteins are a broadly distributed group involved in plant tolerance to water deficit. Most of them belong to the hydrophilins because of their high hydrophilicity and content in small amino acids. Hydrophilins, including LEA proteins, are predicted to be part of a wider group of proteins known as intrinsically disordered proteins (IDPs). By partial dehydration and freeze-thaw *in vitro* assays, it has been shown that some LEA proteins are able to protect other proteins from the effects of water limitation and it was suggested that this might occur by protein-protein interactions. Some other assays suggest their interaction with nucleic acids. Given the unstructured character of LEA proteins, we propose that their flexible nature plays a critical role in the interaction with their partners, allowing them to interact with diverse proteins. We also have considered that this structural flexibility might be modulated by the cell water status, thus promoting selection of specific conformations needed to interact with selected targets depending on the condition. To get insights into their structure and its relation to their function, we have characterized the structural properties of two plant LEA proteins from two different groups: Arabidopsis AtLEA4-5 (groups 4) and *Phaseolus vulgaris* PvLEA6 (group 6). We showed that both are intrinsically unstructured in solution over a wide range of temperatures; however, structure inducers are able to promote secondary structure, mostly a-helix. A decrease in water availability was also able to promote structural order in both LEA proteins. Likewise, conditions inducing molecular crowding led to conformational changes, with a stronger effect on AtLEA4-5. We propose that structural flexibility in these proteins might be involved in the interaction to partners as a requirement for their function during water deficit. Our results suggest that AtLEA4-5 protein might be protecting native proteins from the deleterious effect caused by water limitation and, by contrast, PvLEA6 protein could be acting as RNA chaperone.

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Intracellular localization of the inorganic soluble pyrophosphatase isoforms 5 and 6 in *Arabidopsis thaliana*

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Inorganic pyrophosphate (PPi) is a byproduct of the biosynthesis of carbohydrates, proteins, nucleic acids and some lipids (1). In plants, PPi accumulates in the cytosol, its concentration hardly changes under abiotic stress conditions, and previews reports have given evidence of a strong link between PPi concentration and carbon partitioning (2). Several Mg^{2+}-dependent soluble inorganic pyrophosphatase (PPa) isoforms are present in plant cells, and in *Arabidopsis thaliana* six isoforms are expressed (AtPPa1 to AtPPa6). Transgenic plants expressing AtPPa1 to Atppa5 GFP fusions localized these 5 proteins in the cytosol, suggesting strong redundancy. However, T-DNA insertion mutants lacking isoforms AtPPa2, AtPPa4 and AtPPa5 showed changes in phenotype and each one had a different tolerance to specific types of abiotic stress. The aim of this work was to study the intracellular localization of the AtPPa6 isoform, which has yet to be demonstrated *in vivo*. Transgenic homozygous plants of *A. thaliana* plants expressing an AtPPa6-GFP fusion were selected and confocal microscopy revealed this protein inside the chloroplast. In contrast, the AtPPa5-YFP transgenic present a clear cytosolic distribution pattern. The chromatographic profile of the PPa activity of these plants was compared to the wild type, and surprisingly, all activity peaks showed differential changes in both transgenic plants. Using anti-GFP antibodies allowed immunoprecipitation of these fusion proteins isoforms present in the transgenic plants a differential pattern of bands in SDS-PAGE gels was observed for AtPPa5-YFP and AtPPa6-GFP, not seen in the transgenic expressing an unfused GFP. The data taken together suggest a fine regulation of the expression and the activity of these proteins, possibly through the interaction with other proteins. The identification of these putative AtPPa interactors is in progress.

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Involvement of ABA in salt stress tolerance in the moss *Bryum billarderi*

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Salt stress is a very severe abiotic stress, which affects at least 20 % of cultivated land for irrigation around the world. On the other hand, the plant hormone abscisic acid (ABA) is known as the stress hormone, since in higher plants it increases the adaptation to stresses like low temperatures, UV radiation, pathogens, salinity and water deficit. In this regard, the aim of this work was to characterize a Mexican non-vascular plant tolerant to abiotic stress and determine the possible participation of ABA. To assess the involvement of ABA in the responses to saline stress in non-vascular plants, we used *Bryum billarderi* moss. Protonemal tissues were exposed to different NaCl concentrations with and without an ABA pre-treatment. We performed a photographic record and quantification of photosynthetic efficiency during and after stress conditions. Under salt stress conditions, photosynthetic efficiency decreased at 300 mM NaCl, and was not detectable under higher concentrations. Under recovery conditions (after salt stress), the ABA pre-treated protonematas showed green phenotypes compared to the chlorotic non-pretreated ones, and photosynthetic efficiency reached normal values after 20 days. Additionally quantification of some metabolites supported the salt tolerance during the stress, The differences observed at the phenotypic level, metabolic and photosynthetic efficiency showed that a ABA pre-treatment (10 □ M for 24 h) increased the capacity of the moss *B. billarderi* to tolerate high NaCl conditions, which shows a clear involvement of this phytohormone in the salt stress tolerance in this moss.

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Proline accumulation and ion flux in the roots of two varieties of habanero pepper (*C. chinense* Jacq.) with different tolerance to NaCl

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Accumulation of compatible solutes (eg. proline) in plants and mitigation K^+ efflux are essential for maintaining the water content and K^+ homeostasis in the cell to high concentrations of NaCl[1]. Recently, it has suggested a link between the two mechanisms of tolerance in abiotic stress conditions[1,2]. It has been observed that exogenous addition of compatible solutes reduced NaCl-induced K^+ efflux in the roots[1]. In this study we evaluate the proline accumulation and net fluxes of K^+ and H^+ in two varieties of *Capsicum chinense* which differ in stress tolerance by NaCl; Rex, tolerant variety, and Chichen-Itza (Seminis ®), sensitive variety. The experiment was performed in hydroponic conditions, using 0-150 mM of NaCl as control and treatments, respectively. Also, using the non-invasive microelectrode ion flux (MIFE) measuring technique[3], net fluxes of K^+ and H^+ were measured from NaCl-stressed roots. The proline increased up to 50 times in the roots of tolerant variety (Rex), when was treated with 150 mM of NaCl, compared with the sensitive variety (Chichen-Itza). Also, in the tolerant variety the NaCl-induced K^+ and H^+ efflux was smaller than in sensitive variety. These results suggest a possible link between the proline accumulation and even a role in the regulation of ion transport systems at stress conditions NaCl.

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New players in CLAVATA signaling control shoot meristem size and yield in maize

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Shoot growth depends upon meristems, pools of stem cells that are maintained by a negative feedback loop between the *CLAVATA* pathway and the *WUSCHEL* homeobox gene. CLAVATA signaling involves a secreted peptide, CLAVATA3 (CLV3), and its perception by cell surface leucine-rich repeat (LRR) receptors, including the CLV1 receptor kinase, and an LRR receptor-like protein, CLV2, however the signaling mechanisms operating downstream of these receptors are not fully understood. We isolated the maize *COMPACT PLANT2* (*CT2*) gene, and it encodes the predicted α subunit (Gα) of a heterotrimeric GTP binding protein. *ct2* mutants have CLAVATA-like meristem proliferation phenotypes, and genetic, biochemical and functional assays indicate that CT2/Gα signaling transmits a stem cell restrictive signal from a maize CLAVATA LRR receptor, suggesting a new function for Gα signaling in plants. Heterotrimeric GTP-binding proteins are membrane-associated molecular switches that are commonly activated by ligand binding to an associated 7-pass trans-membrane (7TM) G-protein-coupled receptor (GPCR). Recent studies have questioned the idea that plant heterotrimeric G proteins interact with canonical GPCRs, and our findings suggest that single pass TM receptors act as GPCRs in plants, challenging the dogma that GPCRs are exclusively 7TM proteins. We have also identified new regulators of maize shoot meristem size, *fea3* and *fea4*. These genes will be discussed, as well as their potential role in improvement of maize yields.

On the (epi)genetic control of apomixis: learning from sexual experience.

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Each year plants and animals throw themselves in the most enthusiastic task of repopulating the planet through patterns of courtship and mating that have a unifying and compelling logic: all have evolved to produce offspring. Considering that life of nearly all organisms is organized around sex and breeding, Darwinian thinking has focused more on the struggle for existence than on evolutionary significance of this frantic race to reproduce. Since sexually-derived genetic diversity is essential for the production of offspring, it is often thought that sex is necessary for the perpetuation of a species; however, many organisms are going efficiently about propagating their kind without bothering with meiosis and mating. We have recently found that the regulation of female gametogenesis and seed formation is directed by epigenetic mechanisms that are crucial to control events that distinguish sexuality from apomixis. The PIWI/PAZ domain protein ARGONAUTE9 (AGO9) defines a new regulatory pathway that acts in the ovule of *Arabidopsis thaliana* to restrict the specification of gamete precursors in a non-cell autonomous manner. Mutations in *AGO9* are dominant, and cause the formation of ectopic gametic cells that often differentiate into viable unreduced female gametophytes. AGO9 preferentially interacts with 24 nucleotide (nt) small RNAs (sRNAs) derived from transposable elements (TEs), and its somatic activity is necessary to silence TEs in female gametes. Its expression is necessary to inactivate a significant proportion of long terminal repeat retrotransposons (LTRs) in the ovule, and its predominant TE targets are located in the pericentromeric regions of all 5 chromosomes, suggesting a link between the AGO9-dependent sRNA pathway and heterochromatin formation. Our results suggest a causative link between epigenetic regulation and the natural reproductive versatility found in flowering plants, with important implications for our understanding of the evolutionary forces that shape structural variation and diversity in plant reproduction.

Target of rapamycin is required for root growth and nodule development in *Phaseolus vulgaris* L.

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Target of rapamycin (TOR), a serine/threonine protein kinase is known to function as a sensor of nutritional and cellular energy and a regulator of cell growth. An *Arabidopsis TOR* mutant is embryo lethal and affects plant growth, implicating TOR in an essential role during plant growth and development. Legumes form symbiotic interactions with rhizobial bacteria that lead to the formation of nodules on the roots of the host plant. The nodule morphogenesis involves cortical cell division and differentiation; subsequently rhizobia invade into plant membrane-enclosed compartments (symbiosome) via a tubular structure called the infection thread (IT). Within symbiosomes, the differentiated rhizobia fix nitrogen, to be utilized by the host plant. Here, the involvement of TOR in the *P. vulgaris*-*Rhizobium* symbiotic interaction and its role during the process of nodulation was investigated through RNAi interference silencing approach. RNAi roots that downregulated TOR transcripts showed a clear growth-reduction in root meristem cells resulting in stunted roots with decreased lateral root density relative to the controls. Upon rhizobial inoculation, the IT progression impaired within the root hairs of *TOR*-RNAi furthermore, these roots failed to establish nodule organogenesis as well. These observations were further supported by the decrease in *ENOD40, ERN1* and *NIN* transcript in *TOR*-RNAi roots. The transcripts of cyclins, *CyclinB1-1* (G2/M)*, CyclinD1* and *CyclinD3* (G1/S) involved in phase transition during mitosis were also significantly reduced in *TOR*-RNAi roots relative to controls. Downregulation of TOR revealed neither ROS generation nor *RIP1* gene expression after *Rhizobium* infection in transgenic roots. Together, these results suggest a key role of TOR in root growth, IT formation and nodule organogenesis in *P. vulgaris*.

Wab1 encodes a TCP transcription factor and regulates LG1 expression

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The maize leaf is composed of two major tissues, a distal blade that tilts away from the stem and the more proximal sheath that tightly wraps around the stem. At the junction of blade and sheath, the ligule and auricles are found. The auricles act as a hinge to let the blade lean back and the ligule is a flap of tissue, preventing water from entering into the stem. Our goal is to understand how cells in a leaf primordium differentiate according to position and adopt specific cell types. We are using a number of maize mutants that affect patterning in the leaf. The recessive *liguleless1* and *liguleless2* mutants remove the ligule and auricle, while the dominant mutant *Wavy auricle in blade* (*Wab1*) has ectopic auricle in the blade. *Wab* was cloned by position and shown to encode a TCP transcription factor related to TEOSINTE BRANCHED1. Both *wab* and *lg1* are upregulated in the dominant *Wab-R* mutant. We identified a revertant, loss of function allele for *Wab-R* that has normal leaves. We also discovered it has upright tassel branches and that *lg1* is not expressed. Our results suggest that WAB is needed in the tassel to activate LG1 for proper branch number and angle and in the gain of function leaf to regulate leaf angle. A commonality in tassel branch angle and leaf angle is also seen with other maize mutants suggesting shared mechanisms.

Novel signals regulating chloroplast biogenesis and leaf development

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The acquisition of plastids by plants marks a major impact for the life in this planet. The correct functionality of these organelles depends on a complex and highly regulated differentiation process still not fully understood. This differentiation process occurs in response to specific signals and in coordination to the differentiation of the leaf. The nucleus encodes for the majority of the structural and regulatory proteins that modulate chloroplast development. However, it is well established that the developing plastids also generate signals that regulate the expression of many organelle nuclear-encoded genes. This retrograde feedback mechanism transmits organelle status to the nucleus and coordinates gene expression in both compartments, to ensure appropriate levels of protein complexes required during chloroplast differentiation and function and impacts the overall plant development. The signals responsible for this regulation are largely unknown. In this work we present genetic, developmental and molecular evidences of a novel signal that profoundly affects chloroplast and leaf development. This works highlights the complexity underlying the plastid to nucleus communication. Carotenoids are pigments essential for light capture, photoprotection, precursors of phytohormones and also regulatory signals. Here we demonstrate that a new signal derived from linear carotenoids regulates early chloroplast development and profoundly affects leaf development. Biosynthesis of the signal depends on zeta carotene desaturase (ZDS) activity encoded by the *CLB5* gene of *Arabidopsis thaliana*. Mutants deficient in ZDS (*clb5*) have alterations in chloroplast development and in the leaf development. The expression of many chloroplast proteins nuclear- and chloroplast-encoded is also altered in this mutant. These phenotypes are specific for this mutation and are not observed in other carotenoid deficient albino mutants and also reverted by PDS specific inhibitors. Our data demonstrate that phytofluene or □-carotenoids are substrates for the yet unidentified signaling molecule. Finally our data also demonstrates that the carotene dioxygenase CCD4 is essential element for the generation of this signal. All together these data provides new evidences of the influence that chloroplast functionality has over the developmental fate of the leaves in high plants.

The RAM determinacy versus indeterminacy: developmental programs and their regulation

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Root system formation is important for plant adaptation to its environment and its development depends on root growth and lateral root formation. Root growth in most plant species is considered to be indeterminate. However, primary and lateral roots of *Arabidopsis thaliana* become determinate under phosphorous deficiency and their root apical meristem (RAM) turned out to be completely consumed. Moreover, under these conditions in some species clusters of determinate lateral roots are formed. This developmental pattern represents an induced determinate growth. We have identified a constitutive determinate root growth in Cactaceae. In this plant group the root first behaves as indeterminate with functional RAM and then becomes determinate. How the indeterminacy-to-determinacy switch functions is not well known and this is the main focus of our study. We have identified some *A. thaliana* mutants that show primary root determinate growth (an exhaustion pattern) and a very slow but indeterminate growth of the primary root (a maintaining pattern). We analyze what is the difference between the maintenance of the RAM during indeterminate root growth and the maintenance of the root indeterminacy. We conclude that these two scenarios represent two separate developmental programs. In the mutants affected in either of these developmental programs, a certain level of the RAM disorganization can be found. However, in the mutants with an exhaustion pattern, the stem cells become inactive and the quiescent center (QC) cells start to divide leading to the RAM consumption, whereas in the mutants with a maintaining pattern, all cells of the stem cell niche, including the QC, maintain their activity, while the RAM becomes smaller but is not consumed. Genetic regulation of these processes and other aspects of regulation of indeterminacy-to-determinacy switch will be discussed.

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Chromatin remodeling ATPases at the interface of environment, development and the genome

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The chromatin state of the nucleus is a critical determinant of cell identity and contributes to appropriate responses to environmental cues. One central mechanism for altering the chromatin state is chromatin remodeling, a process that uses the energy derived from ATP hydrolysis to change the interaction between the genomic DNA and the histone octamer in the nucleosome. SWI/SNF ATPases are among the best-studied chromatin remodelers. My lab's investigations have focused on the roles, mechanism of action, and regulation of SWI/SNF ATPases in plants. In *Arabidopsis*, there are 3 classes of SWI/SNF ATPases: SPLAYED (SYD), BRAHMA (BRM) and MINUSCULE (MINU). Like their metazoan counterparts, these SWI/SNF ATPases control both pluripotency and differentiation and are required to overcome polycomb repression for transcription of (floral) homeotic genes. SYD and BRM have unique and overlapping functions. The two MINU factors present in *Arabidopsis* act redundantly and are linked to mitotic epigenetic inheritance. More recently we have investigated how the activity of SWI/SNF ATPase is regulated to enable them to direct correct cell-type and stimulus specific changes in the chromatin state. We have identified families of transcription factors that preferentially recruit SWI/SNF chromatin remodelers to genomic target loci and post-translational modifications that modulate SWI/SNF ATPase activity.

Phosphatidylinositol-4, 5-bisphosphate in the nucleus and its involvement on nuclear myosin 1 function

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Myosins are motor proteins which use ATP to carry cellular cargos along actin filaments. Two nuclear myosin 1 (NM1) and myosin 1C (Myo1C) have been described earlier, they are identical proteins except for 16 extra residues at the N-terminus of NM1. The known cargo molecules that bind to the tail domain of NM1 in the nucleus are DNA, RNA and emerin. Actin and phosphotidylinositol 4,5 bisphosphate (PIP2) are reported to bind to the tail domain of Myo1C in the cytoplasm. PIP2 is a minor membrane phospholipid which is also localizedin the nucleous. We explored if PIP2 interacts with NM1 and Myo1C in the cell nucleus. Here we show that both NM1 and Myo1C bind to PIP2 via their pleckstrin homology (PH) domains in the nucleus. Furthermore, this binding results in slower mobility of NM1 and Myo1C as shown by fluorescence correlation spectroscopy (FCS) and fluorescence recovery after photobleaching (FRAP) methods. Furthermore, NM1 and Myo1C PIP2 interaction include lamin A and farnesylated proteins to the lipo-protein complex. Moreover, several lipid molecules were also found to associate with nuclear PIP2. In addition, nuclear proteins involved in chromatin regulation, transcription, splicing, ribosome synthesis and genomic stability were also found to interact with NM1. Moreover, PIP2 binding with NM1 competes with NM1 association with RNA polymerase (Pol) I transcription machinery. These findings suggest that NM1 and Myo1C are tethered within the nucleus via PIP2 possibly nucleating lipo-protein complexes which function in various nuclear processes other than Pol I transcription.

Non-Mendelian inheritance of epigenetic variation in maize

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In both plants and animals, meiotically-heritable regulatory states of specific alleles can be altered through trans-homologue interactions known as paramutations. This behavior presents exceptions to the laws of Mendelian genetics and challenges basic tenets of evolutionary theory. We have used forward genetics and mutational analyses to discover that paramutations occurring in maize involve components of a small RNA-directed DNA methylation pathway. Our findings have established a plant-specific RNA polymerase (Pol IV) as an important determinant of trans-generational inheritance. Pol IV appears to interfere with Pol II access to LTR retrotransposons (RT) and this has led to models in which expression of specific alleles is regulated by Pol IV through competitions with Pol II. Global run-on sequencing identifies more than 200 such haplotypes subject to transcriptional control by Pol IV. These studies indicate that much of the epigenetic variation defined by Pol IV is due to specific juxtapositions of genic regions and transposons. Our goal is to understand how such a nuclear system creates and maintains epigenetic variation to enable novel strategies for plant improvement.

Factors guiding gynoecium development

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Gene regulation at the level of transcription is crucial for almost all biological processes in a cell or organism. Transcription factors (TFs) are sequence-specific DNA-binding proteins that are capable of activating and/or repressing transcription. Many mutants affected in development have been associated with altered expression levels of TF genes. Therefore, the analysis of TF genes can be the basis for a better understanding of plant developmental processes. Our lab identified various novel TFs affecting gynoecium and fruit development in Arabidopsis. Moreover, we discovered that the hormone cytokinin is important for gynoecium and fruit development. At the moment, we are studying the genetic interactions among them and furthermore, to gain a better understanding about how they function on the molecular level, matrix-based yeast two-hybrid screens are performed with known TFs involved in meristem, flower, and fruit development. The latest results will be presented.

The Impact of Phosphate Deficiency on Carbon Metabolism and Sucrose

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Acclimation to phosphate deficiency by plants involves a wholesale change in root carbon metabolism and shoot carbon fixation. We performed RNA-seq analyses on roots and shoots of control and phosphate deficient white lupin. Based upon an RPKM>3 over 2,000 transcripts were found to be responsive to plant phosphate status. Many of the transcript changes were involved in modified carbon utilization to enhance phosphorus use. Transport of photosynthate to roots was required for phosphate deficiency transcript expression. In efforts to further understand the role of sucrose in the phosphate acclimation response we evaluated an unusual Arabidopsis sucrose transporter 2 (SUC2) mutant. This particular SUC2 mutant had a tDNA insertion in the 3' untranslated region of the gene. Surprisingly plants containing this mutation showed over-expression of SUC2. Results with reporter gene constructs showed SUC2 expression was limited to vascular bundles. The phenotype of the SUC2 mutant plants was very robust growth and seed set, nearly twice that of the control plants. We reconstructed the entire mutant gene and transformed that construct into another SUC2 mutant having no SUC2 expression. Our reconstructed mutant SUC2 gene rescued the null mutant and recapitulated the enhanced vigor and seed set of the original SUC2 3’ mutant. Since SUC2 is the primary transporter which loads sucrose into the phloem our data suggest that enhancing SUC2 expression in vascular bundles may be a viable approach to improving plant biomass and seed production.

Water deficit responses regulated by microRNAs in *Phaseolus vulgaris*

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Common bean (*Phaseolus vulgaris)* is an important legume for human consumption in Mexico. However, water deficit represents a major constraint limiting crop production. In order to contend with different environmental adversities, plants have developed a series of mechanisms at the physiological, cellular and molecular level. To obtain novel insights into the responses to water deficit, we have studied of microRNAs (miRNAs) as regulators of this response at the post-transcriptional level. MiRNAs (small RNAs, 20-24 nts in length) direct recognition of a target mRNA by sequence complementarity, causing mRNA down-regulation by mRNA cleavage or by translational inhibition. We have identified common bean miRNAs that are expressed under water deficit conditions. For their study we have employed different strategies, including high-throughput sequencing of small RNA populations, bioinformatical prediction of targeted transcripts, biochemical analysis of the AGO1 protein and of its interacting RNAs, and deep sequencing analysis of cleaved mRNAs to identify miRNA targets under water deficit conditions. Our results will contribute to better understand strategies used by common bean and other legumes to cope with adverse environmental conditions.

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The role of the phytohormone cytokinin in the design of the plant gynoecium

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Cytokinins play essential roles in plant embryonic and postembryonic growth and development. However, little was known about their role in fruit patterning and morphogenesis, and information about the spatio-temporal localization pattern of cytokinin signaling in gynoecia and fruits was lacking. In this work, cytokinin signaling during gynoecium and fruit development was visualized using the synthetic reporter line *TCS::GFP*. Fluorescence was detected at medial regions of developing gynoecia and, unexpectedly, at the valve margin in developing fruits, and was severely altered in mutants that lack or ectopically acquire valve margin identity. Interestingly, comparison to the phytohormone auxin signaling reporter *DR5rev::GFP* developing gynoecia and fruits showed that the transcriptional responses to cytokinin and auxin were frequently located in complementary patterns during gynoecium and fruit development. Moreover, cytokinin treatments in early gynoecia produced conspicuous tissue-specific overgrowth in gynoecia, while treatment of valve margin mutant fruits restored this tissue. The results suggest that the phytohormone cytokinin is an important player involved in gynoecium and fruit patterning and morphogenesis, playing at least two roles: an early proliferation-inducing role at the medial tissues of the developing gynoecia, and a late role in fruit patterning and morphogenesis at the valve margin of developing fruits.

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New MADS-box genes in the floral transition network

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Flowering is an important trait that depends on gene regulatory networks and their dynamic responses to environmental factors. Arabidopsis initially undergoes a period of vegetative development, subsequently the shoot apical meristem transits to an inflorescence meristem that produces flowers in its flanks. MADS-box transcription factors are key components in floral meristem transitions. Here we present data for *XAL1/AGL12, XAL2/AGL14, AGL19* and *AGL17* that are implicated in floral transitions despite the fact of being predominantly expressed in roots. Mutations in these genes produced a late flowering phenotype under different photoperiod and temperature conditions, and over-expression of *XAL2* and *AGL19* produce flowers with vegetative reminiscences. We have genetic, *in situ* hybridization, RT-PCR and ChIP data that document the dynamic regulation of these MADS-box genes and their important involvment in the gene regulatory network integrating developmental and environmental signals.

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Melatonin regulates *Arabidopsis* root system architecture likely acting independently of auxin signaling

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Melatonin (N-acetyl-5-methoxytryptamine) is a tryptophan-derived signal with important physiological roles in mammals. Although the presence of melatonin in plants may be universal, its endogenous function in plant tissues is unknown. On the basis of its structural similarity to indole-3-acetic acid, recent studies mainly focusing on root growth in several plant species have suggested a potential auxin-like activity of melatonin. However, direct evidence about the mechanisms of action of this regulator is lacking. In this work, we used *Arabidopsis thaliana* seedlings as a model system to evaluate the effects of melatonin on plant growth and development. Melatonin modulated root system architecture by stimulating lateral and adventitious root formation but minimally affected primary root growth or root hair development. The auxin activity of melatonin in roots was investigated using the auxin-responsive marker constructs *DR5:uidA*, *BA3:uidA*, and *HS::AXR3NT-GUS*. Our results show that melatonin neither activates auxin-inducible gene expression nor induces the degradation of *HS::AXR3NT-GUS*, indicating that root developmental changes elicited by melatonin were independent of auxin signaling. Taken together, our results suggest that melatonin is beneficial to plants by increasing root branching and that root development processes elicited by this novel plant signal are likely independent of auxin responses.

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Arabidopsis thaliana MPK6 mutation drives three distinct classes of seed phenotypes, which correlate with alterations in cellular processes that affect root architecture

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Mitogen-Activated Protein Kinase (MAPK) cascades are signal transduction modules highly conserved in all eukaryotes. A typical MAPK module consists of three kinases (MPKKK, MPKK and MPK), which activate downstream targets by sequential phosphorylation. The last kinase of the module (MPK) is able to phosphorylate several substrates, including transcription factors to regulate gene expression. MAPKs are known regulators of various aspects of plant biology including biotic and abiotic stress responses, hormone perception and developmental programs. Functional redundancy is common among MAP kinases and they are proposed to act through common downstream targets and upstream activators. However, the MPK6 loss-of-function mutant displays alterations in the embryo and early root development, indicating that at least for these processes, the function of this kinase cannot be substituted by any other MPK. Several data support the participation of MPK6 in root development, but no relationship has been established between embryo and root phenotypes in mpk6 mutants, neither the impact of earlier root development alterations in the configuration of post-embryonic root architecture. In this work, we provide physiological and molecular evidences that seedlings defective in two independent mpk6 mutant alleles show three distinct classes of seed phenotypes, which correlate with alterations in cell division and elongation processes that affect root architecture. Our data indicate that MPK6 is an essential component of early signaling processes linked to proper embryo development and maintenance of Arabidopsis root system architecture.

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Regulation of ABA-INSENSITIVE (ABI) 4 transcription factor in *Arabidopsis thaliana*.

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The ABscisic Acid-Insensitive 4 (ABI4) transcription factor is a central regulator for many processes during plant life. ABI4 is required for proper ABA and sugar signaling, lipid mobilizations in the embryo, salt tolerance and nitrate-sugar mediated root growth. Recently, ABI4 has also emerged as a central player in chloroplast to nucleus communication. It is known that the ABI4 transcript accumulation doesn't correlate with its protein levels, supporting a post-transcriptional regulation. To understand the mechanism of action and regulation of ABI4, we identified sequence motifs highly conserved among different plant species. These motifs are good markers for ABI4 orthologs (AP2-associated, LRP and PEST motifs). We demonstrated this by isolating one of these putative orthologs from *Theobroma cacao*. Similar to the Arabidopsis ABI4, this gene activates gene expression through the recognized ABI4 binding site. We also showed that *TcABI4* complements ABA, glucose and salt sensitivity of the *abi4* Arabidopsis mutant. The function of these conserved motifs was analyzed through mutagenesis or deletion in the Arabidopsis ABI4 protein, by immunological detecting an ABI4-GFP fusion protein in transient assays with Arabidopsis mesophyll protoplast. Our findings demonstrate that deletion of the AP2-associated motif affects ABI4 transcriptional activity because it is required for the nuclear localization of this protein. The LRP motif is important, but not essential, for the regulation of ABI4 transcriptional activity. Finally, the PEST motif directly modulates ABI4 protein stability via the 26S proteosomal pathway. These results demonstrate that ABI4 is regulated post-transcriptionally through different mechanisms. Recent studies support novel post-transcriptional regulatory mechanisms that involve microRNAs participation. Current advances in this area will be presented. ABI4 is also highly regulated at the transcriptional level. Our current analysis with transgenic plants that express the GUS reporter from different fragments of the ABI4 promoter have permitted us to locate important elements for the expression of this factor during early development of plant.

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BiFC shows that the *S*-determinants from *Papaver rhoeas* directly interact *in vivo* in an *S*-specific manner

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Flowering plants have evolved different recognition-rejection mechanisms to prevent self-fertilization. Self-incompatbility (SI) is a genetic barrier that allows discriminating between "self" and "non-self" pollen and is regulated by the multiallelic *S locus*, which encodes both female (pistil) and male (pollen) *S* determinants. In *Papaver rhoeas*, the field poppy, the pistil *S* determinant, PrsS, is a soluble protein, while the pollen *S* determinant is PrpS, a membrane protein. In an incompatible ("self") pollen, an intracellular signaling cascade is triggered. Major findings include: cytosolic free Ca^{2+} as a second messenger, the phosphorylation of a soluble PPase, activation of a MAPK (p56) and as a hallmark feature, the actin cytoskeleton is rapidly depolymerized. Finally, programmed cell death (PCD) is triggered to provoke the irreversible inhibition of incompatible pollen tube growth. It has been shown that both *S*-proteins are sufficient to produce the same response in *Arabidopsis thaliana* pollen than in *P. rhoeas*. However, direct *in vivo* evidence to demonstrate that the *S* proteins physically interact was lacking. Here, we demonstrate that PrpS localized to the plasma membrane of onion epidermal cells, *A. thaliana* leaf protoplasts and root hairs when transiently expressed fused to GFP. In addition, BiFC using *A. thaliana* root hairs, we show that PrpS and PrsS physically interact *in vivo* and that this interaction occurred in an *S*-specific manner and was located at the plasma membrane. We also provide evidence showing that PrpS:PrsS interaction is functional in vegetative cells. When PrpS-expressing leaf-protoplasts were exposed to cognate allelic PrsS, their viability decreased in an *S*-specific manner. This suggests that PCD appears to be also involved as a final result from PrpS:PrsS interaction in vegetative cells.

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The proteases and proteinase inhibitors game in pollen rejection in *Nicotiana alata*

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NaStEP is an essential gene to self-incompatibility (SI) in *Nicotiana* encoding a Kunitz-type proteinase inhibitor, which is highly expressed in the stigma of SI *Nicotiana* species. NaStEP is taken up by both compatible and incompatible pollen tubes (PT). Its suppression in *Nicotiana* spp. causes SI breakdown. Notably, when NaStEP is suppressed, HT-B protein is degraded on the inside of both incompatible and compatible PT, which is contrary to what happens in SI *N. alata*, where HT-B is only degraded in crossbreeding compatibles PTs, indicating that NaStEP is a positive regulator of the HT-B stability in *Nicotiana* PTs during the SI response. Because it was unknown if NaStEP is a specific inhibitor to subtilisin-like proteases, we evaluated its inhibition specificity. NaStEP purified fractions were immunoanalyzed and those enriched in NaStEP were assayed for proteinase inhibitior activity against trypsin, papain and subtilisin. Our outcomes indicated that NaStEP only showed inhibition activity for subtilisin. In addition, during the purification process we detected that no all the NaStEP purified fractions had inhibition activity. We thought it was attributed to posttranslational modifications on NaStEP such as glycosylation but we did a protein sugar modification assay and we concluded that it was not the reason. A second hypothesis was that NaStEP were being degraded by a protease during the inhibition assay. Results showed that as expected, NaStEP was degraded in those fractions in which a protease activity was present, suggesting that this protease is the responsible for NaStEP degradation. These results have encouraged us to go deeper in the study of proteases and their inhibitors as essential players in the pollen rejection response in S-RNase based SI systems. Thus, one of our future goals is to identify proteases in pistils and PT to evaluate their role in SI by lost of function assays.

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Complexes of cyclins D with CDKS during maize germination: activity and regulation

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The importance of cell proliferation to plant growth and development has been well documented. The majority of studies on basic cell cycle mechanisms in plants have been at the level of gene expression and much less knowledge has accumulated in terms of protein interactions and activation. Two key proteins, Cyclins and Cyclin Dependent Kinases (CDKs) are fundamental for cell cycle regulation and advancement. Our aim has been to understand the role of Cyclins D and type A and B CDKs in the cell cycle taking place during a developmental process as maize seed germination. Results indicate that three maize Cyclins D, D2;2, D4;2 and D5;3, G1-S cyclins by definition, bind and activate two different types of CDKs, A and B1;1 in a differential way during germination. –Whereas CDKA-Cyclins D complexes are more active at early germination times than at later times, it was surprising to observe that CDKB1;1, a supposedly G2-M kinase, bound in a differential way to all Cyclins D tested during germination. Binding to cyclin D2;2 was detectable at all germination times, forming a complex with kinase activity, whereas binding to D4;2 and D5;3 was more variable, particularly that with D5;3, only detected at late germination times. Results will be discussed in terms of cell cycle advancement and its importance for seed germination.

Study of the involvement of jasmonic acid on epidermal cell differentiation processes in *Arabidopsis thaliana*

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Jasmonic acid (JA) is a regulator of defense responses in plants; however, the knowledge about JA functions in developmental processes such as epidermal cell differentiation is limited. In this research, the effect of JA on the differentiation of epidermal cells, namely root hairs and trichomes was investigated in *Arabidopsis thaliana* seedlings. JA promoted root hair formation in a concentration dependent manner in wild-type seedlings, effect accompanied by an increased expression of At*EXP7* and repression of *GL2* gene markers in primary roots. These responses were not observed in the JA-resistant mutants, including *coi1-1*, *jar1-1* and *axr1-3*. It was also found that auxin insensitive mutants *tir1*, *afb2*, *afb3*, *iaa14*, *slr1*, *arf7* and *arf19*, showed resistance to root hairs formation in response to JA. When the mutant lines affected in epidermal cell differentiation processes *cpc, rhd6* and *gl2* were analyzed, it was observed that the JA was unable to restore the formation of root hairs and trichomes. Interestingly, high concentrations of JA activated formation of aberrant trichomes in *gl2* mutant seedlings, which suggests that JA may trigger the formation of these structures through an alternative route to *GL2*. Taken together our results indicate that JA has a key role in the regulation of epidermal cell differentiation, involving auxin signaling through *TIR1, AFB2, AFB3, IAA14, SLR1, ARF7* and *ARF19* loci and genes associated with cell differentiation such as *CPC*, *RHD6* and *GL2*.

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RNA-seq assisted insight into molecular mechanisms of determinate root growth in Cactaceae

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We have recently shown that most species from Cactoideae tribe of the Cactaceae family exhibit determinate growth of the primary root, which implies early root apical meristem (RAM) exhaustion and cell differentiation at the root tip. Besides, we suggested that this type of growth was fixed after separation of the Cactiodeae/Opuntioideae and Maihuenioideae /Pereskioideae lineages. To characterize genes involved in the RAM maintenance and determinate root growth in cardón *Pachycereus pringlei*, we employed mRNA-seq and smRNA-seq. 85 bp mRNA-seq reads were *de novo* assembled into contigs using CLC Genomic Workbench, and annotated by protein similarity. Differential gene expression in primary root tips in the initial growth phase (when RAM is present), and in the terminal phase (when RAM is exhausted), was estimated. For example, homologs of *PIN* auxin efflux carriers were induced in the primary root tip during either initial or terminal growth phases, while most genes involved in cytokinin synthesis and metabolism were induced during the terminal phase. We grouped and annotated small RNAs differentially expressed in the root tip using small RNA analysis tools in CLC Genomics Workbench and the miRBase database. We also identified hundreds of novel, species-specific smRNAs that show differential expression in the two growth phases. Significant conservation was revealed for the amino acid sequence and RNA expression patterns of various proteins of *P. pringlei* and other plant species. The results also suggest that the *P. pringlei* primary root tip after meristem exhaustion performs functions similar to those of the differentiation zone of *Arabidopsis* root.

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Regulation by small RNAs during somatic embryogenesis in maize (*Zea mays* L.)

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Small RNAs (smRNAs) guide RNA-induced silencing complexes to regulate gene expression at transcriptional and post-transcriptional level in response to developmental cues, biotic or abiotic stresses and during embryogenesis. The induction of somatic embryogenesis in maize is accompanied by specific changes in the smRNA population, particularly the repeat-associated small interferent RNA (rasiRNA) and microRNA (miRNA) species. rasiRNAs are known to repress transposable elements (TE) and maintain genome integrity directing DNA methylation at least by two different pathways, whereas miRNAs regulate target mRNA degradation and/or translation. Here we aimed to evaluate the expression regulation by specific miRNAs, as well as the epigenetic changes in the genome regions associated with rasiRNAs induced during embryogenic callus subculture. The results indicate that the most abundant miRNA families in established subcultures are miR156, miR159, and miR528. Interestingly, miR528 is distributed along polysomal fractions suggesting a regulation of its targets at translational level. After a year of subculture, most miRNAs are importantly decreased. In addition, higher cytosine methylation was found in DNA repeated sequences from old calli subcultures compared with recently established embryogenic calli. These findings reveal a temporal-dependent epigenetic regulation by smRNAs in response to callus induction and the length of subculture in maize.

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Functional and phylogenetic analysis of a CBF/DREB gene in *Carica papaya* var. Maradol.

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Carica papaya var. Maradol is one of the most important crops worldwide due to its wide use. It's productivity can be significantly reduced when there are different types of stress such as drought, extreme temperatures and salinity. Therefore in the future it will be necessary to improve crop tolerance of papaya to abiotic stress. In recent years there is evidence of the involvement of signaling elements in response to abiotic stress, some like transcription factors (TF) that belong to the superfamily AP21ERF. The AP21ERF TF's bind to sites cis-DRE1CRT (A1GCCGAC) located in specific regions of the promoters that regulate the transcriptional expression of different genes which play an important role in response to abiotic stress. In this study an ortholog of DREB2C was isolated from Carica papaya var. Maradol called CpDREB2C. Our in silico analysis showed that the gene encodes a protein CpDREB2C containing an AP2 domain (Apetala 2) conserved, so it is located within the group IV of the superfamily AP21ERF. Semiquantitative PCR experiments indicate that the gene CpDREB2C is differentially expressed due to a water deficit and extreme temperatures. Moreover, genetic transformation of tobacco plants which overexpress the CpDREB2C gene showed that they can survive to extreme temperatures and water deficit. Fluorescence experiments indicate that DAPI staining and CpDREB2C::GFP gene is localized mainly in the nuclei of specific organs such as roots and leaves of seedlings tobacco. Our results indicate that CpDREB2C, plays an important role in the signaling mechanism caused by water stress and extreme temperatures in Carica papaya var. Maradol.

Transgenerational epigenetic modifications as a result of *priming* in common bean (*Phaseolus vulgaris* L.)

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Studies on the phenomenon of *priming* (or conditioning of the plant by the presence of an external agent that activates a warning system against a pathogen attack or abiotic stress) have mainly focused on the model plant *Arabidopsis thaliana*. So far, several genes have been described in Arabidopsis that are involved in the phenomenon of priming. Thus, our interest in using the common bean (*Phaseolus vulgaris* L.) in order to determine the genes and epigenetic factors are involved in this phenomenon, is that, unlike Arabidopsis, the common bean has a great economic importance being as it is part of the basic staples in the Mexican cuisine. The results of our investigation show, from an epigenetic perspective, the relationship in gene activation due to the *'priming*' phenomenon when induced by analogues of the salicylic acid, as well as by pathogenic and symbiont bacteria. Also, it has allowed to determine the 'inducer' that confers a greater *priming* response in *Phaseolus.* Finally, by analyzing the F1 generation, we will be able to determine in the common bean the phenomenon of 'transgenerational *priming*' and to establish the degree of 'trangenerational' expression of the genes involves. The advance of our knowledge on this topic will allow us to generate bean lines with a high degree of resistance to pathogen attack, without using transgenes, or even using such genes in other species of agronomical interest.

Interaction between fibrillarin and phosphatidylinositol 4,5-bisphosphate in the nucleolus of *Arabidopsis thaliana*

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The nucleolus is a complex structure inside the nucleus in which transcription, maturation of rRNA and ribosome assemblage takes place. Additionally to these functions, the nucleolus has a multifunctional structure involved in stress response, biogenesis of ribonucleoproteins, gene silencing, cell cycle progress and aging. Furthermore nucleolar activity is also involved in diverse diseases such as cancer and viral infections. Inside the nucleolus we can find a huge diversity of proteins assigned for the functions previously mentioned. One of these proteins and also the most abundant is the fibrillarin. Fibrillarin is a highly conserved protein and among its main functions involve the processing of the pre-rRNA with its methyltransferase activity. We found that inside the nucleolus and the nucleus other molecules like the phosphatidylinositol 4,5-bisphosphate (PIP2) are localize. In the nucleus, PIP2 and other associate factors are necessary for the splicing of the pre-mRNA, inside the nucleolus, PIP2 interaction may change the activity of the fibrillarin, either by repressing or enhancing its functions. Here we show the interaction between the fibrillarin and the PIP2 in the nucleolus of Arabidopsis thaliana cells.

amiRNA-based gene silencing of the gene families *WIP* and *ERF B1*

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The *WIP* gene family includes six members that encode zinc finger proteins that act as transcription factors. It has been described that *WIP2* plays a role in the development of the transmitting tract, while *WIP1* is expressed in endothelial cells during seed coat development, and mutations in *WIP6* cause alterations in vein patterning. However, a phenotype for the rest of the family members has not yet been described, most likely due to redundant functions. Moreover, the expression pattern of some of the genes with reported mutant phenotypes suggest that they have broader functions, which may be masked by gene redundancy. In the same way, mutant phenotypes have been reported for some of the members of the *ERF B1* gene subfamily, composed by six genes, of the AP2/EREBP transcription factor family. The single mutants show low penetrance, and severe phenotypes are only obtained in double or triple mutations, suggesting genetic redundancy. Also, as for the WIP2 family, the expression patterns for these family members suggest that they may have more functions than indicated by their mutant phenotypes. In an approach to understand better the function of these transcription factor families, we attempt to silence all the members of the *WIP* and the subset of the *ERF B1* families through amiRNA gene silencing. The silencing will be controlled by an inducible system, a vector containing a glucocorticoid receptor that will let us analyze the effects of the silencing trough the different developmental stages of the life cycle of *Arabidopsis.*

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Piperine, photosynthetic electron transport and vegetal growth inhibitor

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Natural products have been source for the development of many pesticides, used either in direct form as raw extracts or as pure compounds. Additionally, they have led to the discovery of structural leads for the development of pesticides [1J. Piperine (1) is the most abundant alkaloid in black pepper (Piper nigrum) and in long pepper (Piper longum), very widely used spices in human dietary and also as food preservatives and in perfumery. In recent decades, the biological activity of piperine has been studied and it is known its antioxidant activity, so as antimicrobial, antimutagenic and antigenotoxic [2J. In search of potential biodegradable herbicides from natural products, and non toxic to environment, in this work, effects of piperine were assayed in photophosphorylation on spinach isolated chloroplasts, thus as in germination and growth of roots and stems of seedlings of two monocot seeds (Triticum aestivum and Echinochloa crus-galli) and two dicotiledonous seeds (Lactuca sativa and Physalis ixocarpa), with the aim of evaluating its herbicide potential activity.

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Possible relationship between primary and secondary metabolisms in placental tissue of *Capsicum chinense* Jacq.

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Though capsaicinoids are exclusively synthesized in the placenta of hot peppers, the origin of their precursors (phenylalanine and valine) has not been elucidated. In order to define if capsaicinoids synthesis depends on precursors synthesized in the placental tissue, changes in nitrogenous compounds' pools (nitrate, ammonia, total amino acids, phenylalanine, valine and capsaicinoids) were measured at two contrasting developmental stages, along with changes in the activity of enzymes involved in the synthesis of the above-mentioned amino acids. Nitrate and total amino acid pools were higher in placental tissue than in the pericarp. Nitrogen availability in placentas augmented in pods during the maximal capsaicinoid accumulation stage. Nevertheless, phenylalanine content was lower than valine at this same stage. Arogenate dehydratase was successfully measured in the pod, both in the pericarp and the placenta, and in the leaves. Data suggests that there is an activation of primary nitrogen metabolism associated to capsaicinoid accumulation.

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Avocado roots treated with salicylic acid produce phenol-2,4-bis (1,1-dimethylethyl), a compound with antifungal activity.

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We demonstrated the ability of salicylic acid (SA) to induce a compound in avocado roots that strengthens their defense against *Phytophthora cinnamomi*. The SA content of avocado roots, before and after the application of exogenous SA, was determined by High-Performance Liquid Chromatography (HPLC). After 4 h of SA feeding, the endogenous level in the roots increased 15 times the amount found in control roots. The methanolic extract obtained from SA-treated avocado roots inhibited the radial growth of *P. cinnamomi*. A thin layer chromatographic bioassay with the methanolic extract and spores of *Aspergillus* showed a distinct inhibition zone. The compound responsible for the inhibition was identified as phenol-2,4-bis (1,1-dimethylethyl) by gas chromatography and mass spectrometry. At a concentration of 100 µg/mL, the substance reduced germinative tube length in *Aspergillus* and radial growth of *P. cinnamomi*. A commercial preparation of phenol-2,4-bis (1,1-dimethylethyl) caused the same effects on mycelium morphology and radial growth as our isolate, confirming the presence of this compound in the root extracts. This is the first report of the induction of this compound in plants by SA, and the results suggest that it plays an important role in the defense response of avocado.

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Developmental regulation of valine decarboxylase in *Acmella radicans*

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Para tener un panorama más amplio sobre la síntesis de la afinina en Acmella radicans, se llevaron a cabo una serie de experimentos para conocer el comportamiento de la Valina descarboxilasa (ValDC), enzima involucrada en la síntesis de éste metabolito secundario. Se encontraron las siguientes características: la Km es 44 mM y la vmax 303 1M1mg de proteína1min; pH óptimo de 8 a 10; tiene un amplio rango de temperatura, de los 25 a los 42 oC, sin variaciones considerables dentro del mismo; desde los 10 minutos de reacción es cuantificable; es dependiente de PLP y cataliza la reacción con los enantiómeros L y D-valina. En cuanto a los pasos de pre-purificación, se encontró que: no es necesario añadir coenzima durante el desalado y la cuantificación de la actividad se reduce a la mitad tras éste proceso, indicando que no existen inhibidores en el extracto crudo; la concentración recomendada para precipitar con $(NH_4)_2SO_4$ a la enzima es de 75% y la realización de una diálisis post-precipitación es necesaria para contrarrestar la pérdida de actividad ocasionada por la misma. Por último se cuantifico la producción de afinina y la actividad de la valina descarboxilasa durante el primer mes de desarrollo de la planta. Se observo una mayor actividad de la enzima un día antes del incremento en la producción de afinina.

Virus-induced silencing of a putative capsaicin synthase (AT3) gene affects the expression of genes related to the capsaicinoid biosynthetic pathway in chili pepper fruits (*Capsicum annuum* L.)

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Capsaicinoids are very important secondary metabolites that are restricted to genus *Capsicum* and that result from the acylation of the aromatic compound vanillylamine with a branched-chain fatty acid by the catalysis of the enzyme capsaicin synthase. In this work, we found that virus-induced gene silencing of the *AT3* gene encoding for an acyltransferase (probably capsaicin synthase) produced effects on the expression of genes related to the capsaicinoid biosynthetic pathway. Chili pepper plants infected with the construct derived from the *Tobacco rattle virus* (pTRV2:*AT3*) bearing a partial sequence of the *AT3* gene showed not only a significant decrease in its expression itself (81.07%), but also a significant reduction in the expression of some structural biosynthetic genes of the capsaicinoid pathway, such as a putative aminotransferase [*pAmt* (89.41%)], a branched-chain amino acid transferase [*BCAT* (68.85%)], a ketocyl-ACP synthase [*Kas* (90.4%)] and an acyl carrier protein [*Acl* (58.58%)]. These results suggest the existence of a negative feedback regulation involving possibly the accumulation of intermediate metabolites in the pathway. This study contributes with information about the regulation of the capsaicinoid biosynthesis.

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The evolution of biodiversity in plants: Classic questions - new approaches and paradigms, with special reference to studies of Mexican diversity

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Mexico is considered a hotspot of plant biodiversity, containing about 10% of the total diversity of Earth. This is a significant proportion if we consider that Mexico represents only the 1.35% of the total emerged land of the planet. Different estimates of the total number of angiosperm species in Mexico range from 22,000 to 31,000, making it the fifth country with the highest angiosperm biodiversity (after Brazil, Colombia, China and Indonesia). Also, the diversity of other plant species in Mexico is remarkable. In this talk we will briefly describe the diversity of plant groups in Mexico and explore the theory that tries to explain the causes and patterns of this biodiversity. Then, we will review recent advances in describing this diversity using phylogenetic and molecular evolution studies, including molecular clock approximations, and also discuss different studies describing the genetic variation within species of Mexican plants, including both older population genetic studies and recent phylogeographic and genomic analyses conducted in our lab and in other laboratories in Mexico. Among other studies, we will briefly describe recent studies in the phylogeny and/ or population genetics of the genera *Agave*, *Zea, Bursera*, *Fouquieria*, *Acacia , Abies*, the tree fern *Alsophila* and of the families or subfamilies Cactaceae, Agavoideae, Bombacoideae, Fouquieriaceae and Cyathaceae, including molecular clock calibrations, comparisons of the diversification rates and detailed phylogeographic studies. We will see that the causes and patterns of the botanical diversity found in Mexico are complex, as in some cases we have that diversification is very recent, while other Mexican plants are very old, and have had low extinction rates.

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A Metacalibrated Relaxed Molecular Clock Analysis of Flowering Plants

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Flowering plants (Angiospermae) resulted from an evolutionary radiation that produced extraordinary species-richness, morphological and functional diversity, and the dominant producers of modern terrestrial ecosystems. Available estimates of the onset of the diversification of angiosperms are incongruent, because those derived from molecular clocks are different from each other, and most are substantially older than the oldest unequivocal angiosperms fossils, from the Early Cretaceous. The goal of this study is to date the angiosperm phylogenetic tree using a relaxed molecular clock method, including a comprehensive taxonomic representation, incorporating a large number of fossil-derived temporal constraints, and implementing minimum and maximum bounds to the angiosperm crown node derived from a quantitative method that considers the fossil record across the phylogenetic tree. The phylogenetic tree includes *ca.* 800 terminals that represent 371 angiosperm families. A thorough literature-based review yielded a data base of the angiosperm fossil record, from which we filtered those that reliably represent the oldest reliable report of well-supported clades, spanning from genera to orders. The selected fossils were implemented as minimum age constraints to particular nodes in the tree. The angiosperm crown node was circumscribed to a time interval bounded in the Early Cretaceous. Dating was conducted with penalized likelihood using TreePL and r8s, and with an uncorrelated relaxed clock method implemented in BEAST. The obtained timetree represents a reliable chronological framework not only of the diversification and morphological evolution of angiosperms and its major clades, but also of the origin and evolution of modern terrestrial biomes, and the possibe codiversification of different biological groups, for example, ferns, lycopsids, micorrhizal fungi, as well as diverse types of pollinators.

Beyond natural selection in phylogenomics: uncovering in genes with functional importance

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Understanding the genetic and genomic basis of plant diversification has been a major goal of evolutionary biologists since Darwin first pondered his "abominable mystery," the rapid diversification of the angiosperms in the fossil record. Determining which genes have a significant functional role in the evolution of species is a major goal of modern evolutionary genomics. The large number of genes in the genomes of organisms, even in the smaller microbial genomes, often makes the task difficult and daunting. We develop and deploy a functional phylogenomic approach that helps identify genes and biological processes putatively involved in species diversification. It goes beyond usual measures of positive selection and into an integrative view of evolutionary processes influencing gene function. We assembled a matrix of 22,833 orthologs from 150 species to reconstruct seed plant phylogenetic relationships and to identify gene sets with a unique evolutionary signal. Our analysis of overrepresented biological processes in these sets narrowed down possible genetic mechanisms underlying plant adaptation and diversification. We highlight a few examples and talk briefly about the importance of sampling in large datasets. Our functional phylogenomic approach can be applied to any taxa with available sequences to enhance our knowledge of the evolutionary processes underlying biodiversity in general.

Predicting climate maladaptation in forest trees: perspectives for Mexican conifers

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Forecasting evolutionary responses driven by climate change is one of the main challenges for preserving biological diversity. Successful predictive models depend on the integration of species adaptive information, including that derived from genomic studies. Here, we show an example on the Mediterranean conifer maritime pine (*Pinus pinaster*), where carefully selected single nucleotide polymorphisms (SNPs) were successfully used to forecast the fate of populations under a scenario of maladaptation to climate. Then, we discuss some breakthroughs for performing similar researches on Mexican conifers, by using species of the genus *Abies* as models. For maritime pine, we first selected a set of potentially adaptive SNPs that showed significant genotype-environment correlations. Then, we used climate data from a test site with strong environmental conditions (similar to those forecasted under climate change) to calculate the optimal allele frequencies of these SNPs for this location. On the field, we observed an increased mortality for populations with allele frequencies that departed from these predicted optima, suggesting that a limited number of carefully selected gene-based markers may suffice to forecast the fate of populations under future climate change scenarios. For the Mexican *Abies*, we selected some particular genes that have shown adaptive trends in other conifers and correlated their sequence variation with ecological niche models. Although more research is still necessary on Mexican conifers (particularly at the genomic level and on plantation test sites), perspectives are good for the years to come, especially once the information generated from next-generation sequencing technologies are fully integrated into the predictive models.

Tracing Population History with Haplotype Data

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A haplotype is the combination of adjacent alleles on a stretch of DNA sequence. It is straightforward to infer the number of mutational states that separate two haplotypes and to measure linkage disequilibrium among haplotypes, so haplotype data contain information on the history of mutational and recombination within a population. This information reveals much about the historical connections among populations of a species. We illustrate this use of haplotype data by considering the history of introduction of weedy *Ipomoea* species from Mexico into what is now the Southeastern United States and by investigating the hybrid origins of modern avocado cultivars.

Evolution and domestication in grasses

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The advent of widespread genome sequencing is allowing genetic researchers a much broader choice of models for their own systems of interest. Nowhere is this truer than in the grasses, where data from at least five completed genomes spanning 60 million years of evolutionary history creates a phylogenetically meaningful dataset for examining the evolution of genomes, genes, and phenotypes. We have used this framework to investigate the relationship between natural diversity and the domestication phenotypes of vegetative architecture, shattering and flowering time, and find evidence for both conserved and novel genetic pathways. Using recombinant inbred lines derived from a cross between domesticated foxtail millet (*Setaria italica*) and its wild progenitor green millet (*S. viridis*), we also report changes in gene-by-gene (epistatic) and gene-by-environment (GxE) interactions between wild and domesticated alleles of loci involved in domestication phenotypes, and suggest that such changes are the result of selection during domestication.

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Genetic Architecture of Flowering Time in Sorghum

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Sorghum is a tropical C_4 grass that has been adapted to produce food, feed, and fuel in both tropical and temperate environments. Flowering time is a key determinant of sorghum adaptation to different environments and end uses. Classical genetic studies predict four major flowering time or "maturity" loci in sorghum *(Ma1-Ma4)*. Two of the underlying genes have been identified: *Ma1* encodes a floral repressor (PRR37) that requires coincident light and clock signals for expression, and *Ma3* encodes phytochrome B. Two additional loci, *Ma5* and *Ma6*, show a complementary dominant interaction and are used by the bioenergy industry to produce photoperiod-sensitive (PS) hybrids from two photoperiod-insensitive (PI) parents. However, the relationship between the *Ma1-Ma4* model and the *Ma5-Ma6* model is not clear. Our group is using three related approaches to study flowering time in sorghum: association mapping in large panels of temperate and tropical lines, linkage mapping in (temperate x tropical) biparental populations, and introgression mapping in exotic lines that have been adapted to the temperate zone through backcrossing with selection. I will present evidence for a series of functionally distinct alleles at *Ma1,* and a compelling candidate gene for the *Ma6* locus. Our results suggest that multiple complementary dominant interactions could be exploited to produce PS hybrids from two PI parents.

Strategies for conservation and sustainable use of Mexican maize landraces.

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Mexico as the center of origin and domestication of maize, is home to an enormous diversity of landraces which have been selected and maintained by local farmers in different regions of the country based on specific cultural and environmental needs. The greatest current challenge is to identify rare genotypes and ensure their conservation either *in situ* or in germplasm banks. We have developed a strategy to aid this goal based on microsatellite marker analysis and newly designed statistical methods. The implementation of the strategy in key regions of diversity will aid in determining and monitoring landrace materials and in decisions for conservation of specific accessions. In conjunction we have also initiated a detailed analysis of a specific drought resistant landrace (Michoacán 21) in comparison to a commercial cultivar (B73) in terms of expression patterns of specific genes under drought stress and the genotypic differences which putatively underlie these differences with a view to implement molecular breeding strategies based on the results obtained.

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Exploring the *Physcomitrella patens* genome for the two main enzymatic nitric oxide-producing mechanisms: nitrate reductase and nitric oxide synthase

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Nitric oxide (NO) is a small gaseous molecule with important roles in the control of growth, development and physiology of land plants. In higher plants NO is produced by two main enzymatic mechanisms: 1) nitrate reductase (NR) and 2) a nitric oxide synthase (NOS)-like activity whose neither genes nor proteins have been identified. In green algae NO is produced by NR and not by NOS-like activity, but there is no information about NO production in non-vascular plants. Such information will help to understand the development of enzymatic sources of NO during plant evolution. In order to determine whether NO producing enzymes are present in non-vascular plants we searched for the NR and/or NOS genes and proteins in the moss *Physcomitrella patens* genome. We demonstrated that: 1) there are not *nos* genes in the *P. patens* genome; 2) *P. patens* have a family of three genes (*ppnia* genes) that encode for canonical NR enzymes and 3) although *P. patens* NR conserves the three domain structure common to all plant NRs, the motive (K/R)(S/T)XS*(T/S)XP—the target of phosphorylation and binding of 14-3-3 proteins that down regulates the enzyme activity—is absent, indicating that this regulatory mechanism appeared after the divergence of bryophytes and tracheophytes .

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Functional diversity of plant-soil relations in maize and wild relatives

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The domestication of cultivated maize (*Zea mays mays*) from wild Balsas teosinte (*Zea mays parviglumis*) has become a text-book example of morphological evolution under selection. The domestication and radiation of maize landraces required, however, not just morphological change, but also adaptation to new environments as the plant spread from south-western Mexico to all parts of the country and, subsequently, to all corners of the world. Significantly, within a few thousand years, cultivated maize had moved well beyond the range of its teosinte ancestor. Among the many challenges faced by maize was adaptation to new soils, distinct from those found in the ancestral environment. Here, I will present preliminary results concerning phosphorous relations in maize and teosinte, an element poorly available in the acidic volcanic soils of the central Mexican highlands. Furthermore, I will discuss our general approach to study functional diversity teosintes by using of near isogenic lines. I will comment on the use of landrace maize and teosintes as a source of agronomically useful genetic variation, specifically with respect to the improvement of phosphate use efficiency, a key target of modern tropical and sub-tropical breeding programs in light of dwindling phosphate reserves.

Traditional and genetically improvement of sugar cane

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Sugarcane improvement, from selection of existing variation in pre-historic time to the current bi/multi-parental crossing and subsequent use of non-conventional techniques, has concentrated mostly on improving the yield. Nowadays, incorrect eating habits which highlights the excessive consumption of carbohydrates has led health problems such as increased diseases as diabetes mellitus type 2, obesity, metabolic syndrome and dyslipidemia. Sucrose is the primary sweetener or food additive used today, and perhaps contributes to metabolic disorders. It is advisable to substitute this sweetener for other with lower caloric income. However, the candidate should be soluble in food, stable at different intervals of temperature and pH, and tolerate various conditions and types of processes that are employed, should not have any adverse effect on the consumer, and particularly having a sweetness which is similar or superior to that of sucrose. Isomaltulose is a natural sweetener that has half value in calories than as sucrose, yet their physiological effects varying the sucrose. Due to the economic and nutritional importance of carbohydrates. To achieve this attention should be focused to gene arrangement and expression model to understand how genes interacts with his environment.

Assessment of genetic diversity in Mexican strains of phytopathogen *Clavibacter michiganensis* subsp. *michiganensis*

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Bacterial wilt and canker of tomato caused by actinomycete *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is considered one of the most destructive diseases of tomato worldwide. Previous report showed that *Cmm* pathogenicity trait could vary among tomato cultivars under specific greenhouse conditions, which could be correlated with the level of genetic diversity of *Cmm* strains field isolates found in several countries, however little is known about the genetic and evolutionary basis of *Cmm*. To gain novel insights on the genetic diversity and shaping forces driving evolution of Mexican strains of *Cmm,* we performed the analytical evaluation of genetic diversity and pathogenicity trait through molecular fingerprinting analysis (PFGE) and multi-locus sequence analysis (MLSA), in addition to phenotypic approaches. This study highlights the importance of assessing the biodiversity of wild phytopathogens field isolates, as a novel tool for epidemiological surveillance of crop diseases under environmentally conditions.

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Opaco2 mutant gene and phylogenetic relationships of quality protein maize

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The increase in the essential amino acids lysine and tryptophan in the grain of maize by using the mutant gene opaco2 is a helpful option to develop varieties whose seeds possess protein quality and contribute to the reduction of the chronic malnutrition prevalent in marginalized populations whose diet is largely based on this cultivated specie. Therefore, it was started the development of such modified maize varieties by breeding. Seeds of six inbred lines of maize classified as quality protein maize (QPM) and 15 of its direct single crosses, was subjected to the determinations of tryptophan, lysine, quantity, quality of protein, and also the genetic advance in the crosses in relation to their parental lines mean. The experiments were conducted under a randomized block design with two replications of 100 seeds, the mean comparison was made by the Tukey method, and the genetic advance was analyzed with the ¿t¿ test. It was detected as quality protein maize the M2, M3, and M6 lines. In the crosses, M2 X M6 highlighted by its genetic gain (¿ = 0.01) in protein quantity (2.3%), protein quality index (- 0.5 %), as well, the increases in protein and amino acids levels had an impact on quality index. M1 X M4 and M4 X M5 showed genetic advance acceptable for tryptophan, lysine and quality index, in this case likewise the amount of amino acids affected the protein quality, in contrast with M2 X M4, M3 X M6 y M4 X M6 crosses, with positive genetic gain but only in lysine, tryptophan and protein. It's important to state that by the hybridizing process there was a genetic advantage for essential amino acids and protein, but the lines M2, M3, and M6 well-maintained theirs QPM properties.

Isolation and characterization of a superfamily of candidate disease-resistance genes of the nucleotide binding site (NBS) type from *Cocos nucifera* L.

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Coconut palm (*Cocos nucifera* L.), is a plantation crop ecologically and economically important in tropical regions of world. Unfortunately, is subject to attack by several pathogens, as lethal yellowing disease caused by '*Candidatus* Phytoplasma palme'. Therefore, the characterization of disease resistance genes (R genes) in coconut opens the possibility for developing disease resistance in this crop. The largest known family of plant R-genes encode proteins with nucleotide-binding site (NBS) and C-terminal leucine-rich repeat (LRR) domains. In this study, degenerate primers were used to amplify genomic NBS-type sequences from coconut ecotypes resistant (Malayan Yellow Dwarf and Mexican Pacific Tall 2) and susceptible (Mexican Atlantic Tall) to Lethal Yellowing Diseases. The nucleotide sequence analysis revealed 11 different classes of NBS-type sequences that were identified and designated as resistance gene candidates (RGCs). The predicted amino acid sequences showed that coconut sequences contain all the conserved motif characteristic of the majority of other known plant NBS-LRR resistance genes. Phylogenetic analysis grouped the coconut RGCs sequences with the non-TIR-NBS-LRR receptor subclass on NBS-LRR genes. RGC-specific primers based on non-conserved regions of the NBS domain were developed from a coconut sequence representative of each class. The expression of the RGCs was assessed by qRT-PCR in plantlets treated with salicylic acid (AS). The results revealed a constitutive expression profile and low levels of transcripts in plants untreated, as well as changes in expression in response to AS. This is the first large scale analysis of NBS-LRR in *Cocos nucifera* and are a valuable resource for R-gene discovery, and in the future can be used for development AFLP-RGCs markers applicable for genetic map development and marker assisted selection for defined traits such as pest and disease resistance.

Ecological genomics of the interaction cyanobacteria-cycads in Mexico

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Our interest is to study the symbiotic association between cycad roots and their cyanobacteria. This relationship is not obligated, so that different species of nitrogen-fixing cyanobacteria in soil can be hosted by the plant and probably change with changing environments. In wild Mexican cycads nothing is known about the genetic profile or species identity of their cyanobionts, or their ecological relationship to the ryzhosphere. We have isolated and identified with 16s rRNA cyanobacteria in soil and coralloid roots of two related Mexican cycads species; *Dioon caputoi* and *Dioon merolae* with different distribution and habitat. We hypothesize that different cyanobionts will be found in each species and local environment, or similar species with different metabolic profiles. We isolated 22 cyanobacterial strains from soil and coralloid roots, which correspond genetically to the genera: *Nostoc, Calothrix, Tolypothrix, Synechococcu, Fischerella* and *Microcoleus*. We will discuss findings of traditional classifications subsections of cyanobacteria I to V in our phylogeny, in contrast to reports in literature of only subsection IV.
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Molecular genetic analysis of the mating system of annatto plants (*Bixa orellana* L.) cultivated under different agricultural conditions in the state of Yucatán

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The *Bixa orellana* L. or annatto crop is well-known as the principal source of bixin, a natural pigment accumulated mainly in the seeds. This colouring is used in large quantities by the food industry. However, annatto plants present high quantitative and qualitative phenotypic variability between them, including bixin content and seed numbers. This is likely due to a high rate of outcrossing. Studies of their mating system are therefore required to design improvement strategies, given that this plays a central role in the determination of the genetic structure of a population and is decisive for preserving crop variability. The results of previous studies by our research group on outcrossing performed in an open-pollinated annatto population suggest a high level of allogamy (t_m= 0.748). This study focuses on three agricultural cultivation systems: *solar*, *milpa* and monoculture, in order to evaluate the multilocus outcrossing rate (t_m) in the progenies of annatto (*B. orellana* L.) varieties at each site through the use of Sequence Related Amplified Polymorphism (SRAP) molecular markers. The preliminary result of a rate of t_m=0.872 was greater than obtained previously, which indicates that the outcrossing rate increases as the cultivated annatto plants are closer together. These results will provide information as to what degree this reproductive system is facilitated in the different forms of cultivation and agricultural intensification favouring the maintenance of species variability, thereby contributing to the genetic improvement programme for annatto.

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ISTR markers in the study of genetic variability in cultures of *S. Edule*

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Chayote cultivation is widespread in Mesoamerica. Its introduction in the West Indies and South America took place between the eighteenth and nineteenth centuries, in fact, the first botanical description which mentions the name Sechium is due to P. Brown in 1756, and relates to plants grown in Jamaica. At this time, the chayote was introduced in Europe, from where he was taken to Africa, Asia and Australia, while its introduction in the United States dates from the late nineteenth century. This allows us to corroborate that *S. edule* is a species that was domesticated certainly within the Mesoamerican cultural area, and precisely in the region between southern Mexico and Guatemala (Newstrom, 1990). Chayote is currently at a disadvantage due to the high incidence of diseases, so that aim of this work is to identify the similarity between individuals and populations as it is useful in breeding programs.

Identification of presence absence variation in the landrace Palomero Toluqueño

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Recent increase in the availability of genomic data has revealed the high occurrence of a particular genome structural variant, presence absence variation (PAV; a sequence present in some individuals and missing in others) in maize, and it has been hypothesized that PAV may be functionally significant, possibly playing a role in adaptation to specific environments. In our work, we are studying PAV in the Palomero Toluqueño (PT) maize landrace with respect to the inbreed maize line B73. The aims are 1) to demonstrate the existence of PAV as novel sequences in PT relative to the B73 reference genome 2) to investigate the distribution of PAV sequences within PT accessions and more broadly within the genus *Zea* and 3) to establish tools to map and link PAVs to phenotypic differences in PT. To identify candidate PAV sequences from PT, a subset of contigs has been selected from the transcriptome of a B73 x PT F1 hybrid individual on the basis of its absence from the B73 reference genome and further maize genomic data-sets. From this subset, 12 sequences have been confirmed as high-confidence PAVs on the basis of genomic PCR analysis. The distribution of these high-confidence PAVs was characterized in further PT accessions, other landraces and teosintes (*parviglumis* and *Mexicana*). The analysis of the distribution of these sequences allowed identifying some of them that only belongs to one or other of the subspecies of teosintes and the identification of three PAVs sequences that appears in more frequency in the highland varieties (that grow over 2000 m.a.s.l.) compared with the other non highland varieties of maize tested in this study. B73 x PT mapping populations are also being developed to allow genetic mapping of PAV loci and to investigate linkage to phenotypic traits of agricultural and evolutionary importance.

Characterization of a maize Celaya landrace mutant midrib brown

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Midrib brown mutants have appeared spontaneously or by chemical mutagenesis in maize, sorghum and pearl millet. Generations of hybrids leverage this feature without compromising yield. The forage of these mutants is more easily digestible for livestock, although they are less productive. Another possible application is in the production of ethanol from biomass, as well as a substrate for mushroom cultivation. The mutants loci in maize (*bm1*, *bm3*) and sorghum (*bmr6* and *bmr12*) encode to cinnamyl alcohol dehydrogenase (CAD) and caffeic O-methyl transferasa (COMT). A midrib brown maize mutant from Celaya landrace has been characterized, showing a different composition of the foliage, as well as the relationship lignin-cellulose: wild type 50% cellulose and 50% lignin; the mutant type 38% cellulose and 62% lignin. Components were separated with NaOH. Analysis of extracts on thin layer chromatography also gives a distinct pattern between the wild and the mutant type. Resistance to penetration was wild type stem (culm) 2952 N at start measure and 10933 N at the end; mutant green stem (culm) 2255 N and 7990 N; mutant red stem (culm) 315 N and 13051 N. At present time we have mutant plants S_3 inbred lines.

Literature
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Chen *et al*., 2012. Theor. Appl. Genet 125:1223-1235.

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Biomarker discovery using bottom up analysis: Differential protein accumulation in barley seeds from five Mexican varieties grown under field conditions

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Barley (*Hordeum vulgare*) is an important crop used for the food and beer industries. However, beer production and its quality are affected in part by the barley yield and problems during malting. It is known that both aspects are influenced by the specific protein accumulation during seed development and maturity events. Nonetheless, the knowledge about which proteins cope against these problems is scarce. Thus, identification of proteins related to malting quality will be of great interest for barley breeders and these proteins could be used as molecular markers to select varieties improved for the beer production. Accordingly, five malting commercial varieties were compared at mature seed level. Differences in protein accumulation patterns were assessed by comparative proteomics based on 2D-PAGE and 60 cultivar-differential spots were identified to 42 proteins by nano-electrospray mass. Our results showed that one of the more conspicuous differences among barley varieties were found in the accumulation of proteins such as disulfide isomerase, glutamate descarboxylase and Mildew A proteins. These polypeptides are related with protein folding, nitrogen metabolism and pathogen resistance, respectively. The expression level of these proteins is being further investigated during seed development. According to their function in the above biochemical processes, these proteins represent key players for barley productivity, pathogen resistance and beer production. These results provide a platform for the identification of biological markers as a valuable tool for barley breeders in Mexico.

Variation in environmental conditions leads to an "identity crisis" during bulbil formation in *A. tequilana*

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The perennial monocarpic life-cycle which takes between 5-7 years to complete in *A. tequilana* and the practice of removing developing inflorescences has meant that few breeding programs have been implemented for this species. Traditionally agave plantations are initiated and maintained by planting asexually produced offsets and some selection of useful phenotypes based on somaclonal variation has been carried out. Another abundant source of material useful for propagation and selection purposes are the vegetative bulbils produced on the agave inflorescence when sexual reproduction fails. A deeper knowledge of the mechanisms controlling bulbil formation and development could provide a basis for more cost-effective use of bulbils for propagation purposes and the development of phenotypically variable materials which could be exploited for selection and improvement of *A. tequilana*. By induction of bulbil formation it has been shown that the plant hormone auxin is a key player in bulbil formation and that variation in environmental conditions can lead to changes in gene expression which determine the formation of either vegetative bulbils or determinate non-viable floral structures.

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Integrating the signalling networks that trigger programmed cell death in self-incompatible *Papaver* pollen

Noni Franklin-Tong

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Self-incompatibility (SI) is a genetically-controlled mechanism used by many angiosperms to prevent self-fertilization and inbreeding. A multi-allelic *S* locus allows discrimination between "self" (incompatible) pollen from "non-self" pollen on the stigma. Interaction of matching pollen and pistil S-determinants allows "self" recognition and triggers rejection of incompatible pollen. The S-determinants for *Papaver rhoeas* (poppy) are PrsS and PrpS. PrsS is a small novel protein that acts as a signalling ligand that interacts with its cognate pollen S-determinant PrpS, a small novel transmembrane protein. Interaction of PrsS with incompatible pollen stimulates increases in cytosolic free Ca^{2+} and influx of Ca^{2+} and K^{+}. ROS and NO signals are also implicated. Downstream targets include the cytoskeleton, a soluble inorganic pyrophosphatase, and a MAP kinase, PrMPK9. The major focus for SI signals is initiation of programmed cell death (PCD). I will provide an overview of our understanding of how PCD in this system operates, focusing on how the signals and components are integrated. I will also discuss our recent functional expression of PrpS in *Arabidopsis thaliana* pollen.

CICLINAS DE Gl EN MAIZ. CICLINAS D Y LA GERMINACION

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Las ciclinas tipo D son proteínas fundamentales ya que permiten que las señales extracelulares, presentes en el medio ambiente en que las células se desarrollan, sean interpretadas intracelularmente en términos de la capacidad para proliferar. Todos los organismos pluricelulares contienen ciclinas tipo D, aunque su número puede variar. Mientras que las células de mamíferos contienen tres tipos diferentes (con opciones de splicing alternativo), las plantas pueden contener múltiples, llegando al caso de maíz que contiene al menos 17 genes diferentes. Nuestros estudios han mostrado que la gran mayoría de esos genes se expresan en diferentes tejidos de la planta, y lo hacen de manera diferencial. El desarrollo de anticuerpos contra varias de las proteínas tipo ciclina D ha permitido estudiar su ontogenia, las asociaciones que mantienen con las proteínas cinasas dependientes de ciclina (CDKs), que son esenciales para la regulación y avance del ciclo celular, así como una activación diferencial de la actividad de cinasa en estos complejos durante el proceso germinativo. Dicha activación podría depender del estado de fosforilación que mantienen las CDKs, sea en residuos que estimulan la actividad de cinasa, o bien en residuos que la reprimen. Estudios preliminares han establecido que se requiere de la fosforilación de las CDKs para desarrollar actividad y que existe un estado diferencial de fosforilación de los diferentes residuos a lo largo del proceso germinativo, que podría variar dependiendo de la ciclina D con que se asocie. En este sentido, no pareciera que las diferentes ciclinas D realicen una función redundante.

Poster Sessions

Odd numbers Tuesday 22

Even numbers Thursday 24

PLANT MICROBE AND INSECT INTERACTIONS

1.

Expression and silencing of the gene CABPR-1 in *Capsicum annuum* Morelos 334 during interaction with *Phytophthora capsici*

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Pepper (*Capsicum* spp.) is a vegetable which has a great tradition in the diet and culture of the Mexican population. Mexico ranks second in global production of this species. Culture of *Capsicum annuum* is affected by multiple pathogens, such as insects, viruses, bacteria, fungi, Oomycetes, causing total losses in the production of this vegetable. Among the main pathogens highlights the oomycete *Phytophthora capsici*, causal agent of the disease known as "wilt of pepper". This disease was reported by Leonian (1922) in New Mexico, and it is characterized by a premature death of the plant, the infection occurs in the roots or the base of the stem, especially in irrigated fields. The first symptom is a wilting of the leaves without changes in its color, which are finally hung the petioles; the base of the stem shows a greenish brown spot. *Capsicum annuum* Morelos 334 (CM-334) is resistant to the disease caused by *P. capsici*, without knowing which genes are involved. It was reported that *CABPR-1* encodes for a protein related in the pathogenesis (PR) in chili. It is believed that the main role of *CABPR-1* is related to the biotic and abiotic stress tolerance, this gene was expressed in transgenic plants of *Nicotiana tabacum* cv. *Xanthi*, giving an increase of heavy metal tolerance and also to the pathogen *Phytophthora nicotianae* and bacteria *Ralstonia solanacearum* and *Pseudomonas syringae* pv. *tobacco*. The study of genes *of C. annuum* Morelos 334 (CM-334) which confer resistance to *P. capsici* presents an alternative to the development of resistant commercial varieties. The objective of the study is to silence *CABPR-1* in CM-334 pepper plants in order to demonstrate its role in resistance to *P. capsici*. T o accomplish that aim, we have been used (V-VIGS) based on PHYVV vector.

2.

Contribution of long chain bases to the pattern-triggered and effector-triggered immunity in *Arabidopsis thaliana*

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Long chain bases (LCB) participate as signaling molecules during the programmed cell death in defense against pathogens (Saucedo-García *et al.*, 2011 New Phytol. 191:943–957). In the context of mechanisms of plant defense, it is known that there are two types of immunity. One, the Pattern-Triggered Immunity (PTI), is elicited upon the perception of pathogen associated molecular patterns (PAMPs) and leads to a basal defense response. Other is the Effector-Triggered Immunity (ETI) in which the detection of pathogen effectors by resistance proteins of the plant cell elicits very specific and robust defense machinery associated with the Hypersensitive Response (HR) (Morel and Dangl, 1997 Cell Death Differ 4:671-683). The objective of this study was to determine the contribution of LCB as signaling molecules to both types of immunity. In order to address this question, we used Arabidopsis mutants impaired in the expression of transducers of the signaling network mediated by LCB. These mutants were exposed to FB1 or to the avirulent and virulent strain of *P. syringae* or to the PAMPS flagellin/flg22 and xylanase. These treatments were evaluated by the analysis of the phenotypical lesion, determination of bacterial growth, detection of H_2O_2 and analysis of defense gene expression. Phenotypic analyses and bacterial growth experiments showed that LCB are implicated in the ETI immunity. Moreover, the exposure of the different mutants to the PAMP xylanase revealed a possible role of LCB in modulating a signaling pathway that involves MPK6 and ERO as signaling elements, and therefore, implying LCB with the PTI. The analysis of gene expression suggested that genes associated with the jasmonic/ethylene pathway are involved in the response to LCB accumulation. These results indicate that LCB are implicated in both types of immunity, however more experiments are needed to elucidate the magnitude of this contribution in each case.

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3.

Detección y caracterización de bacterias nitrificantes en frijol común, para la elaboración de un biofertilizante en la Zona Noreste de México

Jesús Gerardo García Olivares[1], José Luis Hernández Mendoza[1], Amanda Alejandra Oliva Hernández[1], Jesús Di Carlo Quiroz Velásquez[1], Cecilia Vázquez Rodriguez[2], Edgar Gómez Olivares[2].

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The bean (*Phaseolus vulgaris L.*) is a plant of great importance in the diet of the Mexican population. Is considered an production alternative within a crop rotation and it is estimated that at the end of the growing season, this provides 30 % residual nitrogen in the soil. The chemical fertilizer decreases the activity of nitrogen fixation bacterias wich nodule the root of the plant and can be native or incorporated by the employment of biofertilizer. Were selected bean root nodules from Flor de Mayo, were macerated and were made dilutions with agar Luria-Bertani (LB), at a concentration of 1x109 cfu/mL-1. At 24 h, were placed in plastic trays with substrate (Peat Moss) sterile. Heights and weights were taken from the root plant, 15, 30 and 45 days after sowing and seeded in Pikovskaya's agar, with purple Bromcresol to select by assimilation of phosphorus. The statistical analysis was performed using SAS 6.03. Morphological detection and molecular screening was performed by PCR amplification of ribosomal 16s. The bacterias formed nodules selectively, in Flor de Mayo CFJM-5, 6, 14, 17 and 18 in Pinto Saltillo CFJP-3, 4, 7, 8 and 10. Were detected significant differences between isolates and production parameters and phosphorus assimilation. The molecular identification showed wich bacteria sequences common bean, amplified belong to the genus Pseudomonas sp. which have the property of producing different substances whose main advantages are to encourage germination of the seeds, accelerating the growth of plants particularly in its early stages, induce root initiation and increase the formation of roots and root hairs, as found in the present investigation.

4.

Isolation and characterization of filamentous fungi associated to metal resistance plant rhizosphere

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The mine tailings are a contaminated soils as result of the extraction processes of mining, this soils containing toxic metals such as Cu, Zn, Pb, Cd, Co, Ni , Cr, Mn and metalloids as As. Plants growing in this mixture of heavy metals and low organic matter can release CO^2 and root exudates increasing the metabolic microorganism activity on the plant and soil. The rhizospheric fungi can help to plant, mobilizing nutriments and produce molecules for enhance the growth plant and heavy metal resistance. The objective of this work is study the diversity of rhizosphere fungi from native plants growing on mine tailings, and the possibility for use this microorganism for biorremediation, and biotechnology. Six plants were collected (*Prosopis strombulifera*, *Spharealcea angustifolia*, *Tithonia diversifolia, Flaveria angustifolia*, *Bahia absinthifolia* and *Asclepia linaria*) in 2 zones (zone 1 mine tailing, zone 2 city) in Villa de la Paz San Luis Potosi . The rhizospheric fungi were obtained for serial dilutions in PDA media. For enzimatic characterization, amylases, pectinases, cellullases, xylanases, phosphate solubilazing and salt and pH tolerance were evaluated. The Heavy metal resistance assays was evaluated with Castañeda media added the metal in three concentration (5, 12.5 and 25 mM). Seventy four rhizospheric fungi were isolated, 36 for zone 1 and 38 for zone 2. The principal components analysis (PCA) for enzimatic activity shown the zone 1 presents the major activities than zone 2. The fungi isolated of *Spharealcea angustifolia* have the principal activities in both zones. The fungi isolated from zone 1 presents high resistance for all heavy metals than zone 2.

5.

The Diguanylate Cyclase (DGC-E) modulates bacteria growth in *Azospirillum brasilense*

Adriana Gamboa-Pérez[1], Alberto Ramírez-Mata[1], Araceli Romero-Pérez[2], María Luisa Xiqui Vázquez[1], and Beatriz Eugenia. Baca[1,2]

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In the last 35 years there has been a special interest in studying the mechanisms of interaction between microorganism-plant that promotes the growth of the latter. *Azospirillum brasilense* is a bacterium associated with plants, this one knowns how plant-growth promoting rhizobacterium (PGPR) that provides beneficial effects on plant growth. To take advantage of positive effects, the bacterium intimately attaches to root surfaces and promotes root colonization. The formation of complex bacterial communities known as biofilms begins with the interaction of planktonic cells with a surface in response to appropriate environmental or endogenous signals. Cyclic di-GMP (c-di-GMP) is a broadly conserved, intracellular second-messenger molecule that regulates biofilm formation by many bacteria. The synthesis of c-di-GMP is catalyzed by diguanylate cyclases (DGCs) containing the GGDEF domain, while its degradation is achieved through the phosphodiesterase activities of EAL and HD-GYP domains. To determine the role of an ORF which shows GGDEF domain in cellular physiology, a null mutant was constructed, inserting a kanamycin-GusA resistance cassette. DgcE (diguanylate cyclase E) is a multidomain protein with two N-terminal receiver modules arranged in tandem and HPT domain, as well as C-terminal domain apparently serving as an output module harboring a GGDEF domain. Here we present evidence that a novel-type response regulator contributes to in cell morphology through the production of a novel signaling molecule, the cyclic bi-GMP. Finally the affectation of DgcE can effect on the regulation of c-di-GMP, and for consequence this could influence in the process of bacterial colonization.

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6.

Interaction study of tomato-*Ralstonia solanacearum*-φITL-1 and evaluation of the stability of bacteriophage in different substrates and water sources

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Bacterial wilt caused by Ralstonia solanacearum, is one of the most destructive bacterial diseases of major economic importance, affecting more than 200 plant species and is endemic to countries in tropical and subtropical areas. The proposed management practices have had little success, given the genomic complexity that has the bacteria. The lytic bacteriophages are a viable alternative to the management presenting variants of the disease and the difficulty of knowing the behavior of the bacteria The aim of this study was to examine the interaction tomato-*R.solanacearum*-ΦITL-1 under the conditions of production in Morelos. An experiment was conducted to determine the preventive and curative effect of the addition ΦITL-and analyzed the characteristics of the substrate and the source of irrigation water. The results showed that the optimal time for maximum concentration during viral infection ΦITL-1 was 5 hours after inoculation of *R. solanacearum* with an MOI = 1.On the substrate as the substrate was found to be inactivated peat moss phage possibly the effect of pH (4.68). The water source influences phage viability, obtaining a title in distilled water in tap water.

7.

Cloning and partial characterization of a putative NIK1, a two component histidine kinase from *Mycosphaerella fijiensis*

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The fungus *Mycosphaerella fijiensis* is the causative agent of black Sigatoka, one of the most destructive diseases of banana plants. Infection with this pathogen results in death of leaves thus causing important decreases in fruit production. The two-component histidine kinases (HKs) regulate responses to environmental stimuli in bacteria and eukaryotes, including yeasts, plants, slime moulds and filamentous fungi. In filamentous fungi, HKs are classified into 11 groups based on the protein sequence, and only members of HK class III have been associated with responses to high osmolarity; these HKs include Os-1 in *Neurospora crassa*, NikA in *Aspergillus nidulans*, Bos1 in *Botrytis cinerea*, Nik1 in *Alternaria brassicicola* and *Cochliobolus heterostrophus* and Hik1 in *Magnaporthe oryzae*. In addition, mutations in class III HKs can result in fungicide resistance and morphological defects. As we were interested to test if *M. fijiensis* perceives fungicides as osmotic agents besides toxic compounds, we used a 4% NaCl treatment to induce and clone the putative NIK1 gene from *M. fijiensis*. After DNA sequencing, assembling of contig and blastn in the database of *M. fijiensis* genome, it was observed a strong homology with MycFi located in scaffold 4, corresponding to one histidine kinase (E-value 0.0). The blastx with the amino acids translated *in silico* against NCBI gene database revealed a high homology (E-value 0.0; identity 93%) with MycFiDraft, an hypothetical protein from *P. fijiensis* CIRAD86. Expression analysis of the putative NIK1 treated with NaCl or benomyl (a fungicide), showed that the fungus strongly induces the gene expression in response to osmotic stress but only at basal levels against the fungicide.

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8.

Endophytic and epiphytic microbial diversity assessments in *Citrus limon* var eureka

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To know the changes that occur on endophytic microbial diversity in plants constitutes one of main challenges involved with physiological and agro economical analysis of important crops. Assessment of these microbial community have been very limited since most methods are nor optimized for a reliable DNA microbial isolation with regarding to huge plant DNA amounts associated. *Citrus limon* var Eureka is the second economically main crop cultivated in Tamaulipas, Mexico (SAGARPA 2004). Therefore, this study deals on the establishment of an endophytic/epiphytic analysis to assess the microbial community associated to this important crop. For this, it was evaluated by directly and microbial enrichment cultivated methods the microflore associated to different plant tissues (leaves, fruits and flowers). UFC counts were analyzed at different dilutions ($1x10^{-0}$, $1x10^{-1}$, and $1x10^{-2}$, $1x10^{-3}$, and $1x10^{-4}$) by using different broths (MRS, AST, PDA, AN) directly for fungus and in different plant homogenized for bacteria. These last were building by using supernatants and pellets of different speed centrifugations (600, 15,000 and 100,000g). Finally, direct and enrichment microbial sequencing analysis was carried out. The enrichment was conducted by take pool scraped colonies, which were re-suspended (0.5 g) in 1 ml of TEN and purified by silica affinity (Rojas-Herrera *et al*., 2008), and then amplified using universal COM primers to make SSCP analysis (Schwieger and Tebbe 1998). Direct automatic sequencing analysis was conducted in ABI 377 DNA sequencer. UFC/ml counts obtained by direct homogenization (endophytic) as for sterile tissue washed (epiphyte) were clearly compared at $1X10^{-1}$ dilution for leaves and flowers ranged of 100 to 2000. Counts in fruits were conducted without dilution with values of 10 to 100 colonies of according to assayed broth. Fungal colonies recovered from $1x10^{-1}$ dilution in leaves and flower, and directly in fruits were re-isolated and purified to sequencing analysis. Differently, bacterial cultures were enrichment by previous different centrifugations being pellets (at 600, and at 15,000 g) the more reliable. For either fungal as bacteria culture the AN broth was discarded since it has a limited colony number. In general, it was recovered more UFC counts for epiphytic microorganism with until three times more than endophytic fraction. So far, current study has preliminary allowed identify bacteria of the genus *Pantoea* sp., *Erwinia* sp., *Azospirillum* sp., and other *Proteobacteria* sp. which have already been reported in other citrus specie and sugar cane (Araújo *et al.*, 2002;. Polanczyk and Alves, 2003).

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9.

Target of rapamycin downregulation impairs arbuscular mycorrhizal colonization in *Phaseolus vulgaris*

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Target of rapamycin (TOR) kinase is a master regulator evolutionarily conserved from yeasts to plants and human, that integrates nutrient and energy signaling to promote cell proliferation and growth. TOR regulates numerous biological processes, including transcription and translation of ribosomal components, which collectively contributes to cell growth. Arbuscular mycorrhizal fungi (AMF) a plant symbiotic partner contributes to P and N uptake and TOR being a nutrient sensor it was necessary to address the role of TOR in the symbiotic association. In the current report we assessed the role of TOR during AMF symbiotic association between *Phaseolus vulgaris*-*Rhizophagus irregularis* utilizing the RNAi approach in *P. vulgaris* hairy root system. The results obtained indicate that hairy roots with downregulated TOR transcripts significantly increased the infection units of AMF in mycorrhized roots. Furthermore, these roots also displayed extensive extra and intra radical hyphae. Interestingly, the intra radical hyphae fail to differentiate into fully developed (mature) arbuscles. Moreover, the expression levels of *PvPT4* and *PvAMT* transcripts were also decreased significantly in mycorrhized TOR-downregulated roots. Thus, we concluded that TOR acts as a positive regulator of mycorrhizal symbiosis and it is required for the arbuscule maturation.

10.

Molecular cloning and characterization of a novel NPRl-like gene from coconut palm (*Cocos nucifera* L.)

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The coconut (*Cocos nucifera* L.), as a portable source of food , water, fuel, and construction materials, played a fundamental role in human and development of civilization across the humid tropics. It is widely cultivated in Mexico in about 80,000 ha. At present, lethal yellowing (LY) disease has destroyed the plantations in Mexico. However, plants have evolved complicated defense mechanisms against various pathogens. One of such responses is known as systemic acquired resistance (SAR), often triggered by a local infection, can provide long-lasting resistance throughout the plant and to be effective against a broad range of pathogens, including fungi, bacteria, and viruses. Multiple studies in both monocots and dicots have shown that salicylic acid (SA) play a central role as a signaling molecule in the activation of SAR, this activation correlates with the expression of the pathogenesis-related (*PR*) genes. The non-expressor of PR genes (*NPR1*) functions as the regulator of SA-mediated SAR. *NPR1* gene encodes a protein containing ankyrin repeats and a BTB/POZ domain, both of which mediate protein-protein interactions. Previous studies have documented that *NPR1* belong to a multi-gene family in the genome of many plant species (six *NPR1*-like genes in *Arabidopsis* and five in rice genome). *NPR1* and its homologous genes widely exist and confer resistance to pathogens in many plant species. In this study, we report the molecular cloning and characterization of *Arabidopsis NPR3* homolog from *Cocos nucifera* L. (designated as *CnNPR3*), which is a *NPR1*-like gene. The analysis of the deduced amino acid sequence showed *CnNPR3* gene contains an ankyrin repeat domain and BTB/POZ domain. Phylogenetic analysis showed that CnNPR3 belongs to the NPR1-like subgroup in which AtNPR3 in *Arabidopsis* is its closest homologues. Taken together, these results suggest that *CnNPR3* could be a *NPR1*-like gene of *Cocos nucifera* L. In addition, these similarities in protein structure suggest that CnNPR3 may also share the same function as AtNPR3 during plant defense response.

11.

Activation of the ethylene signaling pathway impairs the disease resistance of *Capsicum chinense* against *Fusarium oxysporum*

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The specificity of plant responses against pathogens is exerted by the cross talk between the major defense-related hormones, Salicylic and Jasmonic acids (SA and JA, respectively). For instance, activation of the SA pathway generally blocks the JA pathway, and *vice versa*. Ethylene (Et) is an important modulator of this specific cross talk. We demonstrated that previous activation of the Et pathway turned the habanero pepper resistant against the deadly pathogen *Phytophthora capsici*. Because the turning-on of a specific pathway could lead to the shutdown of antagonic pathways, we wanted to know the effect of this Et "protection" over non-pathogen microbes. For that purpose, we sprayed pepper seedlings *in vitro* with ethylene and evaluated its effect over the inoculation with *Fusarium oxysporum,* a non-pathogen microorganism. The inoculation of habanero pepper seedlings with a non-virulent strain of *Fusarium oxysporum* caused no observable symptoms in more than 10 days; however, if plants were previously sprayed with ethylene, the pathogen developed a systemic infection that killed the seedlings in less than 14 days. The analysis of a homologous set of defense-related genes demonstrated that habanero pepper displayed a defense response against F. oxysporum, which is blocked by the action of ethylene, coinciding with the repression of most of the evaluated genes. These results suggested that the naturally resistance of *Capsicum chinense* could be a SA-mediated response, which is antagonically blocked by the action of ethylene.

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12.

Immunoassays of relevant epitopes of *Bordetella pertussis* produced in plants

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Bordetella pertussis is the causative agent of pertussis. To reduce the transmission of pertussis is necessary to improve the current vaccine to produce a long-lasting antibodies response. Two constructs were designed with optimized synthetic genes to be expressed in plants, which encode protein relevant epitopes of pertactin, pertussis toxin, filamentous hemagglutinin and a histidine tag for purification. The sequences were optimized for stable expression in cytoplasm via *Agrobacterium tumefaciens*, as well as for chloroplast transformation via biobalistic. The synthetic gene for nuclear transformation has in addition an adjuvant sequence and a signal peptide, and low nicotine transgenic tobacco was used for transformation via *A. tumefaciens.* The putative transgenic material will be used for future analyses. In addition, this construct was cloned into a Magnifection vector for transient expression in tobacco plants; the material was analyzed by PCR, Western blot, and it will be used for mice immunization. One line was obtained from tobacco chloroplast transformation and was confirmed by PCR assay. In order to produce a control, chloroplast synthetic gene was also expressed in a bacterial system and the protein was purified and detected by Western bolt assay.

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13.
Fungicides on mycorrhizal colonization in soybean and bean seedling

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Fungicide treatment practice on seed can affect mycorrhizal fungi effectiveness. The effect of fungicides on mycorrhizal colonization of soybean (Vernal) and bean (Pinto Americano) seed was studied in greenhouse. Seeds were applied with commercial doses of chlorothalonil, quintozene+thiram, metalaxyl, carboxin+thiram, benomyl, quintozene, captan and mancozeb, and inoculated with *Rhizophagus intraradices* (40 spores/g) at doses of 1, 2 and 3 kg/ha, and control (absolute). Fungicides affect mycorrhization for soybean and bean. For soybean only clorothalonil, metalaxyl, carboxin+thiram and benomyl are recommended for treatments at one dose, while for bean chlorothalonil at three doses.

14.

Arabidopsis thaliana, an experimental host for two papaya viruses: potexvirus (PapMV) and potyvirus (PRSV).

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Papaya mosaic virus (PapMV) and *Papaya ringspot virus* (PRSV) belong to the poty and potex genera respectively with positive ssRNA polyadenylated at the 3' end. At the 5' end, PapMV is capped whereas PRSV has a **v**iral **p**rotein linked to the **g**enome (VPg). Several families have been experimentally infected with PapMV and PRSV. Among them are the *Caricaceae* and *Cucurbitaceae* infected with PapMV and *Cucurbitaceae* and *Amaranthaceae* infected with PRSV. Other species as *Catharanthus, Zinnia, Gommphrena* and *Nicotiana* can be infected with PapMV and finally, *Thumbergia* and *Momordica* only with PRSV. Cooper *et al* in 2003 reported *Arabidopsis thaliana* as a susceptible plant for *Potato virus X* (PVX), a *Potexvirus*. In order to use *A. thaliana* as an experimental host for our PRSV and PapMV studies of infection and resistance mechanisms, we used members of the family of plant translation initiation factors eIF4E and eIF(iso)4E. These are key elements for the infection of RNA viruses and are being used as resistance elements in the control of the latter. These factors contact and bind to the *Cap* structure of the mRNA (or the VPg), and recruit the components necessary for the translation initiation complex. *A. thaliana* transgenic lines on a Col-0 ecotype background over-expressing the papaya eIF4E or its isoform, were tested for susceptibility to both viruses. Also, the ecotypes Col-0, *Landsberg erecta* (Ler), C24 and a null mutant line for *A. thaliana* eIF(iso)4E-1 were also tested. Viral cistrons were detected by RT-PCR for PapMV in wild ecotypes. However, the wild ecotypes were resistant to PRSV infection, in contrast to our transgenic lines over-expressing papaya eIF4Es, which resulted susceptible. This study demonstrates that *A. thaliana* Col-0 over-expressing papaya eIF4E or eIF(iso)4E could be use as an experimental host for PapMV and PRSV to understand molecular aspects of host-virus interactions in single and mixed infections.

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15.

Incidence of single and mixed viral infections in strawberry fields in central Mexico

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Mexico is the fifth strawberry producer worldwide, however single and mixed viral infections, among other causes, are lowering fruit yields. Strawberry mottle virus (SMoV) and Strawberry crinkle virus (SCV) were found for the first time in 1989 in strawberry fields of Irapuato and Zamora counties in Guanajuato and Michoacan States, respectively. Later, Strawberry latent ringspot virus (SLRSV) was also found in 2004. Symptoms associated to viral disease such as: stunting, mild chlorosis and reddening, wrinkled, curled, and deformed leaves that may exhibit mottling, and chlorotic spots were shown in strawberry plants collected in April and December 2007, July and December 2008, and July 2013. The amplification by RT-PCR and sequencing of viral fragments revealed the presence of seven viral species in the samples collected. These were SCV, SMoV, Fragaria chiloensis cryptic virus (FClCV), Fragaria chiloensis latent virus (FClLV), Strawberry pallidosis associated virus (SPaV), Strawberry necrotic shock virus (SNSV), and Strawberry mild yellow edge virus (SMYEV). The last five were found for the first time in strawberry production fields in Mexico. A couple of susceptible strawberry lines were used as indicators plants. All seven viruses were detected in single or mixed infections, where SMoV was the most commonly found mixed with other viruses. This work is the first report of five viral species in strawberry fields in Mexico. In future work viral diversity that affects strawberry production, will be studied.

16.

Development of a VIGS vector from tomato mosaic virus and its use to determine the importance of SUMO E2 conjugating enzyme (SCEI) and Pleiotropic drug resistance protein (PDR) in resistance against *Clavibacter michiganensis* subsp. michiganensis in *Solanum* spp.

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Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is a causal agent of bacterial wilt and canker disease in *Solanum lycopersicum*. *S. peruvianum* and *S. habrochaites* are wild type relative species resistant to *Cmm*. The SUMO E2 conjugating enzyme (*SCE*I) candidate gene was identified by cDNA-AFLP and may be involved in *Cmm*–resistance in *S. peruvianum.* Also in *S. habrochaites* we identified the Pleiotropic Drug Resistance gene (*PDR*). *SCE*I from *S. peruvianum* and *PDR* from *S. habrochaites* were cloned into a novel developed virus-induced gene silencing (VIGS) vector based on the geminivirus Tomato Mottle Virus (ToMoV). The utility of ToMoV for VIGS in *Solanum spp.* was demonstrated by introducing the vector by biolistic and silencing the magnesium chelatase gene as a control, resulted in leaf bleaching. The *SCE*I and *PDR* silenced plants were challenged with *Cmm*, and 15 days post-challenge, the *S. peruvianum* plants showed disease symptoms as unilateral wilting and eventually died. In conclusion *SCEI* and *PDR* might have an important role in the resistance against *Cmm* in tomato.

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17.

Functional analysis of SpRAP2.4 gene in *Solanum peruvianum* and its potential function in bacterial canker defense

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Virus-induced gene silencing (VIGS) is a reliable system to down-regulate gene expression and study genes involved in defense against pathogens. *Clavibacter michiganensis* subsp*. michiganensis (Cmm)* is a causal agent of wilt and canker in *Solanum lycopersicum. Solanum peruvianum* is a wild tomato specie resistant to *Cmm.* A cDNA-AFLP fragment of SpRAP2.4 gene encoding a putative ERF transcription factor was cloned into a novel VIGS vector, and analysis of silenced *S. peruvianum* plants was performed in order to test whether this gene is involved in defense mechanisms against *Cmm*. SpRAP2.4-silenced plants were challenged with *Cmm* and displayed disease symptoms and eventually died. SpRAP2.4 might participate in resistance against *Cmm* by inducing defense-related genes to suppress *Cmm* growth *in planta*.

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18.

Analysis of two expressed genes in the avirulent interaction between *Rhizoctonia* sp. binucleate-*Capsicum annum*

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Capsicum annum (chili or hot pepper) is an important crop in Mexico that is attacked by several phytopathogens, causing a decrease in the productivity and increasing the production costs. Damping-off is the most lost generating disease of *C. annum*, it is caused by a phytopathogens fungus consortium that including *Rhizoctonia solani*. *R. sp.* binucleate has been proposed for damping-off biological control. Since plants triggers changes in signaling pathways and in gene expression in response to beneficial and pathogenic organisms, to understand the benefit relation *C. annum*-*R. sp.* binucleate, a bioinformatic analysis searching genes related to these biological processes in a subtracted cDNA library was done. It was found two candidate genes, a WRKY-like transcription factor (WRKY; pfam03106) and a putative LRR receptor protein kinase (LRR; cd00180). Molecular modeling of WRKY domain and catalytic kinase domain of LRR protein suggested that those proteins can be functional. Additionally, *C. annuum* plantlets of 15 days old were subjected to inoculation in the root with an avirulent isolate of *R. solani,* and the expression patterns of a WRKY and an LRR-like genes by using qRT-PCR at 8 and 16 hours post-inoculation (hpi) were analyzed. Under normal growth conditions (control), WRKY-like gene expression appeared to be with moderate level expression and a decrease of 28% from 8 to 16 hpi, whereas the R-like gene showed 1.8 fold increase at 8 hpi in root-microorganism interaction compared to control condition, and later at 16 hpi the level expression descends to 11%. These findings suggest that at least LRR-like protein has a predominant role in protective effect of *C. annum* triggers by *R. sp* binucleate against *R. solani*.

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19.
Expression of endocytic genes in *Phaseolus vulgaris* root hairs

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Formation of nitrogen-fixing nodules, as a result of the symbiotic interaction between legumes and rhizobia, is a tightly regulated process. Nodulation is initiated when bacteria belonging to the Rhizobiaceae family and legume roots establish a specific molecular dialogue that triggers a series of coordinated, although complex molecular, cellular and physiological responses in root hair cells. It is well known that only actively growing root cells are susceptible to interact with rhizobia and initiate the bacterial invasion process that leads to nodulation. Polarized growth of root hairs is a highly coordinated process, which is restricted to the apical zone of the cell and depends on a dynamic activity of the apical cytoskeleton, vesicular trafficking, exocytosis and endocytosis. To gain insights into the relationship between root hair endocytosis and the molecular dialogue that initiates the nodulation, we have investigated the expression of a battery of genes involved in clathrin-mediated endocytosis in *P. vulgaris* root hairs. An exhaustive i*n silico* analysis of the *P. vulgaris* genome sequences allowed us to identify a group of genes of interest to further assess their expression by RT-qPCR. As some of the endocytic genes are multigenic, we have extended our analysis to RNA samples from root apex and stripped root (with no root hairs, no apex).

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20.

Effectivness of an arbuscular mycorrhizal fungi consortium from a geothermal area on growth of native maize under drought stress.

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This study explored the influence of a consortium of arbuscular mycorrhizal fungi (AMF) from a geothermal area located in Ixtlán de los Hervores, Michoacán on drought tolerance of native maize plants var. white 117 in terms of plant growth. The treatments were: 1) routine water supply, 2) moderated drought stress (70% water hold capacity of soli WHC) and 3) severe drought stress (80 % WHC), in plants treated or not with AMF consortium. Biomass, leaf chlorophyll and proline N, K, and P contents were determined after ten weeks after sowing. The results showed that plants inoculated with AMF under moderated drought stress, had a bigger biomass than the non-inoculated plants under the same irrigation regime. Proline and clorophyll contents were greater in plants with AMF than those in non-inoculated plants. Nutrimental content of plants under drought stress were similar in all treatments. In conclusion, the inoculation of this native maize with the AMF consortium from a geothermal area could lead to grow properly this crop under drought stress.

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21.

Analysis of Pvu-miR1514a and its function during drought stress in *Phaseolus vulgaris*

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miRNAs are small non-coding RNAs (19-24 nt) that post-transcriptionally regulate transcripts by inducing their degradation or by repressing their translation in plants, so its characterization is essential to understand the mechanism of gene regulation in legumes under drought. We focused on common bean because is the second most important crop worldwide and is the first supply of protein in the Mexican diet population, and the drought is one of the mayor causes of crop losses. The Pvu-miR1514a is a non-conserved microRNA present in *Phaseolus vulgaris* (common bean) and other legumes species that are induced in drought stress response. We have seen Pvu-miR1514a has a differential expression levels in roots during drought, in two common bean cultivars with different drought tolerance levels; *P. vulgaris* var. Pinto Saltillo is more resistant to drought stress than *P. vulgaris* var. Bayo Madero. A bioinformatic analysis predicted that the NAC-family Transcription Factor phuv.010g120700 should be a target of Pvu-miR1514a and this interaction could generate NAC-derived ta-siRNAs. We have seen that Pvu-miR1514a accumulates in drought stress and the NAC-family Transcription Factor phuv.010g120700 is down-regulated. Furthermore when we over-express the Pvu-miR1514a in transgenic hairy roots, we also detect the differential accumulation of NAC-TF and NAC-derived ta-siRNA by qPCR. At present, we are confirming that phuv.010g120700 is targeted by Pvu-miR1514a and exploring whether the ta-siRNAs are functional by using genetic approaches to understand their function in roots of *Phaseolus vulgaris* during water deficit induced under drought conditions.

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22.

Different expression patterns of Pb-inducible genes between shoots and roots, when the Pb-hyperaccumulator aquatic plant *Salvinia minima* is exposed to Pb

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Using a suppression subtractive hybridization (SSH) library, we were able to identify 365 sequences that expressed differentially when *Salvinia minima* plants were exposed to 40 uM lead nitrate for 12h. From those sequences, 6 genes were selected according to bioinformatic criteria and their putative function, to further analyze their expression in different plant parts and during different times of exposure to Pb. This time, primers were designed for the 6 selected genes and the expression patterns (using RT-PCR) were analyzed separately for roots and shoots, when plants were exposed to Pb during different exposure periods. Important differences occurred in the expression patterns of some of these genes between shoots and roots. For instance, in roots the genes encoding selenium binding protein (SmSeBP) and glutamine synthetase (SmGS1) were not expressed in control plants, but they were expressed in just 30 minutes after being exposed to Pb, while in shoots, this gene showed a constitutive expression. On the contrary, in leaves, the gene encoding a SmABC-transporter was not expressed in control plants, but it was rapidly expressed in response to Pb exposure, while in roots, this gene showed a constitutive expression. This might reflect a coordinated mechanism between roots and shoots to tolerate high concentrations of Pb, while maintaining an uptake of the metal, in this Pb-hyperaccumulator plant

23.
Accumulation of Iodine in tomato plants

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Iodine is not an essential element in plants, but is an essential element for humans because is an important component in thyroid hormones. Nearly $2X10^9$ people worldwide still receive low levels of dietary iodine causing iodine deficiency disorder (IDD). To decrease the deficit in the consumption of iodine have been used several techniques as salt, oil and bread iodization, but these methods have not eliminated IDDs. There are several recent advances in the area of iodine biofortification in different crops; the objective is to carry the iodine to editable parts of plants. Vegetables can accumulate iodine, when it is applied to soil or foliar spray, but the results depending on the concentration and chemical form in which this element is applied. Were applied two concentrations of iodine in tomato plants: 10^{-4} M of KI and KIO_3 every 15 days by foliar spray and 10^{-6} of KI and KIO_3 daily applied to soil by the irrigation water. Iodine accumulation was monitored in leaves, steam and roots tissue. The greatest iodine accumulation in tomato plants was found with the application of KIO_3. The foliar spray resulted in the accumulation of iodine in leaves and stem, while when applied in soil by irrigation resulted in the accumulation in fruits and stems.

24.

Tissular localization of a stress protein induced by water deficit in *Arabidopsis thaliana*: group 4 LEA protein

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LEA (*Late embryogenesis abundant*) proteins play crucial roles in dehydration tolerance in plants. They accumulate during the last stages of seed development and in vegetative tissues in plants under water deficit. The focus of the research on LEA proteins has been mostly directed to expression analyses; however, little is known about their function and location in the plant organs and tissues. Our previous work uncovered the relevance of a group-4 LEA protein (AtLEA4-5) in the plant tolerance to water deficit. Of particular interest was the finding that this protein seems to play a role in the protection of floral buds in plants under water deficit. To get insight into the function of this protein, in this work we focused on the localization of the LEA4-5 protein in Arabidopsis plants subjected to water deficit conditions. Protein was localized by immune-localization techniques using specific antibodies. Results in this work, obtained from stem tissues and from floral and axillar buds, show that AtLEA4-5 protein localize to all tissues, more abundantly to vascular and epidermal tissues. Consistent with our previous data, we found accumulation of this protein in floral and axillar primordia, particularly in the tunica cells. At the cellular level, this protein was found widely distributed in the cytoplasm, in some cases it was detected in granular structures. Interestingly, AtLEA4-5 protein was also located to nuclei of most cell types, being more evident in sclerenchyma cells. The wide distribution of this protein in the different cell types of plant tissues implies a central role for this protein in the protection of different cell functions during water deficit.

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25.

Label-free quantitative proteomics of tonoplast from *Mesembryanthemum crystallinum* leaves to aid in the identification of mechanisms important for plant salt tolerance

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The Ice plant, *Mesembryanthemum crystallinum,* is a widely used model plant for studying tolerance to salt stress. This halophyte can successfully accumulate up to 1 M NaCl in the aerial tissue, most of which is stored in the large central vacuole present in cells of the leaf. Vacuolar Na^+ accumulation is thought to be mediated by Na^+/H^+ exchangers, energized by the vacuolar H^+-ATPase (V-ATPase), however, relatively little is known about the molecular mechanisms involved in transporting Na^+ across the vacuole membrane and how these may be regulated. Previously we used 2D-DIGE of highly pure leaf free flow electrophoresis tonoplast fractions to identify salt-responsive membrane proteins and identified the glycolytic enzyme aldolase as a key regulator of the V-ATPase under salt stress (Barkla *et al*., 2009). To complement this work and help identify novel salt-regulatory mechanisms we are now employing label-free LC-MS/MS proteomic approaches. This approach is more suitable for the study of membrane proteins which are difficult to resolve in 2D gels and limits problems associated with labelling, while increasing the number of proteins that can be quantitatively compared. Initial results from this study will be presented and discussed.

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26.

Effect of aluminum on primary root growth of coffee seedlings cultured in vitro. Morphological and biochemical studies

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C. arabica (coffee) is a woody species growing in acid soils in which the availability of aluminum (Al) could affect their growth[1]. The roots are the first organ contact and the primary site of action of Al when are present in the soil, causing the inhibition or stimulation of growth[2]. In coffee it is unclear what effect of this metal on the root. Therefore, the aim of this study was to determine Al effect on the growth of the primary root (PR) of coffee seedlings. The experiment was performed on *in vitro* model with Gamborg half its ionic strength (B5/2) at pH 4.3, which supplemented with different concentrations of $AlCl_3$ and the seedlings were treated for 30 days under these conditions. The effect of Al was dose-dependent; 100 and 300 µM of Al stimulated coffee RP growth and K and Ca accumulation in this organ, while 500 µM of Al damaged root tips and inhibited RP growth. Also, we reported as a cause of Al toxicity their association into cell nuclei of the meristematic region of the root. Moreover, we reported the possible involvement of a signal transduction pathway in the beneficial effect of aluminum.

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27.

Computational analysis and function prediction of a stress-related hypothetical protein in *Arabidopsis thaliana*

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Arabidopsis thaliana possesses one of the smallest known genomes in the plant kingdom. Despite the extensive research devoted to the functional characterization of this genome, the molecular function of about one fifth of the genes is still annotated as "unknown". Proteins encoded by these genes lack currently defined motifs or domains, and no molecular function has been assigned based on experimental evidence. These proteins are commonly classified as proteins with obscure features (POFs), and their functional characterization is one of the main challenges in Plant Biology. Bioinformatics provides useful tools for inferring biological function of POFs, which can be further demonstrated by experimental approaches. Here, we present the preliminary characterization of one POF potentially involved in the responses to different types of environmental stress, based on transcriptome profiling. Multi-species sequence comparison shows this protein is present in all land plants for which the complete genome sequence is currently available, but it is not found outside this group of organisms. Amino acid sequence analysis of this POF and homologous proteins revealed two evolutionarily conserved motifs. Secondary structure prediction shows that one of the motifs is predominantly alpha helical, and the particular arrangement of amino acids results in a putative amphipathic helix. Amphipathic helices are structural features found in many proteins able to interact with membranes, thus potentially assigning that role to this POF. This hypothesis is further supported by gene co-expression analyses, showing that the corresponding gene has similar expression pattern to genes involved in membrane binding and vesicle transport. Interestingly, the amphipathic helix predicted for this POF exhibits similar characteristics to that present in alpha-synuclein, a membrane-binding protein not found outside of vertebrates. This last finding suggests this POF could be an example of convergent evolution at the molecular level. Efforts toward the experimental characterization of this POF, including sub-cellular localization to demonstrate binding to membranes, will be discussed.

28.

Characterization of vesicle trafficking in the Habanero pepper (*Capsicum chinense* Jacq.) root apex in response to exogenous nitrate

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Plants integrate information from the environment to trigger responses to lead to the formation of new roots and shoots. For example, when root systems are exposed to heterogeneous conditions of nitrate, root proliferation is observed in the segment exposed to the nutrient, whereas inhibition is detected where there is deficit. However, the effects of nitrogen on root system architecture are complex and depend on several factors such as plant species. The root response to nitrate has largely been characterized in *Arabidopsis* and cereals, but there are very few studies in other families. We demonstrate that Habanero pepper responds to nitrate; heterogeneous nitrate application stimulated the formation and elongation of lateral roots in the segment where the nutrient was present. Interestingly, when Habanero pepper seedlings growing in hydroponic conditions were exposed to nitrate, the transcript levels of *vps26* increased in root apex. Vps26 is a component of retromer, a pentameric protein complex which binds to post-Golgi organelles and is required in plants for the targeting of vacuolar storage proteins and the recycling of endocytosed proteins like PIN proteins. The role of endocytic network in root response to nutrient has not been previously studied. The objective of this work was to evaluate the changes in vesicle trafficking in root apex from seedlings submitted *in vitro* to heterogeneous conditions of nitrate. To characterize the response to nitrate, we treated the roots with some inhibitors of vesicular transport such as brefeldin A and wortmannin, and FM4-64 dye was employed to visualize and to track the dynamic of membrane trafficking in root apex.

29.

A multistage gradual nitrogen-reduction strategy for lipid production and nitrogen removal in wastewater using *Chlorella vulgaris* and *Scenedesmus obliquus*

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Nitrogen limitation has been widely proposed as a method to increase lipid content of microalgae in biodiesel-oriented processes. However, this is typically accompanied by a reduction on the growth rate, and as a result, the overall lipid productivity does not necessarily increase. In this study a novel multi-stage nitrogen-reduction process is proposed, in order to promote a balance between growth rate and lipid accumulation which could result in a net increase of lipid-productivity in microalgae, while simultaneously reducing nitrogen concentrations in wastewater. *Chlorella vulgaris* and *Scenedesmus obliquus* were grown initially in nitrogen-rich (90 mg L^{-1}) artificial-wastewater medium, followed by sequential dilutions (50% v/v) in fresh medium with $N\text{-}NH_4$ concentrations of 60, 40, and 20 mg L^{-1}, respectively. The overall lipid productivity was compared to those obtained in various two-stage nitrogen reduction processes, wherein the nitrogen-rich culture was followed by a 50% v/v dilution in fresh medium containing 30, 20, or 10 mg L^{-1} $N\text{-}NH_4$ in the second stage. Increased net lipid-productivity was observed for both species in the two-stage mode, although nitrogen depletion was not achieved in these cases. On the other hand, in the sequential mode only *C. vulgaris* exhibited a net lipid-productivity increment. The highest lipid productivities occurred in the two-stage mode for both *S. obliquus* and *C. vulgaris* (194.9 and 133.5 mg L^{-1} d^{-1}, respectively). The lipid productivities achieved in this study are among the highest reported in the open literature to date, and the fatty-acid profiles are adequate for biodiesel production.

30.
Capacity of *Salvinia minima* baker to tolerate and accumulate nickel

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Aquatic plants have been demonstrated an effective strategy to decontaminate residual waters. They have been used since the seventy years in phytorremediation to solve contamination problem. For this reason, in this research project we used *S. minima* as an option to reduce the problem of water contaminanted with nickel. Nickel is one of the five most abundant metals finding in the water bodies. Furthermore, nickel is highly required in the steel industry, petrol refinery, textile industry, alkaline batteries, etc. In all these activities, the waste of the industries is deposited in the soil and in the water bodies, and eventually can induce cancer disease in humans, fish toxicity, ecosystems damage, and negative effects in the environment. For this reason, the main focus in this research was to evaluate the capacity of absorption of nickel in leaves and roots of *S. minima* when the plants were exposed to different Ni_2 concentrations and at different time of exposure. *Results obtained showed that* S*. minima* are capable to take up high quantities of nickel in both tissues. It was observed that in 20µM of Ni, plants absorbed 1.75 mg of Ni·g^{-1}DW (BCF=922.79), in 40 µM of Ni 2.13 mg of Ni·g^{-1}DW (BCF=541.86), and for 80 µM of Ni 2.86 mg of Ni·g^{-1}DW (BCF=541.86) and at 160 µM of Ni 3.3 mg of Ni·g^{-1}DW (BCF=210.1). Likewise, Physiological parameters were evaluated; these include Photosynthesis, chlorophyll fluorescence and electrolytes leakage. Results showed than photosynthesis decrease according to the Ni concentration was increased, the PSII values decreased in the same tendency and the electrolytes leakage was evident in the high concentration of Ni. The photosynthetic apparatus and the membrane of the cells in tissues of *S. minima* were affected in presence of Nickel stress.

31.

Analysis of primary root growth and lateral roots in *Nicotiana tabacum* L. under stress generated by Cr (VI)

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Both *A. thaliana* and *N. tabacum*, generate new lateral roots and the primary root is inhibited, when the plants are transferred from metal-free medium to medium supplemented with heavy metals. To determine the difference of tolerance of primary and lateral roots in *N. tabacum* to the stress generated by metals and provide preliminar information about whether this tolerance can be acquired or it is innate in lateral roots, in this study we analyzed the growth of tobaco plants *in vitro* by adding Cr(VI). Seeds were germinated and grown on MS medium without the metal for 5 days and then were transplanted to MS medium with Cr(VI) 100 or 200 μM and the control MS medium without the metal. We analyzed the growth of primary root and lateral roots at 5 and 8 days, the latter on an individual and global-handle also considering the whole plant. Considering the results of this work globally and their comparison with those in Arabidopsis that have been made in our laboratory, we can conclude that plants have a plasticity to adapt to stress conditions created by heavy metals such as Cr(VI) and depending on plant species there are variations in stimulatory or inhibitory concentrations of the growth of root types (primary, lateral pre-existing and new laterals), with the constant that new lateral roots are always more tolerant than existing primary and laterals.

32.

A novel effect for glycine on root system growth of habanero pepper: a role for ethylene in the root response to glycine

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It is known that low nutrient availability restricts plant growth in many environments, especially in places that are extremely poor in nutrients such as the tropics. Due to its important role in metabolism, the availability of nitrogen (N) is one of the key factors that limits crop productivity. Amino acids represent a major fraction of the low molecular weight organic N that is dissolved in the soil and their presence and concentration vary greatly from one ecosystem to another. It has been recently suggested that amino acids may be sensed in the root tip by specific receptors and act as signaling molecules that indicate the presence of nutrient-rich patches in the soil. Recent studies of regulatory interactions between amino acids and root growth have been conducted in *Arabidopsis*. However, reports of amino acids affecting the root growth of Solanaceae family members, which includes habanero pepper are scarce and inconclusive, since they present limited data or contradictory effects. The objective of this study was to evaluate the effect of glycine on the primary root (PR) growth of habanero pepper (*Capsicum chinense* Jacq.). Glycine inhibited cell elongation but not cell number, stimulated root hair growth, and induced a significant accumulation of starch grains in the root apex. The aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, reversed the glycine-mediated reduction in habanero pepper PR growth. Also, glycine treatment increased the expression of aminocyclopropane-1-carboxylic (ACC) oxidase, a key enzyme in ethylene biosynthesis pathway. Together, the results of our pharmacological and molecular approaches suggest that the inhibitory effect of glycine on habanero pepper PR growth is likely the result of elevated ethylene production. We suggest that these changes in the root apex in response to exogenous glycine could be an important adaptive response that allows plants to efficiently access the fluctuating availability of nutrients in the soil.

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33.

Elements of the RNA-directed DNA methylation pathway are involved in the response to water deficit in *Arabidopsis thaliana*

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Because their sedentary life, a wide arrangement of strategies have been selected in plants along evolution to cope with stressful conditions, leading to a complex response involving various levels of control. This response includes the modulation of gene expression without changes in the DNA sequence, formerly know as epigenetics. Very recently, the involvement of heterochromatic small interfering RNAs (hcsiRNAs) in the modulation of the genome epigenetic state was discovered. However, the participation of hcsiRNAs in control of the plant response to water deficit still remains unknown. In this study, we focused on the participation of the RNA-mediated DNA methylation (RdDM) pathway in this stress response. This pathway controls the transcription of many elements in the genome – mainly transposons – through DNA methylation and histone tails post-translational modifications. Using a genetic approach, we found that the absence of different proteins of this pathway leads to a distinctive plant response of to water limitation. The observed phenotype correlates with the altered expression of stress responsive genes under optimal and stress conditions. A preliminary analysis suggests that some of genes with altered expression did not show association with transposon sequences, siRNAs or methylation patterns. These observations suggest that some of the proteins involved in the RdDM pathway may participate in an unknown stress regulatory system.

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34.

A snapshot of the *Phaseolus vulgaris* microRNA activity during drought stress through deep sequencing of processed transcripts

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Plants are subjected to adverse environmental conditions and thus have developed a variety of mechanisms ranging from organismic to molecular levels in order to contend with stress. Common bean (*P. vulgaris*) is one of the most important legumes in the world used as a food source. In plants, microRNAs function primarily as negative regulators at the posttranscriptional level and their participation during stress conditions has been revealed. Complete understanding of miRNA action depends on the identification of its target transcripts. In this context, we identified miRNA-mRNA targets through high throughput sequencing from common bean seedlings during optimal irrigation or water deficit. We found 65 targets, including some that showed changes under stress conditions. To explore the roles of miRNAs under these conditions, we selected nine miRNA-mRNA target pairs: three in which the target messenger showed cleavage evidence only in optimal irrigation conditions, three present only in water deficit conditions and three without changes; then, the relative abundance of the miRNAs and their targets was evaluated by RT-qPCR. Results showed that the abundance of the miRNA and target messengers do not reflect a negative correlation as expected from data provided by degradome analysis. We suggest that the resulting transcript abundance depends on multiple factors, including but not limited to, miRNA regulation

35.

Degradome sequencing analysis reveals novel targets for conserved miRNAs in *Phaseolus vulgaris* L. in response to drought

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MicroRNAs (miRNAs) are small endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level in plants and animals, they play important roles in development, signal transduction, biotic and abiotic stress responses. Regulatory mRNA targets of plant miRNAs have been computationally predicted, based on their extensive sequence complementarity to the miRNA and many of these target predictions have been confirmed by the isolation of the cleavage products directed by miRNAs. In particular, we are interested in the identification of miRNA targets in *P. vulgaris* which is one of the most important grain legumes in Mexico and Latin America where it constitutes one of the primary sources of protein in human diets. In order to identify miRNA targets in common bean, our research group previously applied a deep sequencing approach to identify processed transcripts which directly detects the cleaved mRNAs targeted by a miRNA, a strategy commonly known as Degradome analysis. Based on the results obtained from this strategy, we are currently focusing our efforts at characterizing novel targets for conserved miRNAs which have not been previously identified in other plant systems. The analysis of these targets will provide new information and will advance our understanding about how the miRNA network is involved in response and adaptation to drougth in common bean and other legumes.

36.

Functional analysis of group 4 LEA proteins in *Arabidopsis thaliana*

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Upon water deficit, plants express different classes of proteins that allow them to survive and reproduce under low water availability. LEA proteins, highly accumulated in late stages of embryogenesis, also accumulate in vegetative tissue under diverse water deficit conditions. These proteins, considered as intrinsically disordered (IDPs), are grouped according to their sequence similarity and distinctive motifs in seven different families. *Arabidopsis thaliana* has three members of group 4 LEA proteins. A member of this group, AtLEA4-5, prevents the inactivation of enzymes (LDH or MDH) under water scarcity and freezing. The sequence similarity among the proteins in this group supposes the existence of genetic redundancy. Nevertheless, it has been shown that deficiency in any of the three LEA4 proteins leads to susceptibility to water deficit. These observation has led to two different hypothesis: i) group 4 LEA proteins have similar functions but express in different tissues and/or at different times; or ii) group 4 LEA proteins carry out different functions and their expression patterns are similar. To differentiate between these possibilities and get insight into the group 4 LEA proteins possible functional redundancy, we designed recombinant constructions to express different LEA4 proteins from the AtLEA4-5 promoter and look at their ability to complement the water deficit susceptibility of the *AtLEA4-5* null *Arabidopsis* mutant. A negative result will indicate different functions, whereas a positive complementation will imply similar functions and different expression patterns. These data will contribute to a better understanding LEA protein functions in the plant response to water deficit.

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37.

Analysis of legume-miRNAs present in *Medicago truncatula* in response to water deficit

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Nowadays water deficit is considered as a severe environmental constraint to plant productivity. Legumes represent the second most-important crop family, and a key source of biological nitrogen in agriculture. Recently microRNAs (miRNAs) have been identified as important stress-responsive regulatory molecules in plants, including their participation during responses to water deficit. For this reason we are interested in the characterization of legume-especific miRNAs which might reveal novel strategies unique to this group of plants. Our group has started the characterization of novel legume-specific microRNAs, however we have been thwarted by the limited availability of genetic resources in *Phaseolus vulgaris* (common bean). To overcome this situation we have turned our attention to the analysis of drought responsive microRNAs in the model legume *Medicago truncatula*, which offers the advantages of a fully sequenced and annotated genome, availability of global expression analyses and mutant lines as well as its susceptibility to genetic modification. We will present our results with the use of the legume *M. truncatula,* which has helped to bring legumes into the position of being able to access many of the contemporary tools of genetics, as a model for the study of legume- specific miRNAs. Currently we are establishing growth conditions to study legume-specific miRNAs in the context of water deficit.

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38.

A dicistronic microRNA precursor encoding miR398 and miR2119 responds to drought in legumes

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Plant microRNAs are commonly encoded in a transcript containing a single precurs
microRNA. Processing by DICER-LIKE 1 and associated factors results in the production
a single microRNA:microRNA* duplex of 20-24 nucleotides in length, followed by selecti
of one strand of the duplex and its incorporation into a AGO-containing silencing compl
to direct silencing of a mRNA containing a complementary target sequence. Particul
microRNA loci have been described to contain more that one precursor stem-lo
structure, thus encoding more than one microRNA in the same transcript, such as t
miR395 family, encoding up to eight precursors in a single transcript in rice. We found
unique case where the evolutionary-conserved miR398 is encoded in the same transcri
as the legume-specific miR2119. In *Phaseolus vulgaris* (common bean) mature miR3
and miR2119 accumulate in response to drought as revealed by Northern blot and qP
and we demonstrate they are both functional as they target the mRNAs for CSD1 a
ADH1, respectively, in response to drought. The dicistronic arrangement found
common bean was also observed in other legumes. In addition, legumes possess a seco
locus encoding miR398 that represents an independent transcription unit, similar to th
of other plant species. Our results indicate that the combined expression of miR398 a
miR2119 is utilized to down-regulate the mRNAs encoding CSD1 and ADH1 in response
drought in common bean and other legumes as well, suggesting that reactive oxyg
species and fermentation metabolism are closely coordinated under stress conditions.

39-b

Root growth as susceptibility or tolerance marker to aluminium between different plant species

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The plant root system is in contact with biotic and abiotic factors in the soil[1]. All these factors have an impact on primary root growth and the formation of lateral roots, so it is not surprising that plants alter the spatial and temporal development of their root system in response to a variety of signals in the soil solution [2]. In acid soils, aluminum (Al) is one of the main environmental factors that modify root system growth. In plants aluminum sensitivity varies among species even among cultivars[3]. Because root system is the primary site of contact with Al, its simple structure and eases of cultivation this is a good model for the study of root plasticity between species due to environmental factors. The aim of this study was to compare Al effect on the root system of *Arabidopsis* (*A. thaliana*), coffee (*C. arabica*) and pepper (*C. chinense*). The results showed a differential root response between these species when were exposure to 0-500 μM $AlCl_3$: in *Arabidopsis* a morphological damage in the root apex and root growth inhibition were observed when was treated with dose higher to 100 μM of Al; in coffee Al had two effects on root growth, a stimulatory at ≥100 μM Al and a inhibitory at 500 μM Al, while in pepper primary root growth was not affected until 500 μM of this metal.

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39.
Structural analysis of a group 6 LEA protein

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LEA (Late Embryogenesis Abundant) proteins are a sub-group of hydrophilins, a set of highly hydrophilic proteins that contain a high percentage of glycine and other small amino acid residues. These proteins are also preferentially expressed under water deficit. Recent results of our group indicate that these proteins play a relevant role in plant adaptation to water deficit. To gain insight into the functional mechanism of these proteins, in this work we analyzed the structural organization of a group 6 LEA protein, PvLEA6, from common bean (Phaseolus vulgaris L.). The analysis was carried out by circular dichroism (CD) spectroscopy and nuclear magnetic resonance (NMR). The results showed that in aqueous solution this protein is in equilibrium between two conformations (poly-proline type II helix and extended strand). In the presence of trifluoroethanol (TFE) some segments of the protein adopt an ¿-helix conformation, indicating that the physicochemical properties of the protein allow the formation of secondary structure. The molecular crowding and reduction of water availability produced by addition of poly-ethylene-glycol (PEG) and1or glycerol to a PvLEA6 protein solution induced some degree of secondary structure. In addition, the capacity of this protein to form oligomers was investigated. The results showed that PvLEA6 protein is able to form homo-oligomers of different sizes, being the dimer the most abundant form under native conditions. The relevance of the structural organization of this protein on its function will be discussed.

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40.

An altered hydrotropic response (ahr2) mutant of Arabidopsis is tolerant to drought stress

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Plants are constantly exposed to environmental changes like poor light, gravity, obstacles and low water content in the soil and they have developed a morphological plasticity to adapt to those changes. One of the ways in which plants contend with the fluctuating environment is by developing tropisms. Plant roots show positive hydrotropism in response to moisture gradients, a feature that is important in controlling their growth orientation for obtaining water. The hydrotropic response has an important role in establishing the structure of the root system, and thus, has implications on the ability of plants to survive under limiting water conditions such as drought stress. In addition, it has been reported that ABA, a water stress hormone, is a modulator of the mechanisms that integrate the hydrotropic response. However, the mechanism of moisture gradients perception and response has not been completely elucidated. Using a screening system with a water potential gradient generated with glycerol (NM→WSM; NM, normal medium and WSM, water stress medium), we isolated the *ahr2* semi-dominant mutant of Arabidopsis that displayed altered hydrotropic response in the test system. Moreover, in a system with a mixed water potential gradient (NM→WSM→NM), the *ahr2* roots grew for about 19 days vertically towards the higher water potential sector of the plate (NM) placed at the bottom showing higher hydrotropic response than the wild-type roots that grew only for 7 days and do not reach the first frontier between NM and WSM. Furthermore, the *ahr2* mutant showed higher sensitivity to exogenous ABA on germination and seedling establishment compared to wild-type. Consistent with this result, the *ahr2* mutant grown under drought conditions showed improved tolerance compare to the wild-type. Therefore, we propose that the *ahr2* mutant could help us to establish the relation between ABA signaling with hydrotropic response and drought tolerance.

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41.

Comparative analysis of the sorghum genome among related species with emphasis on drought stress

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Sorghum is of national agricultural importance. An abiotic factor that reduces its productivity is drought. At present, several genomes have been sequenced, such as corn, rice and other related species, and in 2009 the sorghum genome sequence was released. In order to find genes associated with tolerance to drought stress, a comparison of genomes among sorghum, maize and rice was performed using the Biomart program. We have found 8,580 differentials genes. These sequences were analyzed against with Blast database nr of the NCBI gene bank, with non-significant results. The function of 8,580 genes was searcher using the Suite Blast2go program, with also non-significant results. Therefore, a Psiblast analysis against the nr database was realized, we obtained homology between Sorghum and *Vitis vinifera* sequences with 1,274 homologous. But results showed that, several products are annotated as proteins of unknown function or hypothetical, however, just 32 of these genes were associated to some biological process. From these 32 homologous sorghum genes, 24 presented associated functions with heat stress tolerance like flavonoid synthesis pathway, phenylpropanoids, fatty acids and redox processes, agreeing with those reported at 2010 by Lui *et al*.

42.

Proteomic analysis of a basic protein fraction (pI 6-ll) in response to water stress in mycorrhizal sorghum plants

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Arbuscular mycorrhizae (AM) are the most widespread mutualistic symbioses between the roots of most land plants and a phylum of soil fungi. AM are known to influence plant growth by improving mineral nutrition, protecting against pathogens and enhancing resistance or tolerance to biotic and abiotic stresses. It is well known that sorghum is a cereal crop tolerant to hot and drought conditions, and its roots are able to associate to mycorrhizal fungi. There are many proteomic research works about the response to water stress or mycorrhizal colonization in plants. In a previous work, to know the impact of mycorrhization on sorghum plants subjected to water stress, we analyzed the changes in expression of acid proteins (pH 4-7) by a proteomic approach. In the present work, we extended the analysis to a basic (pH 6-11) enrrichment protein fraction. Total leaf protein was extracted with the phenol method and a basic (pI 6-10) enrrichment fraction was obtained by prepative liquid isoelectrofocousing, using a ZOOMR-IEF fractionator (Invitrogen). Two-dimensional gel electrophoresis of this fraction was performed for four independent biological experiments by combining IEF (pI 6-11) using an IPGphor system (Amersham BioSc), with a sodium dodecyl sulphate gel electrophoresis. Comparison of 2D protein electrophoretic profile of inoculated with non-inoculated plants under water stress, allowed to detect quantitative and qualitative changes in the expression of 23 proteins. Among these, 20 proteins increased while 3 decreased. The identification of these poteins by mass spectrometry is in progress. We will discuss some aspects related to the function of these proteins and their possible beneficial effects for the plant.

43.

Antioxidant responses of *Jatropha curcas* L. under short-term cold stress

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Jatropha curcas L. is a plant with great potential for the production of biodiesel; however low temperatures are limiting factor in their growth and spread in subtropical regions. Cold stress induces the overproduction of reactive oxygen species, as H_2O_2 creating a metabolic imbalance, which could result in oxidative damage. Therefore, our objective was to characterize the antioxidant responses of *J. curcas* var. Chiapas under cold stress. Pots with 45d old plants were exposed for 48 h at 10 °C (control at 30 °C). Leaves subjected to cold for 12 h, increased ascorbate peroxidase (APx) and peroxidase (POD) activities by 3-fold. At 24 h of exposure the level of H_2O_2 was regulate by catalase and peroxidase activities. The increased observed in H_2O_2 after 48 h of cold exposure was associated with a low superoxide dismutase activity. As a result of oxidative damage at 48 h, leaves were H_2O_2 positive with NBT and DAB staining, and accumulated 2-fold the lipid peroxidation product, malondialdehyde. Plants showed a slight decrease in the leaves weight. However, an increase in the total content of chlorophyll (1.13-fold) and flavonoids (1.3-fold) were found. Flavonoids are located in the stomata, suggesting the possible involvement of abscisic acid (ABA) in their induction under cold stress.

44.

Isolation of Arabidopsis mutants affected in mercury toxicity response

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Plants, which are sessile organisms, are afected by abiotic cues as water, nutrient availabilit and heavy metals (HM). HM toxicity in plants is known to cause widespread damage. The survival of plants depends on the plasticity of its developmental and defense mechanisms. Mercury (Hg) a HM, is released to the environment in significant amounts by both natural and anthropogenic processes, however, little is known about molecular pathways involved in the plant adaptation to Hg polluted environments. In our work, we found when *Arabidopsis thaliana* grows media added whit HG, their post-embryonic root development is affected. It shows a primary root growth inhibition and reduction in lateral root formation. To gain knowledge on the genetic mechanisms that regulate plant wogth responces to Hg, we screened for *A. thaliana* mutants: a) insensitive to Hg which fail on primary root growth inhibition and increased lateral root formation, and b) oversensitive, strongly affected its development. We isolated 3 plants that seem to be insensitive and 5 oversensitive to $HgCl_2$, which may be useful for cloning genes underlying such responses.

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45.

Ectrophysiological characterization of point mutants from the AMT1;1 transporter of *Phaseolus vulgaris*

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The importance of the family Ammonium transporter-Methylammonium Permease-mammalian Rhesus proteins (AMT/MEP/Rh), is demonstrated by the presence of these proteins in all domains of life. Members of this family of transporters share several properties, among them the high affinity (*Km* in the μM range), saturation ammonium concentrations ≤1mM and high selectivity for ammonium, transporting exclusively ammonium and its methylated analog, methylammonium (MeA); however, the ammonium transport mechanism still remains without being completely elucidated (it is unclear whether the transporters use NH_4^+ or NH_3 as a substrate, or are coupled to H^+ transport or other ions). For this reason, recent research has focused on the characterization of AMT1; 1 transporters in different plant species. Transporters and ion channels have been considered structurally and functionally distinct; however, data coming from fluorescence microscopy and electrophysiology, suggest that conformational changes predicted by the alternate access model in the co-transport, can be smaller, and therefore, more similar to the gates of a channel. It has also been observed that the transporters may exhibit large currents, and in some cases, forming pores comparable or similar to channels that transport ions and/or substrate, as proposed for AMT1 transporters. Based on the properties of some mutants of the *Phaseolus vulgaris* AMT1;1 transporter, it is suggested that point changes are responsible for the modification of the action mechanism of the protein, to convert it from a co-transporter to an ion channel.

46.

Influence of organic agricultural management on metabolomic profiles of various organs of tomato plants

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Agronomic management is defined as the set of cultural practices that are implemented to improve crop production. The organic agricultural management avoids the use of chemical industry inputs Currently, the organic product segment is the fastest growing food sector globally. Mexico is an exporter of organic crops being the tomato one of the main. The tomato is one of the most important crops in the world and particularly in Mexico. One aspect of the plant phenotype that is very flexible and highly influenced by the environment is the metabolome. Previous studies suggest that the environment promotes effects on specific metabolites. They also suggest the influence of organic and conventional agricultural management on specific compounds in some plant tissues. However, no studies had analyzed the complete metabolomic profiles of the different organs of the plant: Fruit, leaves, stem and root under organic and conventional management. The aim of this study is to analyze the differences in metabolomic profiles from different plant organs (fruit, leaf, stem and root) under organic and conventional crop management and analyze the influence of genotype and planting site (greenhouse or open field) in these changes. Agronomic variables we analyze are: Different fertilization regimes, pesticide application, harvesting time in a wide range of genotypes of tomato. Experiments were performed at different levels (field, greenhouse and laboratory). Numerous analytical platforms are used as DIESI-MS, HPLC, GC, UV-Vis and enzymatic assays for the study of samples. So far we have results that suggest that tomato tissues grown in our greenhouse under a variety of agronomic conditions, including different fertilizers and pesticides show differences in their metabolomic profiles, identifying specific molecules that present major changes, so far these results are encouraging in the search for "metabolomic markers" to identify organic crops and to provide knowledge about the influence of agronomic management on plant metaboloma.

47.

Evaluation of genes involved in fruit maturation of Guava (*Psidium guajava*)

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En este estudio, se presentan análisis bioinformáticos y estudios de la expresión de cuatro diferentes clonas de cDNA que codifican una poligalacturonasa (*pgPG*), la ácido 1-aminociclopropano-1-caboxílico oxidasa (ACCo) y tres α-expansinas (α-Exp) en guayaba. La secuencia putativa de aminoácidos de *PgPG1* contiene regiones características y conservadas de las PGs en plantas superiores. *PgACO1* mostró características presentes en todas las ACCo y está relacionada con la maduración. *pgEXP1*, *PgEXP2* y *PgEXP3*, con parte de los dos dominios presentes en expansinas, están agrupadas filogenéticamente con las α-expansinas. Estudios de expresión mediante Dot blot, mostraron que *PgPG1* fue visible en todos los estadios de maduración del fruto pero fue mayor durante el estadio maduro; *PgACO1* fue visible en los cinco estadios de maduración del fruto y presentó su expresión más alta durante el estadio de transición. *PgEXP2* se expresó en todos los tejidos, con un incremento a partir del estadio verde 2 al sobremaduro, similar al comportamiento reportado a frutos no climatéricos. La expresión de *PgEXP3* fue visible en cuatro estadios de maduración del fruto y en pedúnculo, sin embargo, fue mayor en el estadio maduro que en todos los demás. *pgEXP1* se expresó en fruto maduro, pero no en hoja, raíz o tallo. Mediante Northern blot se analizó la expresión en ocho estados diferentes de maduración del fruto, y se encontró principalmente en los últimos tres estados. El análisis de estos resultados muestra que el *PgEXP1* es específico de la maduración del fruto de guayaba.

48.
The hidden energetics of ligand binding to Wheat Germ Agglutinin

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Wheat germ agglutinin 1 (WGA1) is a protein that recognizes and binds in a reversible way to N-acetylneuraminic and N-acetylglucosamine residues. It belongs to the lectin family, a well known group for its carbohydrate binding properties. WGA1 not only plays an important role in the wheat (*Triticum vulgaris*), protecting it from invading insects, yeast and bacteria, but it also has shown many other functions of biomedical relevance. These have opened the possibility to develop drug delivery systems containing WGA1. In solution at neutral pH, WGA1 exists as a homodimer with a molecular weight of approximately 34 kDa. However, the protein dissociates into monomers in acidic media. Each subunit is composed of four hevein-like domains. Crystallographic evidence shows that WGA1, while in its dimeric form, presents eight binding sites. A binding site is formed by each hevein domain via a cluster of three conserved aromatic residues. Six of eight of these binding sites are traditionally termed "primary" binding sites because they´re complemented by polar residues from an adjacent domain of the other chain. In the remaining two binding sites, the polar residues are absent and are therefore known as "secondary binding sites". Until now, it has not been possible to accurately determine the energetics of ligand binding to WGA1. In order to measure these interactions, we propose to perfom ITC experiments upon dimeric and monomeric species of WGA.

49.

Evaluating the role of subunit AKINβγ of the *A. thaliana* complex SnRK1 as energy regulator

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Plants are highly specialized organisms that confront and adapt to different environmental and nutritional conditions. Under these circumstances, they trigger signaling pathways to orchestrate several responses. SnRK1 is a heterotrimeric complex with kinase activity that directly modify cell metabolism and gene expression in response to unfavorable energetic conditions. The complex consists of a □-catalytic subunit and two regulatory β and γ. In plants, there is an additional regulatory γ-subunit, AKINβγ, which displays a chimeric structure composed of typically N-terminal Starch binding domain (SBD) and the C- terminal Bateman domains. Overexpressed and null *akin10* plant mutants indicated a direct role of SnRK1 in starch metabolism. Besides, SnRK1 regulates the expression of starch hydrolyzing enzymes such as □-amylase and ADP-glucose pirophosphorylase. Experiments performed in our lab, indicated that both the catalytic and the □ □subunit were *in vivo* associated to starch granules. *In vitro* experiments, showed that point mutation in amino acids directly involved in the interaction with the carbohydrate, modified very little its affinity, whereas mutant proteins lacking the complete SBD domain lost all the ability to bind to the starch. In this work we will evaluate the role of the □ □ subunit and the importanc of the SBD domain in the regulation of SnRK1 activity.

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50.

Modeling deterioration and sensory shelf-life of fresh-cut papaya (*Carica papaya*) var. 'Maradol'

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Changes in consumers' lifestyles and increased incidence of cardiovascular diseases have influenced the need to consume convenient, healthy, and nutritious products; thus minimally processed vegetables are the fastest growing segment of the food industry (Alzamora *et al*., 2000). However, these products are very perishable, since the cutting process drastically accelerates physiological, physicochemical, and sensory changes (Gil *et al*., 2006). Hence, it is necessary to search objective and reliable methods to estimate quality losses during storage and to predict shelf-life limits. Considering that food quality is a dynamic state continually decreasing (Labuza, 2000) and that quality loss can be described by mathematical modeling (Saguy and Karel, 1980; Singh, 1999; Taoukis *et al*., 2000), a kinetic approach was applied to model physicochemical and sensory changes during storage of fresh-cut papayas. Results indicated that zero and first order kinetics models were useful to estimate physicochemical and sensory changes during storage and could be useful to estimate shelf-life.

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51.

The effect of D-amino acids on root growth of Habanero pepper (*Capsicum chinense* Jacq.)

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All amino acids, with the exception of glycine, have two optical isomers: L-and D-enantiomers. D-amino acids are far less abundant in nature than the corresponding L-enantiomers, which are the predominant form occurring in biological molecules. However, D-amino acids occur naturally in bacteria, insects, molluscs, earthworms, mammals, plants, and others organisms, where are synthesized *in vivo* by enzymes such as amino acid oxidases, transaminases, and epimerases. Also, the appearance of D-amino acids in plant tissue can be explained by the uptake from the soil. Some D-amino acids exist in soil organic matter at 10 to 20 % of the concentration of L-enantiomers; for example, D-Ala or D-Asp can be found in amounts of several milligrams per kilogram of soil. Although the physiological relevance of these substances has been documented in some organisms, the importance of D-amino acids in the biological function of higher plants remains unknown. To investigate the effect of D-amino acids on root growth we selected as model an agricultural plant, Habanero pepper (*Capsicum chinense* Jacq.), which grows in Yucatán, México, where more than 90% of these soils contain clay, a high organic matter content, low inorganic nitrogen, and alkaline pH. We tested the effect of 18 D-amino acids on the primary root of aseptically grown seedlings. We also investigated the role of a glutamate receptor in root response to D-amino acids. The presented results indicate that the effect on primary root growth was dependent on the type and concentration of D-amino acids. We discuss here the possible ecological implications of root response to this form of amino acids in soils with low nitrogen.

52.

Dinámica de los CNE en tallos de maíz a través del desarrollo, y en condiciones de sequía y de eliminación de órganos productores-consumidores

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La mayoría de los estudios bioquímicos y moleculares en maíz se han enfocado en los granos y hojas, mientras que el tallo es uno de los órganos menos estudiado. La hipótesis de este trabajo fue que el tallo de maíz además de brindar estructura y ser un conducto de transporte, es un órgano de reserva de carbohidratos. Los Carbohidratos No Estructurales (CNE) pueden ser solubles, como la glucosa (Glc), fructosa (Frc) y sacarosa (Suc); o insolubles como el almidón. Nos preguntamos si los CNE del tallo son afectados por condiciones como la etapa de desarrollo, el estrés hídrico y por la alteración del balance de carbono al eliminar órganos productores (hojas maduras) y consumidores (jilotes). Se realizaron tres experimentos con diferentes genotipos subtropicales de maíz. En todos, se molió el tallo entero, se extrajo el jugo y a partir de éste, se cuantificaron CNE. Los resultados muestran que los principales azúcares solubles en el jugo de tallo de maíz son Suc, Glc y Frc. No se encontraron oligosacáridos ni polisacáridos de la familia de los fructanos. Los niveles de sacarosa incrementaron durante el desarrollo hasta alcanzar los mayores niveles después de floración y luego se mantuvieron; mientras que glucosa y fructosa tuvieron una concentración máxima transitoria alrededor de la floración femenina; por el contrario, los niveles de almidón fueron mayores en etapas vegetativas, y alrededor de floración mostraron sus niveles menores. El tratamiento de estrés hídrico incrementó los azúcares reductores pero disminuyó el contenido de almidón en comparación con las plantas control. La remoción de las mazorcas propició un incremento en la concentración de almidón, mientras que la eliminación de las hojas originó cambios ligeros en los niveles de NSC. En base a estos y otros resultados, nosotros concluimos que los CNE del tallo no son estáticos ni tampoco son una simple consecuencia del transporte de sacarosa a través del floema. Se sugiere que además de funcionar como soporte y transporte, el tallo en maíz podría estar cumpliendo una función de reserva dinámica de carbohidratos modulando el suplemento de azúcares hacia otros tejidos

53.

Characterization of mutants lacking of pyrophosphatase of *Arabidopsis thaliana* under phosphorus deficiency

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The inorganic pyrophosphatases (PPa, E.C. 3.6.1.1) recycle the pyrophosphate (PPi) into inorganic phosphate (Pi). Unfortunately, studies on PPa expression and activity have been scarce under conditions of low Pi availability, despite its obvious relationship with plant PPi content. We are interested in understanding how the six Arabidopsis thaliana's (At) genes encoding classical inorganic soluble PPa are regulated, and what are their individual roles, if any, during adaptation to Pi-nutritional stress. To improve our understanding of individual PPa¿roles, we subjected an Arabidopsis T-DNA insertion homozygous mutant lacking the PPa1 isoenzyme (At¿PPa1) to Pi-starvation and analyzed changes in phenotype, some physiological traits and the expression of Pi-stress related transcripts (IPS1, PEPCK, MYB62, SUC2, SQD1, ACP5, PHO1 and CNCH), as measured by RT-PCR. The changes observed were compared to those found in the At-wt plant grown under the control and Pi-starved conditions. The At¿PPa1 plant was more robust, grew larger leaves, stem and siliquas than the At-wt under both conditions. In addition, a global analysis of the transcriptional activity of the At¿PPa1 plant against the At-wt was done using RNA-seq and further characterization of global changes in the At¿PPa1 mutant under control and stress conditions against At-wt was studied using 2D-SDS-PAGE. There were numerous changes in transcription and more modest but significant changes in the proteome. These changes suggest the existence of non-essential, but important roles for At¿PPa1, which are not completely compensated by the remaining AtPPa isoforms, and are consistent with a link between PPi and abiotic stress responses in plants.

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54.

Elucidating the elements involved in STPl (SugarTransporter Protein l) sugar regulation in *Arabidopsis thaliana*

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Sugars as signals modulate several processes during plant development, impacting the expression of many genes at transcriptional level. Despite of the importance of sugar levels sensing and its transduction for plant development, only some elements in this signaling pathway have been elucidated. In order to identify the elements involved in transcriptional regulation by sugar, we analyzed the regulation of STP1 (Sugar Transporter Protein 1), a gene repressed by glucose in Arabidopsis thaliana. The analysis of STP1 expression by northern-blot in treatments with glucose analogs showed that the regulatory signal is produced only by phosphorilable sugars, excluding the participation of an intermediary metabolite of sugar metabolism. Through the study of mutants in sugar signaling, we showed that STP1 regulation by sugars is conducted by an HXK1 (Hexocinase 1) independent pathway, and analyzed the possible role of other pathways. Moreover, previously we have identified a fragment of 310bp of STP1 promoter that contains the necessary cis elements for sugar repression by glucose, this fragment was used to isolate possible trans factors that recognize this region and the preliminary results are presented in this work.

55.

Advances in the generation of *Physcomitrella p*atens mutants to study glucose-triggered signaling in non-vascular plants

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Sugars can act as signals controlling the expression of genes involved in the growth and metabolism of higher plants; however, morphological complexity has limited the sugar signaling understanding. These signaling mechanisms and response to sugars have not been studied in bryophytes (nonvascular plants) and neither is known about the interaction of these processes with stress responses. Our goal is to generate mutants to study the signaling pathways activated by glucose (Glc) in the moss (bryophyte) *Physcomitrella patens*, since this plant is tolerant to osmotic stress, has its genome sequenced, targeted gene disruption is efficient, aploid phase is dominant and morphological simplicity facilitates the study and understanding of the signaling mechanisms and response to sugars in plants. We will use three strategies to generate *P. patens* mutants. The first one is based on a mutagenized cDNA library obtained protonemal tissue exposed to Glc concentrations that we have shown to cause phenotypic effects The second alternative is to use site-directed mutagenesis in two genes known to be involved in Glc signaling in *Arabidopsis thaliana* (THYLAKOID FORMATION 1, THF1 and G-protein alpha subunit, GPA1). The third is based on mutagenesis of protonemal tissue using ultraviolet (UV) light. Based on recently obtained results we know that the moss *P.patens* can perceive and respond to different Glc concentrations and this has an impact on the phenotype, photosynthetic efficiency and gene expression of two photosynthesis related genes (*RCBS* and *CAB1*). Therefore, generation of mutants affected in responses to Glc will reveal elements of the signaling pathways activated by Glc in bryophytes and evolutionary aspects of the mechanisms involved.

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DEVELOPMENTAL PATTERNING

56.

Arabidopsis homolog of TRITHORAX (*ATX1*) is required for lateral root primordium morphogenesis

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The *ATX1* gene codes for a histone H3K4 methyltransferase that regulates floral organ development and functions as an activator of homeotic genes (Alvarez-Venegas *et al.*, 2003). We studied the role of *ATX1* in the morphogenesis of the lateral root primordia (LRP) and possible interactions with genes involved in the LRP development.
We found abnormal morphology in the 70% of the *atx1-1* lateral roots (LRs) and LRP. The abnormalities were most evident at later stages of the LRP development, suggesting that cell division patterns were affected in the mutant. Next, we examined expression of the *WOX5::GFP,* a quiescent center marker, and *ProSCR::GFP,* a radial pattern marker, in the *atx1* background and found expanded domains of WOX5 and SCR promoters' activities in LRP and young LRs. Using *ProCycB1;1::GUS* reporter, we found that only 20% of the *atx1-1* LRP were GUS positive, indicating that ATX1 is important for cell proliferation during the primordium development. The auxin transport mediated by PIN auxin efflux transporters is essential in determining the number and position of lateral roots. In particular, *PIN1* is expressed during the primordium development and the data of the expression pattern of this marker in the *atx1-1* background will be presented. We also analyzed the expression of genes involved in the LRP development by RT-qPCR and found that *ATHB53* and *PUCHI* mRNA were down-regulated in the *atx1* background when compared to the wild type. Using chromatin immunoprecipitation assays we found that ATX1 was associated with the chromatin of *ATHB53*. Overall our data suggest that ATX1 plays an important role in lateral root primordium morphogenesis.

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57.

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

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Plant LEA proteins accumulate to high levels in response to water deficit imposed during development or by the environment. The close association between their accumulation and water scarcity has led to the hypothesis that these proteins play a role in the adaptation of plants to this stress condition. LEA proteins are classified in seven groups according to their amino acid sequence similarities, the proteins in six of these groups are highly hydrophilic and rich in small amino acids. These physicochemical properties suggest that LEA proteins are intrinsically unstructured (IU), which impose not only structural flexibility but also may lead to functional plasticity. Among the mechanisms that control the functional diversity in some of the characterized IU proteins are post-translational modifications (PTMs), which have been poorly addressed in LEA proteins. Group 6 LEA proteins are a highly conserved set of proteins widely distributed in angiosperms and gymnosperms that present distinctive motifs along their sequences. We have shown that one of the representative proteins of this family (PvLEA6) is unstructured in aqueous solution in equilibrium with an extended conformation. Analyses *in silico* and phosphoproteome data suggest that these proteins may be PTM targets. To get insight into the structural characteristics of group 6 LEA proteins and its relation with their function, we have investigated possible PTMs in a *Phaseolus vulgaris* LEA6 protein (PvLEA6). Results from 2D electrophoresis analyses using native extracts from seed embryos revealed isoforms with different pIs. Alkaline phosphatase treatments indicate that these isoforms are phosphorylation products. The functional analysis of different LEA6 proteins and *in vitro* produced mutants will also be presented.

This work was partially supported by CONACyT (132258-Q) and DGAPA-PAPIIT-UNAM (IN-208212). I.A. and L.Y.R. received CONACYT MSci and PhD fellowships, respectively.

58.

NaTrxh, a protein with a non-orthodox signal peptide that follows the classical ER/Golgi secretion pathway

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NaTrxh is a type *h* thioredoxin (Trx h) with higher expression in self-incompatible *Nicotiana* spp. than in self-compatible ones. NaTrxh interacts strongly with S-RNases *in vitro* and co-localizes with them in the extracellular matrix of the stylar transmitting tissue of *N. alata*. NaTrxh possesses extensions towards both the N- and C-termini, which is a common feature among Trxs h from subgroup 2 and whose role is still not clear. A bioinformatics analysis suggested that secretion information could be accommodated at the NaTrxh terminus. Based on this, the N-terminus was divided into two motifs: Nα (Met1-Ala16) and Nβ (Ala17-Pro27). Through a deletion analysis of NaTrxh by generating GFP fusion proteins transiently expressed in onion epidermal cells, we found that the Nβ motif was responsible for the extracellular localization of NaTrxh. Due to the unique position of this secretion signal and its hydrophilic profile, we investigated the possibility that the protein could be secreted through an ER/Golgi-independent pathway. However, from immunogold labelling and electron microscopy analysis, we found that NaTrxh was mainly associated with cell structures usually related to the secretory pathway (*e.g.* vesicles) as well as the extracellular matrix. Remarkably, a NaTrxh-GFP (KDEL) fusion protein was retained in the ER and the treatment of NaTrxh-GFP-expressing cells with Brefeldin A led to retention of this fusion protein in the Golgi apparatus. Notably, the Nβ domain was also found to play a critical role in NaTrxh tertiary structure stabilization. In contrast, the C-terminus was found to be essential for interaction with S-RNase *in vitro*, providing relevant information about the role of NaTrxh as a modifier protein within the self-incompatibility system of *N. alata*.

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59.

dhm1, an Arabidopsis mutant with increased sensitivity to alkamides shows tumorous shoot development and enhanced lateral root formation

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The control of cell division by growth regulators is critical to proper shoot and root development. Alkamides belong to a class of small lipid amides involved in plant morphogenetic processes, from which *N*-isobutyl decanamide is one of the most active compounds identified. This work describes the isolation and characterization of an *N*-isobutyl decanamide-hypersensitive (*dhm1*) mutant of Arabidopsis (*Arabidopsis thaliana*). *dhm1* seedlings grown in vitro develop disorganized tumorous tissue in petioles, leaves and stems. *N*-isobutyl decanamide treatment exacerbates the *dhm1* phenotype resulting in widespread production of callus-like structures in the mutant. Together with these morphological alterations in shoot, *dhm1* seedlings sustained increased lateral root formation and greater sensitivity to alkamides in the inhibition of primary root growth. The mutants also show significant alterations in both shoot and root development when grown in darkness. When grown in soil, adult *dhm1* plants were characterized by reduced plant size, and decreased fertility. Genetic analysis indicated that the mutant phenotype segregates as a single recessive Mendelian trait. Developmental alterations in *dhm1* were related to an enhanced expression of the cell division marker *CycB1-uidA* both in the shoot and root system, which correlated with altered expression of the auxin-responsive marker *DR5:uidA* as well as with cytokinin-responsive gene markers *ARR5:uidA* and *TCS::GFP*. Pharmacological inhibition of auxin transport decreased LR formation in WT and *dhm1* seedlings in a similar manner, indicating that auxin transport is involved in the *dhm1* root phenotype. These data show an important role of alkamide signaling in cell proliferation and plant architecture remodeling likely acting through the *DHM1* protein.

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60.

The SL gene of *Arabidopsis thaliana* is involved in the primary root apical meristem function, lateral root primordium initiation and morphogenesis

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The proper establishment of the root system depends of the root apical meristem function and continuous lateral root development. In search of new players of the root system formation we isolated an EMS-induced *Arabidopsis thaliana* loss-of-function mutant tentatively named "*short lateral root*" (*sl*). The primary root growth rate in *sl* mutant was significantly lower compared with that of Wild type (Wt). The cellular analysis showed that this slow growth was caused by both, affected cell elongation and disrupted function of the root apical meristem, the latter was shorter in the mutant. Cell production rate was much lower in *sl* compared with Wt and this was related to a two-fold increase in average cell cycle time. The number of cells in the meristem was lower in comparison to Wt and cells in the meristem passed a lower number of cycles. The *DR5rev* transcriptional auxin response in the *sl* background showed no changes in pattern compared with Wt, however *DR5rev* activity increased both in the quiescent center and the protoxylem within the primary root apical meristem suggesting an increased auxin tissue content in *sl*. In support to this hypothesis we found that the lateral root initiation in the mutant increased compared with Wt. Interestingly, while the *DR5* activity in early stage lateral root primordia was similar to that in Wt, it was significantly diminished or not present in primordia at later stages. These changes were accompanied by abnormal lateral root primordium morphogenesis. Overall, our data indicate a complex mode of *SL* action in root system development.

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61.

Role of HSP101 and HSP70 in specific mRNA translation during maize (*Zea mays* L.) germination

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Heat Shock Proteins (HSPs) play an important role as molecular chaperones in the cell. Besides this function, additional roles have been reported in cellular signaling and control of gene expression. Particularly, plant HSP101 and mammalian HSP70 have been reported as regulators of translation, enhancing the translation of specific mRNAs containing certain motif sequences. Proteomic profiling of *Zea mays hsp101*-null mutant germinating embryos suggested specific control of translation by HSP101 during the first hours of germination. The present work aims to indentify mRNAs whose specific translation is regulated by HSP101 or HSP70 during maize germination. A bioinformatic approach identified particular motifs in both, the 5' and 3', untranslated regions (UTRs) of mRNAs whose protein levels are upregulated in the *hsp101*-null mutant. On the other hand, a search throughout maize transcriptome of germinating seeds revealed mRNAs with sequences known to bind HSP101 or HSP70. Translation efficiency of these mRNA candidates will be evaluated *in vivo* under varying HSP101 and HSP70 levels, by quantitatively probing their stability and polysome distribution. This approach is expected to render clues of gene expression regulation triggered by certain HSPs during maize germination.

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62.

Characterization of SERK1 gene ortholog from *Capsicum chinense* Jacq.

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The receptor-like kinase SERK1 is considered one of the best molecular markers of plant somatic embryogenesis (SE). SERK1 is a potential key regulator of the initiation of SE after the differentiation process has been triggered. The objective of this work was to isolate and characterize the SREK1 ortholog (*CchSERK1*) from SE-recalcitrant species *Capsicum chinense* Jacq. (Habanero pepper). The complete cDNA of *CchSERK1* has 2,673 nucleotides, encoding a 629 amino acid putative protein, with a high conservation within the Solanaceae family. The open reading frame possesses all characteristic domains of the reported SERK1 proteins. RT-PCR experiments revealed that CchSERK1 is expressed in seeds through the whole cygotic embryogenesis process, halting in coincidence with the green to orange maturation stage of the fruit. *CChSERK1* transcripts are scarce in the pericarp and the yalk during early stages, but are undetectable after 25 days post anthesis. CchSERK1 is highly expressed in stems and moderately in flowers, but in low levels in leaves and roots. Its expression patterns during SE and in adult organs suggest a broad role in cygotic embryogenesis and also in the control of growth and developmental processes. Cloning of CchSERK1 full cDNA constitutes a valuable tool to study the regulation of cell differentiation in plants and also to understand the recalcitrance of members of the genus Capsicum to the tissue culture *in vitro*.

63.

Folate polyglutamated (vitamin B9)-dependent pathway is important for indeterminate root growth and lateral root development in *Arabidopsis thaliana*

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Indeterminate root growth is maintained by the activity of the root apical meristem (RAM) and is important for the root system formation. During determinate root growth, the RAM becomes exhausted and its cells completely differentiate. We isolated a recessive EMS-induced mutant *moots koom2* (*mko2*), whose primary RAM is completely consumed within two weeks after germination. The point mutation in *mko2* was identified in a conserved region close to the catalytic domain of FOLYLPOLYGLUTAMATE SYNTHETASE (ATDFB/FPGS1), a plastidial isoform of an enzyme that catalyzes folate polyglutamylation. RNA levels of *FPGS2* and *FPGS3* that encode mitochondrion and cytoplasmic FPGS isoforms, respectively, were diminished in the *mko2*. Exogenous application of a folate 5-CHO-THF re-established *mko2* primary root growth and normal RAM organization. Moreover, the *mko2* mutation resulted in a significantly altered folate profiles. Expression of *WOX5, SCR* and *PLT1* markers in *mko2* background was maintained in the RAM before the exhaustion. The RAM disorganization started from the cell cycle activation of the quiescent center cells. Analyses of *DR5, AUX1, PIN3, and PIN4* markers suggested that auxin transport and response was affected in the *mko2* RAM. In spite of the reestablishment of the root growth in the *mko2* in presence of CHO-THF, all markers analyzed had lower activity or expression compared to those in the wild type. Lateral root (LR) emergence and LR primordium morphogenesis, but not LR initiation, were affected in *mko2.* Our data suggest that FPGS1-dependent pathway is the first metabolic pathway proved to be involved in the indeterminacy-to-determinacy switch in the root development. Other aspects of the FPGS1 roles in stem cell niche maintenance will be discussed.

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64.

Molecular and functional characterization of CLB19 (Chloroplast Biogenesis 19) protein in *Arabidopsis thaliana*

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Plant RNA editing modifies cytidines (C) to uridines (U) at specific sites in the transcripts of both mitocondria and plastids. Specific targeting to particular C nucleotide is achieved by pentatricopeptide repeat proteins (PPR) that recognize *cis* elements upstream of the edited C. CLB19 is a member fo the PLS group of PPR proteins that belongs to the E subgroup, and participates in the editing of *rpoA* and *clpP1* chloroplast transcripts. The E domain at or near the C terminus of these proteins has been shown to be essential for editing, and is presumed to recruit the enzyme that deaminates the target C residue. Here we analyzed the functionallity of E domain of CLB19 through the complementation of the *clb19* mutant with a truncated version (CLB19ΔE) and the overexpressing lines of this construct in wild type, both displayed negative dominance phenomenon, showing impairment in the development of the female gametophyte. Furthermore we identified several specific nucleotides at the RNA targets that are important to the recognition and binding to CLB19 protein using Electrophoretical Mobility Shift Assay. Recently, several non-PPR proteins have been identified to mediate editing in plants, these proteins are known as MORFs (Multiple Organellar RNA Editing Factor). In this work we tested CLB19 for protein-protein interaction with several MORF proteins through BiFC assays. We found fluorescence complementation upon CLB19 and MORF2.

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65.

Changes in root development induced by a point mutation in ACTIN2 gene

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Actin plays essential roles ranging from morphogenesis, organellar movements, cytoplasmic streaming and vesicular transport to gene regulation. An important property of actin is its highly dynamic ability to rearrange itself in response to multiple environmental and developmental signals by polymerization and depolymerization of filaments, capable of forming higher-order structures as bundles and ramifications. Transition of actin between the monomeric (G-actin) and filamentous (F-actin) states is regulated by a plethora of actin-binding proteins. *actin2-5* (*act2-5*) has a single point mutation that generates a substitution of Arg-179 to Cys-179. This mutant shows constitutive waving roots with altered microfilament organization. More importantly, it has auxin and brassinosteroids enhance responses (Lanza *et al*., 2012). In this study, we analyze *act2-5* root development at the cellular level, demonstrating that cell proliferation, elongation and lateral root production is altered. We also provided evidence that actin bundling in this mutant can be reconstitute by orthovanadate treatment.

Lanza *et al*., (2012). Dev. Cell *22*, 1275–1285.

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66.

Differential response of cell cycle markers against glucose and sucrose in maize during germination

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In plant cells, sugars can act as signaling molecules. Sucrose (Suc) can be sensed as a signal directly or a signal may arise via its hexose cleavage products glucose (Glc) or UDP-Glc and fructose. Glc triggers mitotic activity in developing tissues, while Suc is rather associated with the regulation of storage and differentiation-related processes. Its effect could be concentration-dependent. Many plant processes are regulated by nutrient availability, among them is the cell cycle, which is controlled by Cyclins (Cyc) and Cyc-Dependent Kinase (CDK) proteins. CycDs can sense internal and external stimuli such as phytohormones or sugars and are key regulators of G1/S phase transition, while CycA and B are expressed during mitosis. In this work we analyzed the effect of Suc and Glc on protein abundance of some CycDs and Bs, and of two CDKs with a role in cell cycle (A and B) in embryo axes imbibed at 12 and 24 h.
Suc showed a positive effect on ZmCycD2;2 regarding protein abundance and a negative impact on ZmCycD4;2 at high levels (120 mM). Glc reduced ZmCycD4;2 and 5;3 levels. In contrast, this sugar increased the abundance of both CycBs analyzed (ZmCycB1;2 and 2;1). The results indicate that sugars can regulate both CycD and B protein abundance. In general, Suc promoted cell growth through control of CycD and thus G1/S phase, while Glc acted more as a mitotic signal via control of CycB in maize embryo axes during germination.

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67.

Cyclin D6;1 has an associated kinase activity and it is located in nuclei during maize germination

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The eukaryote cell cycle is a highly regulated process that is finely orchestrated in time and space along with developmental processes. The main cell cycle regulators are Cyclin-CDK protein complexes. In particular, D-type cyclins drive G1/S transition by phosphorylating specific substrates such as Rbr, thus promoting E2F transcription factor release which then transactivates S phase specific genes. Noticeably, plants have a high number of D-type cyclins genes which are thought to have non-redundant functions. Recently, it has been demonstrated that *Arabidopsis* CYCLIN D6;1 (AtCycD6;1) plays important roles in establishing the proper root cell patterning and in stem cell niche maintenance by driving asymmetric cell divisions, linking cell cycle control with developmental programs; nothing is yet known about its role either in germination or embryogenesis although AtCycD6;1 reporters have been shown to be expressed in both mature and immature seeds. Despite its relevance as a model organism, *Arabidopsis* developmental processes are quite different from those of other crops; on the other hand, AtCycD6;1 has been studied only at transcriptional level. To have an insight into the role of maize CYCLIN D6;1 (ZmCycD6;1) at the protein level, we developed specific anti-Zm CycD6;1 antibodies that have been used to determine the accumulation pattern during germination by western blot, protein interactions and associated kinase activity by immunoprecipitation, and tissue localization by immunofluorescence. Results show that ZmCycD6;1 is present and has kinase activity throughout germination. Furthermore, ZmCycD6;1 gets nuclear localization as germination advances. Additional work has to be done to determine whether ZmCycD6;1 interacts with Rbr protein as D6 is the only D-type cyclin subgroup lacking Rbr binding motif, LxCxE.

68.

The jasmonate receptor COI1 plays a role in jasmonate-induced lateral root formation in *Arabidopsis thaliana*

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Plant growth and development is regulated by different classes of phytohormones. Jasmonic acid (JA) regulates a broad range of defense and developmental responses. COI1 has been recently found to act as JA receptor. In this work, we show that low micromolar concentrations of JA inhibited primary root (PR) growth and promoted lateral root (LR) formation in Arabidopsis wild-type (WT) seedlings. These responses on primary root growth inhibition were caused by alterations of cell division and elongation. It was observed that the *coi1-1* mutant was insensitive to JA on pericycle cell activation to induce lateral root primordia (LRP) formation and presented alterations in lateral root positioning and lateral root emergence. To investigate JA-auxin interactions important for remodeling of root system (RS) architecture, we tested the expression of auxin-inducible markers *DR5:uidA* and *BA3:uidA* in WT and *coi1-1* seedlings in response to indole-3-acetic acid (IAA) and JA and analyzed the RS architecture of a suite of auxin-related mutants under JA treatments. We found that JA did not affect *DR5:uidA* and *BA3:uidA* expression in WT and *coi1-1* seedlings. Our data also showed that PR growth inhibition in response to JA was likely independent of auxin signaling and that the induction of LRP required *ARF7*, *ARF19*, *SLR*, *TIR1*, *AFB2*, *AFB3* and *AXR1* loci. We conclude that JA regulation of postembryonic root development involves both auxin-dependent and independent mechanisms.

69.

Expression of CycD3;1 in maize germination

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Cyclin proteins are regulatory subunits of cyclin-dependent kinases (CDK); together, they play fundamental roles in cell cycle control. Like in mammals and yeast, the plants D-type cyclins are required for re-entry into the cell cycle in response to extracellular signals (nutrients and hormones). The role of CycD3;1 for cell proliferating and development in Arabidopsis and tobacco has been established. However, knowledge in maize is scarce. We found two almost identical CycD3;1 genes in maize, types a and b. The expression of CycD3;1 was studied during maize germination, in the presence and absence of phytohormones and also in three different plantlet tissues leaf, mesocotyl and root tips. Also, the behavior at the protein level was followed in maize germination, using antibodies raised against a 66 aa peptide in the C-terminal. CycD3;1 is present at the same level in the 0- 24 h germination. CycD3;1 co-inmmunoprecipitates with CDKA, CDKB;1 and PCNA during germination, and under these conditions the protein complexes show kinase activity. We will determine if kinase activity is modified by phytohormones.

70.

TOR Kinase activation by insulin stimulates the development of root hairs of *A. thaliana*

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TOR is a constituent of PI3K1S6K1TOR1S6K signaling pathway, this pathway allows eukaryote organisms to regulate growth and cell division. The TOR protein kinase can be inactivated by rapamycin following the formation of a ternary complex between TOR, rapamycin and FKBP12. It has been reported that Arabidopsis is resistant to rapamycin action (due to the inability of FKBP12 to binds rapamycin), which led to the construction of rapamycin-sensitive plants through the expression of ScFKBP12, -such as 25c line overexpressing this gene-. In this study we determined that the root hair development in wild lines (Wt) and 25c was stimulated by insulin dose-dependent manner, as well the production of hydrogen peroxide. The results showed that rapamycin and Torin1 -direct inhibitor of TOR- inhibit the development of insulin-stimulated root hairs in wild line. While the effect of both inhibitors on the growth hairs in the 25c line was dependent of the physiological state at the studied times. The genetic strategy with the use of estradiol-inducible tor transgenic Arabidopsis (tor-es1), confirmed the observations with both inhibitors. Together the above results, strongly suggest that insulin promotes development of Arabidopsis root hairs through activation of TOR.

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71.

Identification of a MAP kinase module involved in *Arabidopsis thaliana* embryo and root development

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Mitogen Activated Protein Kinases (MAPK) conform signaling modules playing important roles to transduce several intra- and extra-cellular signals allowing cells to respond efficiently to a given stimulus. MAPK signaling, conserved in all eukaryotes, involves the sequential action of three kinases, which activates each other by sequential phosphorylation. The first kinase (MPKKK) activates a second kinase (MPKK) and this in turn activates the last kinase of the module (MPK) that phosphorylates several substrates (other kinases, transcription factors, etc.) to regulate cellular responses (Andreasson and Ellis, 2009). In *A. thaliana* genome 60 MPKKKs, 10 MPKKs and 20 MPKs have been found, some playing complementary functions in cell physiology (MAPKGroup, 2002), but most of them still awaiting to be characterized. Recently, we found that a MPK mutant (*mpk6*) produces three genetically stable seed phenotypes (apparently associated with abnormal divisions during early embryo development) each one related with altered root system architecture (Lopez-Bucio *et al*., In progress). Taking advantage of these phenotypes, we performed a mutant screening trying to identify the complete MAPK module also up- and down-stream components involved in these development programs. Our data show that *Arabidopsis* mutants defective on MPKKK4 (*yoda*) and MPKK5 present embryo phenotypes similar to seedlings defective on MPK6. Additionally, we found that mutants on the receptor-like kinase SHORT SUSPENSOR 1 (*ssp1*) and on the transcription factor SPEECHLESS (*spch*) show *mpk6* like embryo abnormalities, suggesting a role for these proteins in the same signaling cascade. We also determined that mutants in MPKK5, as *mpk6*, develop longer primary roots and more lateral roots than WT seedlings. The role of referred proteins in embryo and root development will be discussed.

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72.

Alkamides delay flower senescence in *Lilium asiaticum* cv. Navona.

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Alkamides are considered plant growth regulators, they alters root development, stimulation the production of biomass and longevity in *Arabidopsis thaliana*. In this study, we observed the retardant effect of alkamides, particularly affinin, on flower senescence in *Lilium* cv. Navona. *Heliopsis longipes* root extract was applied during plant development, and the *Lillium* flowers in vase life expand evaluated, considering the time length it took the color to appear, the flower to open, the withe petals in release yellow petals and leaves, considering only three flowers in each stem. It was observed that the concentration 45µM of affinin applied during plant development resulted in a significant difference in the maintenance of bright green foliage; in appearing yellow, that was five days after the control and the wilting of the petals took 3 days longer than the control. Furthermore, compared with flowers grown without alkamides, kept in vases with other components in the solution under the same concentrations. It was found that by keeping the concentration of 45µM affinin resulted in longer vase life for 3 days than the control before it appears wilting of the petals, and the bright green of the leaves remained for more than four days after yellowing of the control. This shows that the alkamides delay senescence in cut flowers of *Lilium* cv. Navona, particularly if applied during the plant growth at concentrations of 45µM, allowing increased vase life of *Lilium* up to 3 days.

73.

A conserved bZIP mRNA involved in the Unfolded Protein Response (UPR) is processed during common bean root nodule ontogeny

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Unfolded protein accumulation in the endoplasmic reticulum (ER) promotes protein folding, peptide degradation and synthesis attenuation to recover homeostasis. This mechanism is known as Unfolded Protein Response (UPR) and remains conserved between yeast and mammals to maintain the optimal polypeptide folding environment, enhancing the expression of diverse chaperones and transcriptional factors. In plants it has been identified at least two signaling pathways for UPR, which are active upon adverse environmental conditions (heat stress, salt stress, pathogenic bacteria and viral infection). Symbiotic interaction between *Rhizobium* and *Phaseolus vulgaris* results in an infection process that presents conditions where ER stress could be active: increased cell division, massive membrane protein synthesis and increased reactive oxygen species (ROS) production. We identified a membrane-associated basic leucine zipper domain (bZIP) transcription factor in *P. vulgaris* genome, which conserves the characteristic 23 nucleotide processing signature related to UPR activity. This processed mRNA is present from 14 days post inoculation (dpi) to 28 dpi, coincidentally with nitrogen fixing activity in root nodules. These evidences suggest that UPR is active in N-fixing stages during nodule ontogeny.

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74.

Cell cycle proteins in landraces maize seeds

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During their ontogeny, the seed assume its higher level of vigor around the physiological maturity, thereafter the vigor gradually decline as well the biological potential of seeds, this process is called senescence which alters synchronous confluence of metabolic events leading to the seed germination, among them is relevant to note the establishment of the cell cycle because it implies a rigorous scrutiny of the biochemical and molecular embryo potential before the first cell division. The aim of this study was to analyze the impact of artificial aging on the initial germination and the presence of cyclins and cyclin dependent kinases in 3 landraces maize seeds (Chalqueño, Oaxaca, and Cuijingo). Total protein was extracted from embryonic axes at 0, 6, 12 and 24 germination hours, and were made western blots with specific antibodies. The findings indicate significant reductions in physiological evaluations in aged seeds compared to their controls (protrusion root radicle, and stem length, dry matter accumulation and formation of normal seedlings), in correspondence the biochemical markers of cell cycle showed a variable behavior, some maintained their levels through germination times evaluated and others not. Finally, it was possible to establish the presence of the protein according to their aging method, genotype and embryo germination time.

75.

Vanilloids affect root development in *Arabidopsis thaliana*

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Vanillloids are a group of molecules which contain a benzene ring and a lipidic chain, within that compounds capsaicin is the compound with highest molecular weight and the lipid chain contains a polar amide group. The topic of the vainilloid action in plants is a new and interesting topic because most of the studies of vainilloid are focused, mainly, in human medicine. In this work we tested the effect of vainilloids hexacapsaicin, octacapsaicin, decacapsaicin and dodecacapsaicin on root development in *Arabidopsis thaliana.* 50μM concentration hexacapsaicin induce primary root length and depletion in lateral root number, while 200 μM reduces primary root length and induces lateral root number. The another capsaicin structures have similar behavior. Then vanilloid are able of regulate root development in *A. thaliana* in a dose-dependent manner.

76.

Enzymatic activity and expression of three polygalacturonases during ripening in mango (*Mangifera indica* L.) cv. Kent

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Polygalacturonase (PG) degrades cell wall pectins in fruits affecting fruit firmness. PG activity has been evaluated and various isoforms have been identified in several fruits. The family of genes encoding PGs is large and it has been identified in plants whose complete genome is sequenced. However, only a partial cDNA clone of *PG* is available for mango fruit in databases. Therefore, in this study partial nucleotide sequences for three mango *PG* cDNAs were obtained and their expression was evaluated during ripening of "Kent" mango as well as *Mi*PG total enzymatic activity. Fruits were harvested at mature green stage and physiological parameters were evaluated. RNA was obtained from mango fruits (1, 4, 7, 10 and 16 storage days), and corresponding cDNAs were synthesized for quantitative gene expression evaluation and for cloning purposes. Moreover, mango pulp protein extracts were made and PG activity determined. Three partial cDNAs were obtained: *MiPG1, MiPG2* and *MiPG3* and their amino acid sequences were deduced. Mango PG sequences were similar to PG*s* from other plants and they contain functional motifs corresponding to the family of polygalacturonases. Gene expression of *MiPG1, MiPG2* and *MiPG3* was differential across the ripening process of mango fruit. The highest levels of expression of the three *MiPG* coincided with the highest mango tissue firmness losses. These events occurred after maximum ethylene production. Likewise, a significant increase in *Mi*PG activity was observed at day 16 of storage. These results suggest that we have discovered three *PG* genes that apparently are associated with ripening of mango fruit.

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77.

Protein pattern of cell cycle markers during the first 36 h of maize seed imbibition

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Maize seed germination (as all orthodoxes seeds), incorporates events that commence with the uptake of water (imbibition) by the quiescent dry seed and terminates with the emergence of the embryonic axis, usually the radicle. It is a time of intense metabolic activity, involving subcellular structural changes, respiration, macromolecular synthesis and cell elongation that drives to cell cycle start. The cell cycle is an important event where cells multiply and thus play a role in the growth and development of an organism, and its regulation strongly depends on phosphorylation and dephosphorylation events. In this work, we have studied some cell cycle markers during the first 36 h of Chalqueño maize seeds imbibition. At 36 h of maize seed imbibition, roots have protruded in 50% of the seeds and embryo axes protein patterns show no changes in the 0-24 h period, but major visible changes are seen in the 24- 36 h period on a SDS-PAGE. Cyclins A1;1, B1;1, D3;1, D4;2, and D6;1, CDKA, CDKB1;1 and PCNA are all present in dry embryo axes; Cyclin A1;1, Cyclin B1;1 and CDKB1;1 do not change in the period 0-36h imbibition while the amount of cyclins B2;1, D2;1, D3:2, D4;2 and D6;1 decrease as imbibition progresses. Contrastingly, PCNA increases with time and CDKA has a major increase at 36 h imbibition; additionally, this coincides with an increase in kinase activity in pulled-down p13suc1-kinase complexes. Results concerning identification of P13suc1-associated cyclins and CDKA phosphorylation at Thr14-Tyr15 and Thr160 residues are discussed.

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78.

Identification and characterization of microRNAs from *Bouteloua gracilis* by high-throughput sequencing and bioinformatics analysis

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The miRNAs are endogenous non-coding RNAs (19-24 nucleotides) have been characterized in plants and animals, in plants these molecules play a important roles in the modulation of different biological processes of development, proliferation and differentiation, as root development, in those include leaf morphogenesis and root and floral organ development. Also found that miRNAs regulate the response of the plants to various abiotic and biotic stresses as nutrient starvation, salinity, drought, oxidative stress, cold, UV-B radiation and virus. *Bouteloua gracilis* is a facultative apomictic, C4-photosynthetic, drought-tolerant, warm-season perennial and important forage grass of the semiarid regions of the United States and Mexico, where it yields abundant and high-quality forage for livestock and native fauna. Due to its economical importance, drought-resistance characteristics and wide distribution this grass is the subject of intensive ecological and physiological research. However to date, not exist information about the population expressed miRNAs in *Bouteloua gracilis*, therefore, is important the identification and characterization of expressed miRNAs as an important step toward understanding the molecular mechanism that regulate these molecules in *Bouteloua gracilis*. In the present work, we performed smallRNAs high-throughput sequencing from chlorophyllous cells of *Bouteloua gracilis* with Illumina Solexa technology. The data obtained were analyzed using bioinformatics tools. Were obtained 3,729,851 mappable unique sequences, of which 654 corresponded to mature miRNAs reported from other species in miRBase and 1005 were predicted as potential new miRNAs in rice, maize, sorghum and *Arabidopsis*. The most abundant sequences were taken into account in the search for target mRNAs and functional analysis included molecular function, biological process and cellular component. This study is the first identification of miRNAs and potential targets in *Bouteloua gracilis*.

79.

The dinoflagellate algae *Symbiodinium kawagutii* encodes three differentially regulated PsbO proteins

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The PsbO protein is a photosystem II component with important environmental implications due to its close interaction with D1 protein, which has been identified as the primary site of damage in endosymbiotic dinoflagellates after thermal stress. We biochemically characterized a 28 kDa protein from *Symbiodinium kawagutii* and identified it as the PsbO homolog (SkPsbO) through single band separation and partial peptide sequencing. Its chloroplast association was confirmed by western blot on photosynthetic membrane preparations and immunolocalization. Analysis of a cDNA library revealed three transcripts with different 5' and 3' UTR regulatory sequences. The coding sequences yielded three PsbO proteins differing by only one amino acid residue. The protein sequences displayed all the characteristic domains and features of PsbO from other sources but with unique dinoflagellate algae amino acid substitutions whose role in regulation, albeit key to PSII function, remains to be determined.

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80.

The family of E2F/DP transcription factors in maize. Genome-wide analysis, phylogeny and gene expression

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E2F transcription factors and their dimerization partners were initially identified as proteins required for expression of essential genes for the entry and progression of S-phase during the cell cycle. More recent studies have shown that S-phase regulation is not their only function and their role in many biological processes that include DNA replication, mitosis, response to DNA damage and repair, differentiation, development and apoptosis has been demonstrated. Some members of the family may function as transcriptional activators, while others show repressor activity. During the cell cycle, the control of E2F activity depends on their interaction with the RETINOBLASTOMA-RELATED (RBR) protein family members, which are strong transcriptional repressors. During the G1/S transition, RBR is phosphorylated at multiple sites by D-type CYCLIN/CYCLIN-DEPENDENT KINASE (CDK) complexes, causing its dissociation from the E2F/DP heterodimers, thus allowing transcriptional activation of their target genes. In maize, we have previously described members of the CYCD, CDKA and CDKB protein families, complexes formed and kinase activity on RBR. In this study, we performed a genome-wide analysis of the E2F/DP families in nine plant species. In all cases grasses had a larger number of family members than dicots. Moreover, the Poaceae group members show the formation of exclusive clades of protein sequences. We identified 12 *E2F*/*DP* genes in the maize genome, which show differential expression patterns in seedlings tissues and during seed germination, indicating a possible role in various processes during maize development.

81.

In vitro culture of polyploid species of Agave: A model for genetic and epigenetic studies on the species

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Agave is a species of economic and ecological importance to Mexico. Karyotype studies have shown that this species may contain diploid to octoploid varieties. Even basics unknown ploidización process in the species and because it is inferred that these demonstrate greater tolerance to biotic and abiotic factors, as has been based for *Brassica* and *Arabidopsis Trapogon,* our research has been to task polyploid develop models in vitro, in order to apply them in the study of basic understanding of genetic and epigenetic events that may be associated to polyploid plants ability to respond better to the above factors. In this work, standardized protocols for obtaining polyploid species *A. tequilana* var Azul (2X), *A. angustifolia* var marginarta (2X), *A. angustifolia* var Chelem Ki (6X), *A. fourcroydes* var Kitam ki (3X) and *A. fourcroydes* var Sac ki (5X). Also evaluated physiological, genetic and epigenetic events positioning the polyploid *in vitro* culture as a powerful tool for clarifying basics ploidización process of the species.

82.

Silent mutation in beta-glucosidase (glu2) from teosinte *Z. diploperennis*

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In maize, two isoenzymes of □-glucosidase known as Glu1 and Glu2 which are encoded by *glu1* and *glu2* genes, respectively, have a 90% identity at the nucleotide or amino acid level. The main function of □-glucosidase in maize is in the defense against pathogens and herbivores by releasing toxic aglycones (hydroxamic acids). We are interested in identifying and characterizing □-glucosidases isoenzymes Glu1 and Glu2 in some species of teosinte using molecular biology techniques. Seeds of teosinte *diploperennis* were germined. At the fifth day of growth, total RNA was extracted using TRIzol® reagent (Qiagen). The cDNA synthesis was performed using the One-step RT-PCR kit (Qiagen), with *glu1* and *glu2* primers reported in maize, the maize actin gene *Mac1* was used as control. The PCR products were analyzed on a 2% agarose gel with electrophoresis conditions of 80V for 90 min. Approximately 100 bp and 150 bp were the amplicon sizes of *glu1* and *glu2* respectively. The sequence of *glu1* amplicon showed 100% identity with the signal peptide of Glu1 maize protein. The sequence of *glu2* amplicon showed 100% identity with the coding sequence of Glu2 maize protein (102 pb) that allowed us to identify a change of a cytosine by a thymine that did not affect the amino acid sequence (silent mutation), Now, we work in identifying more changes in complete coding sequence of *glu1* and *glu2* that allows us to find structural differences. It is thought that defense mechanism in maize has been preserved for more than ten thousand years.

83.

Discovering the involvement of PpPLT genes in determinate growth of primary root of cardón *Pachycereus pringlei* (Cactaceae)

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In most angiosperms primary root grows for an extended period of time. Interestingly, as an adaptative advantage to long periods of drought, the primary root of several species of the Cactaceae family have a of determinate growth pattern (Dubrovsky, 1997; Shishkova *et al.* 2013). In these species growth of the primary root stops when the root apical meristem (RAM) becomes exhausted and cells at the root tip differentiate, and the molecular mechanisms of meristem exhaustion in Cactaceae are still unknown. We are interested in elucidation the role of *PLETHORA (PLT)* genes, encoding transcription factors from the AP2/EREBP superfamily, in determinate root growth in Cactaceae. In *Arabidopsis thaliana* the partially redundant *PLT* genes regulate maintenance of the RAM (Galinha *et al.,* 2007), and *plt1 plt2* double mutant exhibits determinate growth of the primary and lateral roots (Aida *et al.,* 2004). Previously in our group mRNA-seq of cDNA from primary root tips of *Pachycereus pringlei* on the initial (when RAM is present) and terminal (when RAM is exhausted) phases of development was performed; and CLC bio Genomic Workbench program was employed for *de novo* assembly of contigs. This permitted to identify five *P. pringlei* homologs of the *Arabidopsis* PLT transcription factors. Analysis of virtual expression levels indicates that all identified *PpPLT* genes are repressed in the *P. pringlei* primary root tip during the terminal growth phase.

The work was funded by UNAM-DGAPA-PAPIIT (IN204912).

84.

Characterization of the expression conferred by the At4g12640 5' regulatory region in transformed plants of *Arabidopsis thaliana* (L.), Heynh

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The At4g12640 gene of *Arabidopsis thaliana* encodes a Spen type protein whose function is unknown. Spen type proteins have at least one domain that allows them to bind to RNA (RRM) and a SPOC domain that allows them to interact with other proteins. Thus, these proteins are generally involved in gene regulation processes. In this work, we generated three gene constructs in which segments of 1500, 1000 and 500 base pairs of the 5´ regulatory region were fused to the *uidA::GFP* reporter gene using the Gateway® technology. *A. thaliana* plants were transformed by the modified floral dip method obtaining efficiencies between 1 and 1.2%. Expression associated to the vascular tissue was observed in the T1 transformed plants in the following structures: root, stem, cotyledon leaf, true leaf, stamens and gynoecium. These results suggest that the function of the At4g12640 gene might be associated with vascular tissue and in cells that will give rise to this tissue.

85.

Partial characterization of a mango (*Mangifera indica* L.) MADS-type transcription factor: expression at various fruit developmental stages and molecular modeling

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MADS-box genes are a large family of transcription factors initially discovered for their role during organ identity of flowers and fruit ripening. In this work, a MADS-box cDNA (*MiMADS1*) from mango was obtained and its expression was evaluated in mango fruit at various developmental stages. The MiMADS1 gene product was compared and its DNA-binding domain was modeled. Relative expression of *MiMADS1* was evaluated by qRT-PCR and the 18S rRNA gene was used for data normalization. Hand harvested mango fruits cultivar Keitt from the different developmental stages (45, 75, 105 and 135 days post-anthesis or DPA) were used for total RNA extraction and cDNA synthesis. The Phylogenetic analysis of MiMADS1 was performed using proteins from the SEP and AGL subfamilies from several fruits. The structure of the N-terminus of MiMADS1 was modeled as a homodimer and it was also docked to a double strand DNA fragment. The *MiMADS1* cDNA is 765 pb long and the coding protein of 254 amino acids has a molecular weight of 29.7 kDa. *MiMADS1* mRNA steady state levels remained constant during mango fruit development and were up-regulated when mango fruits reached physiological maturity (135 DPA). The MiMADS1 transcription factor is very similar to others MADS proteins expressed in fruits like kiwi, tomato and strawberry and probably belongs to the SEP clade of MADS-box proteins. The N-terminus of MiMADS1 was predicted as a loop, which has several residues that interact with the DNA minor groove and the phosphate backbone. MiMADS1 could have a role during development and ripening of this fruit MiMADS1 and it is an interesting model for understanding DNA binding for transcriptional regulation.

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86.

Chromatin dynamics during plant cell proliferation.

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Plant organogenesis occurs continuously over the entire lifespan and required a coordinated balance between cell proliferation and cell differentiation. Cell proliferation depends on a highly regulated series of events of which the transcriptional control and DNA replication play essential roles. In both process, chromatin modifiers such as DNA methylases, histone modification enzymes and non-coding RNAs regulate the recruitment of DNA replication proteins or transcriptional factors to DNA. In the present work, we determine that some Arabidopsis genes encoding chromatin remodeling factors, such as Histone methytrasferases (HMT) and Histone acetyltrasferases (HAT) are preferentially expressed in proliferating cells, even some of them are cell cycle regulated as cyclins. This expression pattern suggest that some epigenetic modifiers play essential roles in proliferative cells, in fact we found that initiation of DNA replication requires an increase in acetylation of histone H3 and H4, as well as in trimethylation of histone H3 at lysine 4 (H3K4me3). Furthermore, HAM1 a HAT also acts during cell proliferation. HAM1 is a nuclear protein, which interacts with DNA replication proteins and its expression is restricted to proliferating cells, where it behave as transcriptional regulator. This work provides some evidence of epigenetic implications on cell proliferation.

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87.

Transcriptional and protein-protein networks in gynoecium and fruit development in Arabidopsis

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Gene regulation at the level of transcription is crucial for almost all biological processes in a cell or organism. Transcription factors (TFs) are sequence-specific DNA-binding proteins that are capable of activating and/or repressing transcription. Many mutants affected in development have been associated with altered expression levels of TF genes. Therefore, the analysis of TF genes can be the basis for a better understanding of plant developmental processes. Our lab identified various novel TFs affecting gynoecium and fruit development in Arabidopsis. Moreover, we discovered that the hormone cytokinin is important for gynoecium and fruit development. At the moment, we are studying the genetic interactions among them and furthermore, to gain a better understanding about how they function on the molecular level, matrix-based yeast two-hybrid screens are performed with known TFs involved in meristem, flower, and fruit development. The latest results will be presented

88.

Analysis of genetic regulation of the reproductive structure in *Arabidopsis thaliana*

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In *Arabidopsis thaliana*, like most angiosperms, a flower has four sepals, four white petals, six stamens and two fused carpels. Specific combinations of homeotic proteins determine the identity of the floral organs. However is still unknown how these combinations select their target genes. Interestingly for some homeotic proteins have been shown that their targets genes could depend on the developmental stage. Then one biology question is how these different combinations occur and which group of genes are target of each. Genes have been identified, which when expressed have a specific role in the development of carpels and stamens, as are *SEPALLATA1* (*SEP1*), *SEP2*, *SEP3*, *APETALA 3* (*AP3*), *PISTILLATA* (*PI*), *CRAWSCROW* (*CRC*), *SHATTERPROOF 1* (*SHP1*), *SHP2* and *AGAMOUS* (*AG*). And some combinations have already been reported as for carpel development is AG, AP3, PI and SEP3 whereas for stamens is AG, AP3 and PI. In order to identify the genetic network regulated by AG and AP3, we performed a ChIP analysis coupled to microarray (ChIP-chip), using different genetic backgrounds. So far, we have identified around fifty genes as putative targets genes of the homeotic complex AG-AP3. Currently, we are confirming the interaction between AG and AP3 with the upstream intergenic region of a group of putative genes target, for this we are using ChIP with AG and AP3 antibodies.

89.

The ICK/KRP family of CDK inhibitors in maize: genome-wide analysis, phylogeny and expression patterns

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In eukaryotes, the cell division cycle is strictly regulated by cyclin-dependent kinases (CDKs) together with specific cyclin partners. The activity of CDKs can be regulated positively or negatively by transcriptional regulation, binding by other proteins, phosphorylation/dephosphorylation and proteolysis of the cyclin partner. While binding by cyclins activates CDKs, CDK inhibitors (CKIs), generally low-molecular-weight proteins, bind to CDKs and inhibit their activities. In plants, two families of CKIs are known. The first has a conserved C terminus domain that shows a limited similarity to the mammalian KIP/CIP inhibitor $p27^{Kip1}$. This family of proteins in Arabidopsis is referred to as ICKs (interactors/inhibitors of cyclin-dependent kinase), and also as KRPs (Kip-related proteins). Recently, a second family of CKIs was described as the SIM/EL2 family. In maize, we have previously described members of the CYCD, CDKA and CDKB protein families, the complexes they form and kinase activity on specific targets. In this study, we performed a genome-wide analysis of the ICK/KRP family in maize and we identified at least ten genes, although two of them may be pseudogenes. According to the analysis of sequence and phylogeny of the ICK/KRP gene family in maize, these are present in only two of the three classes known; additionally they show differential expression patterns in different tissues of seedlings, suggesting a possible role in various processes during the development of the plant

90.

Analysis of differentially expressed genes during the transition from the vegetative to reproductive stage Agave tequilana

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A. tequilana is a perennial monocarpic species exploited commercially for the production of tequila. Initiation of bolting signals that plants are ready to harvest and this process must be controlled in order to reduce loss of stored carbohydrates. The development of transcriptome analysis based on deep RNA sequencing is a very powerful and reliable tool to perform the identification and quantification of almost all transcripts present in a cell or tissue, either in a specific developmental stage or physiological condition. We are currently carrying out an analysis of genes expressed differentially at four distinct stages during the transition from the vegetative to the reproductive stage of *Agave tequilana*, ,in order to identify the genes that induce and regulate the transition and gain a better understanding at the genetic and biochemical level of this process in *Agave tequilana,*
Issuer ID 00016, Topic Plan developmental process.

91.

Effect of HDAC and HAT inhibitors on pathogenicity in *Phaseolus vulgaris* L. by the fungus *Macrophomina phaseolina* (Tassi) Goid.

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Epigenetic regulatory mechanisms are responsible for the establishment of the phenotype in eukaryotes. DNA methylation and histone modifications control regulation of information stored in the genome, throughout the entire series of biological events that in time lead to specialization of a cell and its differentiation; pathogenicity involves cell differentiation. The use of chromatin remodeling enzyme inhibitors is a strategy to inquire about the biological consequences of chromatin modifications in organisms. *Macrophomina phaseolina* (Tassi) Goid., is a plant pathogenic fungus with a broad host range (more than 500 plant species) including common bean (*Phaseolus vulgaris* L.). Common bean is the most important grain legume crop for human consumption in the world. We used the histone deacetylases inhibitor trichostatin A (TSA), the histone acetyltransferases inhibitors CPTH2 and garcinol to assess their effects on growth and development of *M. phaseolina*, as well as fungal pathogenicity in *P. vulgaris*. TSA, CPTH2 and garcinol affected both daily growth rate and macroscopic morphology of fungal colonies in solid minimum medium as well as microsclerotia diameter. In greenhouse experiments, pathogenicity of *M. phaseolina* in V3 growth stage plants was significantly decreased by TSA. These results show the importance of acetylation/deacetylation in cellular differentiation process of *M. phaseolina* and also suggest that this mechanism of epigenetic modification is closely associated to regulation of genes involved on pathogenesis of the fungus in *P. vulgaris*.

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92.

Molecular analysis and identification of genes from the light signaling pathway, the circadian clock and crassuleacean acid metabolism in Agave tequilana

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Agave genus comprises monocotyledonous, monocarpic, succulent plants, whose principal feature is their capability to tolerate and develop under extreme environmental conditions such as drought, high salinity, excessive irradiance, between others. Agaves withstand these harsh conditions by their physiological, morphological and metabolic traits, which includes the crassuleacean acid metabolism (CAM). However until recently few molecular and transcriptomic data were available to understand these biological process, but the *Agave tequilana* 35,000 EST database has opened a path to study these magnificent plants. To understand agaves physiology in an holistic and comprehensive way, we have searched the database for genes involved in CAM metabolism, light reception and the circadian clock. The later has been related to plant performance, stress tolerance and homeostasis, while the light is a key factor in entraining the clock in temperate plants. However the role of the circadian clock in subtropical plants is widely unknown, particularly in CAM plants. Furthermore the clock can be modulated by metabolic signals derived from carbon metabolism, thus CAM metabolism in *Agave* may override the clock transcriptional control. Currently, we have identified key components of the light signal transduction pathway including blue (*CRY1*, *CRY2*, *PHOT1*, *PHOT2*) and red light (*PHYA*, *PHYC*) photoreceptors and key enzymes from the CAM metabolism (PEPC, PPCK, NADP-ME, PEPCK). From the circadian clock machinery, we found core components as *LHY*, *GI*, *TIC* and members of the pseudo-response regulator family, as well as the F-box family *ZTL*, *LKP2* and *FKF1*. The study of the transcriptional behavior of these set of genes under different environmental conditions may allow us to understand their role in environment sensing, and ultimately the role and interaction between the circadian clock and CAM metabolism.

93.

Antioxidant response and triterpene production by jasmonic acid elicitation in Jatropha curcas cell suspension cultures

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Jasmonic acid (JA) is a linolenic acid-derived, which has a dual function as phytohormone and signal molecule when is used as elicitor of secondary metabolite production and oxidative defense. It has been reported that *Jatropha curcas* plants tolerate different types of stresses, so their response to JA was investigated. Cell suspension cultures of *J. curcas* elicited with 400 M of JA for 198 h resulted in a threefold increase of ascorbate peroxidase, peroxidase and catalase activities. After elicitation the production of antioxidant Lupane type pentacyclic triterpenes: lupeol and butulinic acid increased 4 and 2 times while hydrogen peroxide and lipid peroxidation (MDA) contents were only slightly high. Our results suggest that in *J. curcas* cell cultures JA modulate the triterpene production concomitant with an antioxidative defense mechanism.

94.

A new experimental model to study primary and secondary metabolism in Capsicum chinense Jacq.: cell suspension from placentas

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Habanero peppers *(Capsicum chinense* Jacq.) accumulate capsaicinoids in the placental tissue in blister-like structures. These compounds are of great commercial interest given their many applications in different industries. Since the location of the enzymes that synthesize capsaicinoids coincides with those involved in the production of their precursors, placentas are considered an excellent model to study the regulation of both primary and secondary metabolisms. A single cell suspension has been obtained from placentas of green immature habanero pods in MS medium without growth regulators. This cell suspension has been propagated for over a period of six months. The characterization of the cell suspension comprised growth parameters, such as fresh weight, dry weight and number of cells per fixed volume, and viability. Growth data indicated that the cell culture accumulated a maximum biomass of 1.34 g after 36 days. Its viability is above 80% and the microscopic observation of a sample showed isolated single cells, with numerous chloroplasts, even after this six-month period.

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95.

Los genes hisCl e hisC2 están involucrados en la producción de ácido indol-3-acético y están altamente conservados en cepas de Azospirillum brasilense

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Some *Azospirillum* strains can produce significant amounts of IAA and can increase IAA production when medium is supplemented with tryptophan (Trp). The biosynthesis of IAA, among other growth regulators, by *Azospirillum* could explain the enhancement of plant root development and the concomitant improvement of mineral and water uptake by roots. Such effects make these microorganisms beneficial as comercial inoculum in agronomical important crops. IAA is a metabolite derives from Trp by several pathways and can also be synthesized by Trp-independent route in plants and bacteria. More that one pathway could be present in bacteria. Physiological evidence for different Trp-dependent pathways for IAA synthesis in *Azospirillum brasilense* has been reported. The indole pyruvate (IPyA) route is one of the main pathways for IAA synthesis from Trp (Trp-IPyA-indole-3-acetaldehyde (IAald)-IAA), catalyzed by the key enzyme indole pyruvate decarboxylase (IPDC); and for the aromatic amino acid aminotransferases (AAT).
We cloned and sequenced the genes encoding AAT1 and AAT2 (*hisC1* and *hisC2* genes, respectively). It was determined production of IAA and AAT activity in the mutant *hisC2::km*^{R} from *A. brasilense*. IAA production was reduced significantly compared with the wild-type production (up to 60% less). Analysis of genes obtained from several strains isolated from different origin show that *hisC1* is highly conserved. Date presented suggested that AAT1 activity plays a role in *Azospirillum*–plant interactions.

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96.

Regulation of Deoxyxylulose phosphate synthase from *Arabidopsis thaliana*

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In plants, there are two metabolic pathways for the synthesis of isoprenoids: the mevalonate pathway and the MEP pathway. The MEP pathway takes place in the chloroplast. The first step of the pathway is catalyzed by the enzyme 1-deoxy-D-xylulose 5-phosphate synthase (DXS) which is a limiting step of the pathway. Previous studies have shown that the expression of the different genes in this pathway is coordinated. In mutants of the pathway the protein levels of DXS increase but the rest of the proteins are reduced. A similar effect is observed in the presence of fosmidomycin, an herbicide that blocks the second enzyme of the MEP pathway. These results have let us to suggest that this constitutes a feedback mechanism in response to the demand of the IPP and DMAPP final products. In the presence of fosmidomycin the half-life of DXS increases to more than 12 hours in comparison to a half-life of 80 minutes in the absence of this inhibitor. We concluded that DXS is posttranslational regulated. To analyze any potential posttranslational modifications that the DXS protein could suffer, we performed 2D- gels protein analysis of wild type plants treated with and without fosmidomycin. Western blot anti-DXS analysis shows, differences in the migration of this protein in the samples treated with the inhibitor, several points were recognized by the DXS antibodies suggesting that posttranslational modifications that change its isoelectric point and migration might probably related to the DXS stabilization. We are interested in wether there are differences between treatments, with and without fosmidomycin, in various aspects. For this purpose we are looking for DXS's interactors in both conditions by coimmunoprecipitations. We also know that DXS is more stable in presence of fosmidomycin, but we want to know if DXS is also more active in those conditions, for that reason we are going to determine DXS enzymatic activity.

97.

Strawberries with high amounts of nutraceutical antioxidant flavonoids

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La fruta de la fresa (Fragaria vesca L.) de la familia de las rosáceas es muy apreciado en general por su sabor y color. Además, cuenta con una cantidad considerable de metabolitos secundarios con propiedades antioxidantes, incluyendo compuestos fenólicos, que están asociados con la prevención de las enfermedades cardiovasculares, para reducir la inflamación y actividad anti-cáncer. El contenido de algunos compuestos fenólicos son modificados por factores tales como las técnicas de cultivo, las condiciones de crecimiento, y el proceso de maduración entre otros. También, la exposición de frutas a condiciones de tensión con varios agentes bióticos y abióticos tales como la radiación UV, agentes patógenos, parásitos, las lesiones y la exposición a temperaturas extremas; afecta directamente el contenido de compuestos fenólicos. Anteriormente, en nuestro laboratorio hemos creado las condiciones para aumentar la cantidad de flavonoides y después de la irradiación UV de antocianina. En este trabajo se presentan los resultados de otros tratamientos como el ácido salicílico, metil jasmonato y la combinación de la radiación UV con éstos en cuanto al contenido en flavonoides frutas fresas. El objetivo es aumentar aún más los flavonoides antioxidantes y estabilizar la respuesta al estrés. Un análisis cuantitativo se llevó a cabo utilizando el espectrofotómetro y HPLC, para medir el contenido total de flavonoides, la actividad antioxidante y la antocianina, así como fisetina, quercetina y pelargonidina específicamente.

98.

In vitro cytotoxicity and antibacterial activities of *Callistemon citrinus* (Curtis) Skeels

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Las especies de Callistemon son usadas en México como plantas ornamentales, sin embargo en otros países son usadas en la medicina tradicional. El presente estudio evaluó las actividades antibacterial y citotoxica de Callistemon citrinus en base a la edad fisiológica y parte de la planta usada, en un esfuerzo para darle un valor agregado a esta planta. Se evaluó la actividad microbiana de extractos etanólicos de hoja y flores de dos edades de la planta C. citrinus. Se utilizo el método de difusión en agar para la determinación antimicrobiana se encontró que los extractos de hoja y flores de una planta de 20 años tienen un efecto inhibitorio sobre las siguientes bacterias: Escherichia coli, Salmonella spp, Klebsiella pneumonia, Staphylococcus aureus, Staphylococcus epidermidis, y Staphylococcus saprophyticus, mientras que los extractos de hoja y flores de una planta de 4 años tuvieron un efecto ligeramente menor que los de la planta de 20 años. Se usó Artemia salina para los ensayos de citotoxicidad, se encontró que los extractos de hoja de ambas edades presentaban actividad citotóxica para Artemia salina (CL50 ¿600 1g1ml), mientras que los extractos de flor de 4 y 20 años no presentaron actividad citotóxica. Se encontraron 17 terpenos en los extractos etanólicos de C. citrinus sin embargo la concentraciones de estos fue diferente dependiendo del tejido y la edad fisiológica de la planta.

99.

Evaluation of *in vivo* antitumour and antioxidant activity of proteins and cytotoxic effect of peptides from *Bixa orellana* L. seeds

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Biologically active proteins and peptides have become important products as a result of new knowledge of their functional and biological activities, such as antihypertensive, hypocholesterolemic, antimicrobial, antithrombotic and antioxidant activities, as well as their possible anticarcinogenic use. Seeds including soy beans (*Glycine max* L.), cacao beans (*Theobroma cacao* L.) and amaranth seeds (*Amaranthus hypochondriacus*) have received widespread study, and have shown proteins with antitumour activity and peptides with cytotoxic effects on cellular proliferation in different cancer cell lines. *Bixa orellana* seeds have a protein content of 11-17% and possess storage proteins which have been shown to possess biological activities, especially albumins, which are water-soluble proteins. These were shown to possess antitumour and antioxidant effects *in vivo* by ABTS and ORAC-FL methods. Furthermore, albumin hydrolysates hydrolysed with digestive enzymes and alcalase produced peptides which were purified by FPLC. Their fraction termed C5 demonstrated 99% cytotoxic activity on cellular proliferation at 10 µg/ml. This fraction was found in both hydrolysates produced with digestive enzymes and those hydrolysed by alcalase. The study was carried out on transformed L5178Y mouse lymphoma cells.

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100.

Effect of chitosan on transcript levels of genes related to defense responses in grapevines (*Vitis vinifera* L.)

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Vitis vinifera is susceptible to several fungal diseases that affect grape production worldwide. The use of chemical fungicides has been the main control method, but it has led to the emergence of resistant strains among other unwanted side effects. Therefore, there is an increasing interest in finding new natural alternatives to induce the natural resistance of plants. Chitosan has been proven to exert antifungal activity against a large variety of phytopathogenic fungi and, in grapevine leaves it is reported to induce an increment in the enzyme activity of pathogenesis-related proteins and their corresponding genes. The aim of this study was to evaluate the effect of chitosan on the genic expression of chitinase (*CHI*), β-1,3-glucanase (*GLU*), phenylalanine ammonia-lyase (*PAL*) and chalcone syntase (*CHS*), and their relation with the induction of resistance in grapevine against the phytopathogenic fungal model *Botrytis cinerea*. We demonstrated that the application of chitosan reduced the infection development in leaves of treated grapevines. Leaves infected 24 and 48 h after treatments application were less susceptible to infection. Chitosan influenced the expression of the evaluated genes in dependence of the concentration and the time elapsed after application. The most obvious changes were observed in the transcript levels of *GLU* and *CHI* with a higher accumulation than in controls. A positive correlation between the expression of *PAL* and *CHS* was observed only in treated plants. Interestingly, the increase on the expression of *GLU* and *CHI* correlates with the decrease in the fungal development in grapevine leaves.

101.

Metabolic characterization during the seed ontogeny of *Brachypodium distachyon* (L.) P. Beav. (Poaceae): an interesting case of replacement of α-glucans by β-glucans as main storage compounds

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Brachypodium distachyon (L.) P. Beauv. is a Poaceae that is emerging as a new plant model for temperate grasses. The *Brachypodium* genome has been sequenced but metabolic information is rather limited. In order to gain knowledge of the seed biochemical composition during development, we measured metabolites (e.g. soluble sugars, total amino acids, starch and (1-3), (1-4)-β -D-Glucan), in 5 different tissues/organs (embryo, endosperm, lemma, palea and lodicules) during seed ontogeny. Reserve carbon in *Brachypodium* seeds is stored mainly as (1-3), (1-4)-β -D-Glucan, starch and sucrose, which varied in concentration and proportion according the developmental stage and the tissue evaluated. The nucellus contained low concentrations of starch (~15 umol/g DW) before fertilization. Embryo starch increased as the seed maturated reaching the highest values at the dry stage (~200 umol/g DW). Unexpectedly, endosperm starch decreased continuously (on weight basis) during maturation until it reached low values (~20 umol/g DW). In *Brachypodium* seeds, the endosperm contained low traces of β-glucans (0-2.17 mgL-1) at the initial stages (few days after fertilization), and then after cellularization, the β-glucan concentration steadily increased till seed maturation when the highest values were recorded (~1,881 mgL-1). Sucrose content was higher in the embryo than in the endosperm. Sucrose levels in the embryo reached the highest values (~200 umol/g DW) at the final stage (dry seed). In contrast to this, in the endosperm were highest after fertilization (75 umol/g DW), and decreased gradually as the seed ripen (~0.5 umol/g DW). Lemma and palea had high concentrations of chlorophylls and carotenoids. In these structures, sucrose, glucose, fructose, total amino acids and starch concentrations were high during early stages of anthesis and gradually decreased during seed development. We conclude that in *Brachypodium* each of the seed structures has a distinctive metabolism during development, which may be related to its physiological function.

102.

Involvement of the octadecanoic pathway in the induction of alkaloid biosynthesis in cell suspensions of *Argemone mexicana*

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Cell suspensions of *Argemone mexicana* (chicalote) accumulate significant amounts of the benzophenanthridine alkaloid sanguinarine without requiring the exposure to elicitors of secondary metabolism. However, when challenged with yeast extract (YE) or methyl jasmonate (MeJa), a limited induction occurred within the first 72 h of exposure. The magnitude of the response increased when both elicitors were sequentially applied. In order to establish if the response to these compounds occurred *via* independent pathways or share common elements, cell suspensions were exposed to YE and diethyldithiocarbamic acid (DIECA), an inhibitor of jasmonates' biosynthesis. Although YE induction of sanguinarine accumulation was abolished by DIECA, its content maintained the same level as in non-induced cultures. Interestingly, MeJa exposure partially reverted the inhibitory effects of DIECA suggesting that *Argemone* cell suspensions response to YE may involve jasmonate participation.
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103.

Alkaloid distribution in *Argemone mexicana* tissues associated to transcript accumulation

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Argemone mexicana (chicalote) belongs to the Papaveraceae family. It produces benzylisoquinoline alkaloids (BIAs), which may explain its use for medicinal purposes. *Argemone* BIAs belong to the benzophenanthridine and protoberberine families. It is one of the few plant species producing both types of BIAs. Alkaloid distribution follows a specific tissue pattern associated to development. In this way, sanguinarine and chelerythrine (benzophenanthridines) and berberine (protoberberine) were differentially accumulated in roots and aerial tissues. In mature plants sanguinarine occurred in roots and seeds; whereas, berberine and chelerythrine were observed throughout the entire plant. On the other hand, in growing seedlings, sanguinarine was early detected, both in radicles and hypocotyls. In contrast to mature plants, berberine in seedlings was detected in roots, but only after hypocotyls had emerged. Interestingly, when the first pair of true leaves was formed, this alkaloid was restricted to the aerial tissues. These results suggest that the biosynthetic ability of the different tissues is modified during the early development and results will be analysed associated to their transcriptional profiles.

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104.

Physiological, biochemical and molecular effects of applying immobilized urease to habanero pepper (*Capsicum chinense* Jacq.)

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Nitrogen is fundamental for plant growth and development. Given that most agricultural soils rarely contain enough N, it must be provided in the form of fertilizers. Their application, though helping to improve productivity, also induces detrimental consequences to the environment. Hence the development of enhanced efficiency fertilizers (EEFs) has acquired a renovated relevance. They extend the duration of N release/availability to soils improving yields. Immobilized enzymes have been used to control N release, so the physiological, biochemical and molecular effects of the use of immobilized urease were studied in habanero pepper seedlings. Seedlings, growing under hydroponic conditions, were subjected for 30 days with one of the following treatments: 1 mM KNO_3, 7 mM urea, 3 mM urea + immobilized urease, 7 mM urea + immobilized urease, and without N. Growth parameters (fresh and dry weights, plant height and leaf number), along nitrogenous compounds (nitrate, ammonia, amino acids and total protein), and nitrate reductase and glutamate dehydrogenase activities and gene expression (*CcNRT2*, *NR*) were evaluated. Results suggest that the application of immobilized urease could be a feasible strategy to improve N usage when urea is used as fertilizer to cultivate habanero pepper.

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105.

Quantification of aloin by HPLC on crops of *Aloe vera* that growing under different environmental conditions at Yucatán State, México

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Aloe vera, which is originated from Africa, it is well recognized by its extremely high utilization and its ability to adapt on various environmental conditions, making it easy to cultivated on other parts of the world. Its exudate (acibar) contain numerous biologically active compounds, the most well known of which is aloin (a drastic laxative). In Yucatán it is cultivated by temporarily (the most common by farmers) and watering conditions. The aim of this study is the quantification of aloin in exudates of *Aloe vera* growth under different environmental conditions (dry and hot seasons) by HPLC. The samples were collected monthly on Maxcanú (sites A and B) and Chicxulub (site C), Yucatán. From august to june. The exudates were filtered by using a C_{18} column and analyzed by Zahn Method in a Flexar Perkin Elemer HPLC on Alltech C_{18} column (250 x 4.6 mm, 5 m) at 27 C . The method used a gradient elution of methanol and water, and the UV detector was settled at 294 nm. 20 l of the sample (1mg/ml) were injected for each collection site with three replicates. Calibration curve was made with sigma aloin at the concentration from 0.01-0.9 mg/ml. We observed that the aloin content in sites A and B was the same trend, Aloin content increase at dry season (November-may) having a highest concentration in april and february, respectively. Site C showed irregular behavior in aloin content in both seasons. All of them presented the best content of aloin under dry or hot seasons.

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106.

Isolation and chemical modification of quinones of *Aloe vera* and their biological evaluation against phytopatogenic bacteria

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Aloe vera is a specie belonging to the *Aloe* genus known for its medicinal and therapeutic properties. In Mexico, the commercialization of this specie is focused on the leaves, being Tamaulipas (4,550 hectares) and Yucatan (280 hectares) the main places of production. In contrast, the root of this specie (rich in phenolic compounds) is discarded. The roots elaborate many interesting secondary metabolites, predominantly; free anthraquinones and anthrones, and their extracts show antimicrobial activities against different pathogenic bacteria. In order to find new applications for this crop, this work focused on the isolation and chemical modification of quinones from roots of *A. vera* and its evaluation against strains of *Xanthomonas campestris pathovar carotae, Pectobacterium carotovorum subsp. carotovorum and Pseudomonas syringae pathovar pisi* which are phytopathogenic bacteria that affect some crops of commercial importance. The root dry material was extracted with methanol and then partitioned with ethyl acetate (EtOAc). The EtOAc extract was fractionated by successive column chromatographic procedures until purification of the quinones aloesaponarin I (4.43%), aloesaponarin II (0.2%), desoxyerythrolaccin (0.63%), laccaic acid D methyl ester (0.59 %) and aloesaponol I (0.49%). Additionally, some derivatives were obtained. The compounds were characterized by spectroscopic and spectrometric techniques. Desoxyerythrolaccin (MIC = 46.87 µg/mL) and laccaic acid D methyl ester (MIC = 93.75 µg/mL) showed antibacterial activity against *X. campestris pv. carotae*. In conclusion, the roots of *A. vera* from Yucatan contain quinones showing antibacterial activity against phytopathogenic bacteria that affect crops of commercial interest.

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107.

Deep-sequencing transcriptome analysis of cycad *Dioon edule*, a non-model species

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The adaptation of species to ecological niches is a common and dynamic mechanism, however the genomic bases that allow the adaptive variability of species are little known. Cycads (*Cycadophyta*) are a group of plants belonging to a lineage of gymnosperms with a 300 million years-old long evolutionary history including major environmental and landscape changes, driven in part by human activity and part by climate change. Recent evidence suggests that cycads are a dynamic group with undergoing diversification. The genome of cycads therefore constitutes the link between the origins of seed plant and gymnosperms diversification under a dynamic environment. We used data from the 1KP Project Consortium (http://www.onekp.com/index.html) to functionally annotate the transcriptome of *Dioon edule*. We explore the contribution of retrotransposons in genome size and evolution in cycads, and the prediction of biosynthetic pathways of secondary metabolites shared with their cyanobacterial symbionts. The application of Next-generation (NextGen) sequencing technologies, such as RNA sequencing (RNAseq), to non-model species like *Dioon edule*, will enable us to get novel insights about the genomic basis of cycads and gymnosperms' adaptive variability, in an ecological, evolutionary and phylogenetic context.

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108.

Metabolic and physiological changes in wheat transformed with a bifunctional gene for trehalose synthesis

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Trehalose, a disaccharide, is widely distributed in nature. In *Saccharomyces cerevisiae* and *Selaginella lepidophylla* trehalose accumulation has been related to survival capacity under extreme dehydration conditions. Recently a promising transformation strategy has been utilized consisting of transformation with bifunctional *tps-tpp* constructs. Our aim was to analyze changes in carbohydrate metabolism and physiology in wheat plants transformed with a bifunctional *ScTPS1-TPS2* construct. Kronstad F2004 wheat variety was transformed by agroinfection using either the *35S::ScTPS1-TPS2* or *rd29A::ScTPS1-TPS2* construct. There was an effect on T_2 plant growth and development: T_2 seed germination was delayed as were time to spiking and anthesis, whether the promoter used was constitutive or induced expression. Of the *35S::ScTPS1-TPS2* transformed plants, 33% had greater root development, which was correlated with higher glucose, sucrose and fructose contents. *ScTPS1-TPS2* transformation, regardless of the promoter used, favors CO_2 assimilation rate (A) and CO_2 assimilation efficiency. Improved water use efficiency (A/gs) was also observed in *rd29A::ScTPS1-TPS2* plants. This may be related to more efficient electron transport in the photosynthetic apparatus, perhaps as result of decreasing photorespiration. Carbon assimilation efficiency is also a consequence of the lower energy dissipation and better quantum efficiency in PSII. These results suggest trehalose metabolism participates in the regulation of wheat development and physiology, and improves photosynthetic parameters.

109.

Opaco2 mutant gene and phylogenetic relationships of quality protein maize

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The increase in the essential amino acids lysine and tryptophan in the grain of maize by using the mutant gene opaco2 is a helpful option to develop varieties whose seeds possess protein quality and contribute to the reduction of the chronic malnutrition prevalent in marginalized populations whose diet is largely based on this cultivated specie. Therefore, it was started the development of such modified maize varieties by breeding. Seeds of six inbred lines of maize classified as quality protein maize (QPM) and 15 of its direct single crosses, was subjected to the determinations of tryptophan, lysine, quantity, quality of protein, and also the genetic advance in the crosses in relation to their parental lines mean. The experiments were conducted under a randomized block design with two replications of 100 seeds, the mean comparison was made by the Tukey method, and the genetic advance was analyzed with the ¿t¿ test. It was detected as quality protein maize the M2, M3, and M6 lines. In the crosses, M2 X M6 highlighted by its genetic gain (¿ = 0.01) in protein quantity (2.3%), protein quality index (- 0.5 %), as well, the increases in protein and amino acids levels had an impact on quality index. M1 X M4 and M4 X M5 showed genetic advance acceptable for tryptophan, lysine and quality index, in this case likewise the amount of amino acids affected the protein quality, in contrast with M2 X M4, M3 X M6 y M4 X M6 crosses, with positive genetic gain but only in lysine, tryptophan and protein. It's important to state that by the hybridizing process there was a genetic advantage for essential amino acids and protein, but the lines M2, M3, and M6 well-maintained theirs QPM properties.

110.

Autophagy promotion by insulin and deprivation of auxin in NT-l tobacco cell cultures

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Upon exposure to stress conditions or during senescence, plants degrade cytoplasmic macromolecules inside the vacuole by a process known as autophagy which allows cells to recycle cytoplasmic intracellular components during development or limiting nutrient conditions. Furthermore, insulin in metazoan activates the PI3K1TOR pathway which regulates growth, gene expression and negatively the autophagy. Previous work in our laboratory showed that NT-1 cell cultures supplemented with insulin stimulates cell proliferation in an auxin-dependent manner and the formation of autophagosome-like structures. As mentioned above, the aim of this study was to determine the mechanism by which the availability of auxin and the addition of insulin induce autophagy. By staining with MDC (monodansyl cadaverine) and the cysteine protease inhibitor E-64c was observed by fluorescence microscopy that both insulin and auxin deprivation promote this process. The expression of several genes (ATG) involved in autophagy showed a strong correlation with the increase in H2O2 levels, suggesting that the mechanism by which it induces autophagy is a process dependent of ROS levels.

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111.

Analysis of contrasting *Bixa orellana* (annatto) morphotypes cultivated in Chicxulub, Yucatán

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Annatto (*Bixa orellana* L.) is a wild plant native to the tropical Americas and of great commercial importance due to the natural pigment bixin, present in the outer coat of its seeds. It is the most important natural colouring for food use after saffron, and it is in high demand by the food, pharmaceutical and cosmetics industries. Large-scale annatto production is problematic due to its high heterozygosity and variations in bixin content. In light of this, 20 adult plants (7 years old) were studied with controlled watering and using the same agricultural practice to select "Elite" parent plants for genetic improvement, as well as to serve as a model for the study of key genes in carotenoid biosynthesis. Fruit morphology was evaluated, including colour, size, weight, shape and dehiscence, as well as the number of seeds, number of fruits per panicle, seed bixin content and germination percentage. For the flowers, the colour, number of stamens and pistil length were evaluated. The results showed differences in morphotypes, such as plants with purple, pink and white flowers. The fruits of all of the varieties with purple and pink flowers were dehiscent and those from plants with white flowers were indehiscent. There were no significant differences in the number of fruits per panicle between all of the varieties. Fruit width was similar for the three flower types, except for white flower varieties. Fruit length of plants with pink and purple flowers was 30% greater than in the other varieties. The average number of seeds per fruit was 41% greater in plants with pink and purple flowers compared to those with white flowers. Bixin content in the varieties with pink flowers was double that in those with purple and white flowers. The number of stamens varied, with the exception of a pink flower variety. Seed germination was greater in moist as compared to dry mature seeds. We currently have an *in vivo* germplasm bank in an experimental 500 m^2 field in Temezón, Yucatán and the plants are being evaluated at the genetic level.

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112.

Partial characterization of genes related to the synthesis of gibberellins and abscisic acid in pecan trees

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The production of pecans in warmer agricultural areas has been threatened by a gradual and consistent increase in the pre-harvest sprouting of nuts. The processes of seed dormancy and germination are in part regulated by hormones, where a high level of abscisic acid (ABA) promotes the latency, and a high level of gibberellins (GAs) stimulates the mobilization of reserves and germination. Therefore, the aim of this study was to isolate and characterize the cDNA of genes involved in the synthesis of GAs and ABA in *Carya illinoensis*. Degenerate primers were designed and used to amplify cDNA synthesized from different pecan tissues (kernel and foliar bud). PCR products were cloned and sequenced. Identification of nucleotide sequences was determined by searching in the Genbank and ORFs were identified by on-line algorithms. The amino acid sequences were deduced and identity and similarity levels calculated. Three partial sequences of 439, 806 and 773 bp length were assembled and identified as *gibberellin 20-oxidase*, *abscisic-aldehyde oxidase 3* and *β-tubulin*, respectively, with an amino acid identity of 69.9, 69.4 and 95.6 % to other plant species. These sequences are novel and will contribute to the knowledge of the pecan transcriptome. The characterization of the genes involved in the synthesis of these hormones will allow determining their function during kernel development in future studies.

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113.

Microsatellites analysis of genomic profiling of *Jatropha curcas* accesions

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Reproduction of *J. curcas* is essentially by cross pollination, which results in a high degree of variation between individuals and allows breeding programs for the improvement of the cross amplified taxa, in which can select the desired characteristics of the parent plants and segregation. Nonetheless this type of genetic improvement based solely on morphological characters are not successful, because they vary in response to environmental conditions. Therefore is necessary to have a system based of non-variable characteristics that enable identification among individuals of a single species, such as Molecular Markers. Since Microsatellites are molecular genetic markers that do not depend on the physiological or morphological information, they are considered neutral markers of the organism, accordingly microsatellites are ideal markers for genetic analysis since they are not affected by natural selection or environmental changes. Microsatellites, are short tandem DNA repeated sequences (2-6 bp), that are found in all genomes of Eukaryotic organisms. These sequences are specie specific, but differ in the number of repetitions between individuals. Microsatellites are widely used, because they are highly polymorphic, these markers identify both homozygous and heterozygous individuals, thus are considered codominant markers. The feasibility of this type of genomic analysis to analyze genetically different genotypes of *J. curcas*, is considered a breakthrough for genetic improvement. The present investigation was undertaken to assess the genetic diversity among 26 selected accessions of *J. curcas* by advantageous characteristic agronomic, that are used as parental for the generation of new varieties. Of 25 primers tested initially, eight were selected for genomic analysis, since amplified a pattern consistent and repeatable, between four and ten bands, the position of each band amplified by each primer is considered as a character for the phylogenetic analysis. Phylogenetic analysis of 52 characters is expressed with a distance matrix and a phylogenetic tree which groups individuals according to their genetic proximity.

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114.

Exploring the *Physcomitrella patens* genome for the two main enzymatic nitric oxide-producing mechanisms: nitrate reductase and nitric oxide synthase

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Nitric oxide (NO) is a small gaseous molecule with important roles in the control of growth, development and physiology of land plants. In higher plants NO is produced by two main enzymatic mechanisms: 1) nitrate reductase (NR) and 2) a nitric oxide synthase (NOS)-like activity whose neither genes nor proteins have been identified. In green algae NO is produced by NR and not by NOS-like activity, but there is no information about NO production in non-vascular plants. Such information will help to understand the development of enzymatic sources of NO during plant evolution. In order to determine whether NO producing enzymes are present in non-vascular plants we searched for the NR and/or NOS genes and proteins in the moss *Physcomitrella patens* genome. We demonstrated that: 1) there are not *nos* genes in the *P. patens* genome; 2) *P. patens* have a family of three genes (*ppnia* genes) that encode for canonical NR enzymes and 3) although *P. patens* NR conserves the three domain structure common to all plant NRs, the motive (K/R)(S/T)XS*(T/S)XP—the target of phosphorylation and binding of 14-3-3 proteins that down regulates the enzyme activity—is absent, indicating that this regulatory mechanism appeared after the divergence of bryophytes and tracheophytes .

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115.

Validation of markers SSAP and AFLP for the study of diversity of *Psidium guajava* L.

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Guava (*Psidium guajava* L.) is a fruit that belongs to the family Myrtaceae, native to tropical America, widely adapted to the environment and of great economic importance in countries with tropical and subtropical areas such as Mexico, Brazil and Pakistan, among others. For this species, although they have developed morphological and molecular markers (RAPD, AFLP, SSR), which have demonstrated a wide genetic variability in both accessions from Mexico and other countries of Latin America; until now, due to the gene pool of the species, the perspective is the development of a molecular marker that allows us to detect more polymorphism and co-dominance among the accessions evaluated. With the precedent that possibly an S-SAP marker in Guava may be more informative in this studied by molecular analysis of 15 accessions of *Psidium guajava* L. of Mexico, we worked on identifying-LTR retrotransposons in the design of SSAP markers. These markers were evaluated and compared with AFLP markers developed for this species. The results indicated that the S-SAP marker resulted in a better marker because this method showed greater polymorphism and lower deep similarity indices indicating interspecific genetic variability.
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116.

Divergence and regulation of 5S rDNA polyploid species of Agave

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Polyploidy is an important event in the evolution of plants. Due to the events of genetic and epigenetic occurred during this process, the species of *novo* acquire specific characteristics that allow a better adaptation to extreme environments and resistance to biotic and abiotic factors in comparison to its predecessors diploides. Due to the advantages that presents polyploid species of interest in the commercial field, currently it has focused on the basic knowledge of this process. *Agave* is a species of endemic, ecological and economic importance of the México. Polyploid species have been described in this genus ranging from diploid (2X) up to octaploides (8X). In species belonging to *Brassica, Arabidopsis*, and recently in the genus polyploid *Agave* studies have been conducted to learn three main aspects in polyploids, these are: 1) how replication affects the expression and function of proteins, 2) what is the evolutionary relation between the polyploid species, and 3) is it possible to increase the productive characters of important commercial crops, through the increase of the genome. In this respect and to contribution knowledge the basic process of polyploid, in this work study divergence and 5S rDNA gene regulation polyploid species of *Agave*. The results obtained in this study indicated the presence of four groups allelic 5S rDNA highly represented in the genome of the species *A. tequilana*, *A. angustifolia* and *A. fourcroydes*. These showed a differential expression at the species level. Similarly, was able to identify the presence of groups allelic and specific haplotypes of each species. The information obtained in this work in the future could be used for the development of markers for genetic dynamism and ploidy of the species; as well as contribute to the improvement of crops of economic importance of the genus species.

117.

Study of genetic changes in redundant genes in polyploid species of Agave

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Currently the polyploid species have ecological, economic and commercial importance because that genetic, physiological, morphological and ecological characteristics that reflect certain capacities of adaptation of these species in response to different biotic stress and abioticos. It is attributed to many of the genetic changes in genes in response to stress are responsible that the polyploid species respond better to different types of stress and be located as a powerful tool in improving important commercial crops.This same approach in this study identified genetic changes in genes in response to biotic stress (CC-NBS-LRR) and abiotic (LEA V) present in the polyploid species *Agave*. The results obtained indicate that possibly these genetic changes found in these genes could be triggering processes of functional specialization, affecting levels of transcription of these genes, which together with other biochemical and physiological mechanisms, can be associated to the capacity of the polyploid species *Agave* to tolerate different environments where the amount of water is a limited resource in plant-pathogen interaction.

118.

Isolation and characterization of DNA satellite in *Agave tequilana*

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The eukaryote genome includes repeated regions like retroelements, ribosomal DNA and Satellite DNA. This repeated DNA plays a role binding proteins, other regulatory elements an increasing the chromosomal DNA content, thus contributing to the variability in plants. This variability is responsible for the evolution and divergence in species. The study of satellite DNA is important to understand the evolution and origin of polyploidy. The origin and evolution of Agave are not fully understand, here we developed a technique for the isolation of satellite DNA in A. tequilana employing self priming, analysis of the obtained sequences confirmed they were satellite DNA, southern blot and in situ hybridization reveled their localization within the genome.

119.

Análisis molecular de la presencia de transgenes en maíces criollos de Sinaloa

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El maíz es el cultivo más importante en México, ocupa la mitad de la tierra cultivable del país y somos el principal consumidor del mismo a nivel mundial. Anualmente se importan más de ocho millones de toneladas de granos procedentes de Estados Unidos, los cuales en su mayoría son genéticamente modificados destinados exclusivamente para su procesamiento. En México no está legalmente permitida la siembra de maíz genéticamente modificado a nivel comercial, sólo se han desarrollado la etapa experimental y plantas piloto. Sin embargo, en Sinaloa se identificaron transgenes en la cosecha de temporal del año 2001 en las localidades de Badiguarato, San Ignacio, Elota y Culiacán. El fragmento amplificado por PCR, clonado y secuenciado corresponde a una homología del 100% y una identidad del 99% al gen marcador NPTII que confiere resistencia a kanamicina. Así mismo, se han analizado mediante inmunotiras cosechas recientes de los lugares en donde previamente se detectaron dichos transgenes teniendo en todas ellas resultados negativos. El presente trabajo continuará con la corroboración de dichos resultados mediante PCR y se usará un control interno de actina para descartar falsos negativos.

120.

Evaluation of qpm hybrids for hihglands valleys of Mexico in relation to best parent

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Seeds of six inbred lines of maize (L1 - L6) classified as quality protein maize (QPM) and 15 of theirs direct single crosses, was subjected to the determinations of tryptophan, lysine, quantity, and quality of protein, and genetic gain in the crosses in relation to the better of them. The experiments were conducted (IPN and UNAM laboratories) under a randomized block design with two replications of 100 seeds, the mean comparison was made by the Tukey method, and the genetic gain was analyzed with the ¿t¿ test. It was detected as quality protein maize to the lines L2, L3, and L6. The cross L2 X L6 highlighted by its genetic advance for the best parent through all parameters, as well, the increases in protein and amino acids levels had an impact on quality index. L1 X L4 and L4 X L5 showed acceptable values in genetic gain for tryptophan, lysine and quality index, in this case also the amount of amino acids had an impact on the protein quality, which was not presented in crosses L2 X L4, L3 X L6 y L4 X L6 with positive genetic gain for parents mean but only in lysine, tryptophan and protein.

121.

Genetic advance in essentials amino acids and protein quality index for qpm hybrids in relation to their parents

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The increase in the essential amino acids lysine and tryptophan in the grain of maize by using the mutant gene opaco2 is a helpful option to develop varieties whose seeds possess protein quality and contribute to the reduction of the chronic malnutrition prevalent in marginalized populations whose diet is largely based on this cultivated specie. Therefore, it was started the development of such modified maize varieties by hybridization in Mexico. Lines of maize with quality protein (QPM) and their direct single crosses, was subjected in our laboratories (IPN and UNAM) to the determinations of tryptophan, lysine, quantity, quality of protein, and also the genetic advance in the crosses in relation to their parental lines mean. The experiments were conducted under a randomized block design with two replications of 100 seeds, the mean comparison was made by the Tukey method, and the genetic advance was analyzed with the ¿t¿ test. It was detected as quality protein maize the M2, M3, and M6 lines. In the crosses, M2 X M6 highlighted by its genetic gain (¿ = 0.01) in protein quantity (2.3%), protein quality index (-0.5 %), as well, the increases in protein and amino acids levels had an impact on quality index. M1 X M4 and M4 X M5 showed genetic advance acceptable for tryptophan, lysine and quality index, in this case likewise the amount of amino acids affected the protein quality, in contrast with M2 X M4, M3 X M6 y M4 X M6 crosses, with positive genetic gain but only in lysine, tryptophan and protein. It's important to state that by the hybridizing process there was a genetic advantage for essential amino acids and protein, but the lines M2, M3, and M6 well-maintained theirs QPM properties.

122.

Linear regression model to predict the agronomic performance of maize plants

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Actually different alternatives in breeding programs are being used around the world attempting to improve crop yield and nutritional value of new maize lines that can be able to grow in stressing environmental conditions such as lower temperatures. Metabolomics is understood as the characterization, identification, and quantification of molecules synthesized in biological organisms. Advances in the development of metabolomics give us analytical platforms we use to investigate interesting metabolites, the quantity and nature, at different stages of plant growth. In this research we used VIS-UV spectrophotometry for carbohydrates determination. To evaluate some metabolites generated in corn grain we planted commercial varieties of white corn: Puma, Leopardo and Oso, and two varieties of yellow corn: 2A120 and 2B150 with normal irrigation and proper fertilization of nitrogen under in a greenhouse. During the flowering stage, maize ears became self-pollinated in order to obtain larger grains with improved nutritional qualities. At the senescence stage we registered some of the most known secondary traits such as stem weight and height, leaves number, tassel weight and height and, corncob weight. Using UV-VIS spectrophotometry we measured glucose, fructose, sucrose, starch and, total carotenoids content in maize grain. The metabolites with the highest level we found are next: 2B150-Fructose, 23.59 mmol1L; 2B150-Glucose, 20.36; Oso-Sucrose, 110.27; Leopardo-starch, 102.00; 2B150-total carotenoids, 9.56 UA1gr dry weight. Tukey test shows genotype significant differences at 95% confidence intervals. Having the 200 weight of kernels from each variety as the response variable, a linear regression model was developed with Minitab 16 1.0 choosing ¿step-wise¿ forward option for finding the agronomic and biochemical predictors that deliver a reliable yield prediction. Statistical fitness linear model report shows that corncob weight and total grain weight variables perform as quadratic elements. The predictors that fit better the model are the secondary agronomic traits.

123.

Expression of antigenic peptides derived from F and G proteins of Respiratory Syncytial Virus in lettuce plants as a first step for a plant-based vaccine

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Respiratory Syncytial Virus (RSV) is the most important pathogen leading to hospitalizations in young children and elderly people. The current work focused on the expression of antigenic peptides derived from RSV in plants as a first step for the development of an edible vaccine. The synthetic gene was optimized for plant expression and encodes for three epitopes from the F protein and an epitope from the G protein. The lettuce transformation was achieved by co-cultivation with Agrobacterium tumefaciens. We demonstrated the presence of the synthetic gene by PCR and Southern blot analysis, and the antigenicity of the heterologous protein were recognized by a polyclonal antibody against F protein and we performed an immunogenic analysis in mice; the transgenic lettuce group showed higher IgG antibodies levels than the wild type lettuce group. This work is a first step towards the generation of an edible vaccine in plant systems against RSV harboring epitopes from F and G proteins.

124.

Transient transformation of tobacco plants to produce an antiviral peptide against human metapneumovirus

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Plants are an ideal expression system for recombinant protein production which can be used as antiviral or vaccines against infectious agents. The etiologic agents that cause respiratory illnesses are mainly bacteria and viruses, the last ones are responsible for 50-60% of acute respiratory infections (IRAs). Human metapneumovirus (hMPV) is responsible for acute respiratory infections and at least for 5-10% of hospitalizations in young children, mainly in children under three years old, the elderly and immunocompromised patients. Currently there is no treatment or vaccine available against hMPV. It has been demonstrated that the fusion of the virus to the host cell can be inhibit by a peptide analogous to F protein. We designed a synthetic gene expressing this peptide named HRA2 and by a transient viral expression system (Magnifection), the recombinant peptide was produced in tobacco plants. The protein antigenicity was tested *in vitro* by a Dot blot and Western blot using a primary antibody against the histidine tag. Its efficacy as an antiviral peptide will be evaluated by replication inhibition assays in Hep-2 line. This approach is an alternative for hMPV prevention and could be a less expensive alternative in a near future to reduce the transmission of hMPV and the incidence of respiratory infections.

125.

Agrobacterium tumefaciens mediated genetic transformation of *Tagetes erecta* L. with the gusA reporter gene

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Tagetes erecta L is a native plant from Mexico with traditional and industrial uses, such as antiparasitic, insecticide as well as carotenoid pigment source. There is great interest in achieving stable transformation of this plant for the introduction of foreign genes of agricultural interest. However, there is only one report of stable genetic transformation with the *gus* reporter gene, using microparticle bombardment. The *Agrobacterium tumefaciens*-mediated transformation of *Tagetes erecta* only has been produced transient results. Several factors could influence the success of *A. tumefaciens* transformation (strains, the presence of acetosyringone, bacterial density, temperature, the use of selective agents, explant type, etc.). However, plant genotype plays a critical role in determining the efficiency of stable transformation. In here, apical meristems from eleven different varieties of *T. erecta* L. were transformed with *A. tumefaciens* strain LBA4404::pCAMBIA 2301, bearing the *gusA* reporter gene as well as the *nptII* gene. The *gusA* gene codes for the β-glucuronidase enzyme, whereas *nptII* codes for neomycin phosphotransferase and confers resistance to kanamycin. Assays for GUS histochemical staining and molecular testing of the presence of *ntpII* by PCR in meristems, buds and flowers confirmed stable transformation in two varieties; Marvel orange and Lady gold. This study opens the possibilities of using *A. tumefaciens* to introduce genes of metabolic pathways leading to terpenoid synthesis, such as *dxs* (DXS/CLA1) that codes for 1-deoxy-D-xylulose 5-phosphate, the enzyme catalyzing the first and the rate-limiting step of the MEP pathway, in an attempt to control the production of carotenoids in *Tagetes erecta* L.

126.

Optimization of genetic transformation of commercial alfalfa cultivar CUFlOl

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Optimization of genetic transformation of commercial alfalfa cultivar CUF101 MarÍa Teresa Esquivel-Contreras, Ana Luz Romero-GarcÍa Luzmila MartÍnez-González and Ángel Gabriel Alpuche-SolÍs* División de BiologÍa Molecular, Instituto Potosino de Investigación CientÍfica y Tecnológica, Camino a la Presa San José 2055, 78216, San Luis PotosÍ, México. *Corresponding author: alpuche@ipicyt.edu.mx

Alfalfa (Medicago sativa L.) is one of the most important crops worldwide and is used as fodder for cattle feed due to its high nutritional quality, high yields and recently it has been used as a bioreactor for biopharmaceutical products. Agricultural productivity is seriously affected by drought and salinity. Either to increase tolerance to abiotic stress of this forage or to express a particular therapeutic or prophylactic protein, genetic engineering approach can be used to introduce a desirable genes and an efficient transformation and plant regeneration system should be optimized. For this purpose, we standardized the transformation and regeneration conditions for the expression of recombinant proteins of interest in this plant system. Five different strains of Agrobacterium tumefaciens (C58C1, C581GV3101, GV3101, GV3010 and LBA4404) harboring the binary vector pBI121 were tested. Furthermore, three different transformation protocols were evaluated using seedlings, mature embryos and mature hypocotyls as starting material. Plant transformation was confirmed by PCR and the glucoronidase activity by histochemical assay. The highest transformation efficiency (30%) was obtained by using A. tumefaciens strain GV3010 and hypocotyls as starting material, thus we conclude that this is an efficient transformation method to introduce genes of agronomic or pharmaceutical importance into alfalfa var CUF101.

127.

Effect of *Bacillus subtilis* in genes associated with the maturation of the fruit of strawberry

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The fruit of strawberry (*Fragaria x ananassa*) is highly perishable, presenting a rapid softening post-harvest, result of its cell wall degradation, resulting in huge economic losses. This degradation is caused by the activity of pectic enzymes: Polygalacturonase (PG), Pectate lyase (PL) and Pectin methyl esterase (PME). It has been shown that microorganisms in the rhizosphere, (PGPR) plant growth promoting bacteria, increase the shelf life of some fruits. We evaluated different strains of *Bacillus subtilis* on the quality of strawberry fruit, resulting in outstanding strain DN. We then determine the biochemical and molecular effect of this strain in fruits of strawberry in vivo. Obtaining a decrease in levels of transcripts for genes *pl*, *pg* and *pme*, and a significant reduction ($P>0.05$) in the enzymatic activity of PG, PL, and PME compared to untreated fruits. It shows the strain DN of *B. subtilis* plays a key role in the regulation of the process of maturation in strawberry fruits. These results support the development of an alternative to environmentally friendly production, to improve the quality of fruits in terms of firmness and shelf life.

128.

Production in plants of Rho A antiviral peptide against respiratory syncytial virus infections

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Antiviral activity against respiratory syncytial virus (RSV) of RhoA-derived peptide has been reported over a decade both *in vitro* and *in vivo* and emerged as a promising alternative to a monoclonal antibody-based therapeutics against RSV infection. However, a cost-effective production platform for this peptide needs to be developed to meet the demand for anti-RSV therapeutics. To address this point, we have engineered and expressed RhoA peptide as fusions to two different carrier molecules, lichenase (licKM) and coat protein (CP) of Alfalfa mosaic virus (AlMV) in *Nicotiana benthamiana* plants to enhance expression and process efficacy. The results shown that RhoA-peptide fused to the C-terminus of LicKM and fused to CP without a signal peptide were efficiently expressed in *N. benthamiana* plants and the RhoA-peptide fused to LicKM and one of the RhoA-peptide-CP fusions without PR signal peptide inhibited RSV growth *in vitro*. In summary, these data demonstrate the feasibility of the transient expression system in plants for the production of antiviral RhoA-peptide and the advantage of the use of carrier molecules to enhance expression and process efficacy.

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129.

Characterization of the expression conferred by the At4gl2640 5' regulatory region in transformed plants of *Arabidopsis thaliana* (L.), Heynh

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The At4g12640 gene of *Arabidopsis thaliana* encodes a Spen type protein whose function is unknown. Spen type proteins have at least one domain that allows them to bind to RNA (RRM) and a SPOC domain that allows them to interact with other proteins. Thus, these proteins are generally involved in gene regulation processes. In this work, we generated three gene constructs in which segments of 1500, 1000 and 500 base pairs of the 5´ regulatory region were fused to the *uidA::GFP* reporter gene using the Gateway® technology. *A. thaliana* plants were transformed by the modified floral dip method obtaining efficiencies between 1 and 1.2%. Expression associated to the vascular tissue was observed in the T1 transformed plants in the following structures: root, stem, cotyledon leaf, true leaf, stamens and gynoecium. These results suggest that the function of the At4g12640 gene might be associated with vascular tissue and in cells that will give rise to this tissue.

130.

Determination of the conditions for genetic transformation in chrysanthemum (*Dendranthema grandiflorum*) var. micromargara, mediated by *Agrobacterium tumefaciens*

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The efficiency of *Agrobacterium* mediated transformation in recalcitrant plants such as Chrysantemum (*Dendrathema grandiflora*) varieties is affected by some factors like bacterial concentration, co-culture period and acetosyringone concentration. It has been reported that interaction of these parameters are important to increase the efficiency of genetic transformation of Chrysantemum explants. In this work, we applied an experimental design 2^3 with three central points, to determine if the statistic interaction between bacterial concentration ($OD_{600} \approx 2$ and $OD_{600} \approx 1$), co-culture period (1 or 3 days) and acetosyringone concentration (100 µM or 50 µM) increase the efficiency of genetic transformation in *D. grandiflora* var. micromagara. We used Chrysanthemum var. micromagara calli as plant material, and LBA4404 strain of *A. tumefasciens* harboring pBI121 and PAL4404 plasmids for transformation experiments. Putative transformants were screened by GUS histochemical assay and uidA and NPTII expression through RT-PCR. PCR was conducted using specific oligonucleotides for uidA and NPT II. Preliminary results suggest that the most effective conditions for Chrysanthemun var. micromargara transformation were bacterial concentration of Optical density ≈2, three days of coculture and acetosyringone concentration of 50 □ M.

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131.

Construction genetics of a vector chloroplast of *Chrolella vulgaris*

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Algae (*Chlorella vulgaris*) are being used for the production of biofuels, bioethanol, biomethane. The first challenge presented to genetically manipulate algae, is that you do not have a suitable natural vector possessing the desired elements, allowing insertion of foreign DNA directly to chloroplast DNA. In addition there are several reports in which protein recombinants of application pharmaceutical, agricultural, cosmetic, vaccines, antibodies have been obtained and chloroplast transformation via. This paper proposes the construction of a specific vector for chloroplast for the genetic transformation of algae as an alternative biosecure for the subsequent production of various products, such as biofuels, that allows obtaining high levels of biofuel and overproduction at low cost, without destroying the crops, without emission of harmful gases and with control of the transmission of the genome without fear of the impact of the content of transgenes, since there is no transmission through the pollen.

132.

Induction of genetic variability by mutagenesis in *Paulownia elongata*

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Natural populations of living things have a lot of genetic variation, and therefore is a common phenomenon in nature, this is what allows populations to adapt to unfavorable conditions, demonstrating an essential condition for the evolution species. Mutation induction is a very interesting technique that has been used in the past decades because it allows hereditary characteristics lead to improved germplasm, higher performance, and resistance to diseases and pests. This reduces the excessive use of pesticides, preventing damage to human health and the environment. Another advantage is to confer specific varieties improvements without significantly altering its overall performance in a short period, the achievement of features not found in natural variability, the most common is resistance or increased production of a particular metabolite. In this study we evaluated the variability induced by chemical mutagenesis in plants regenerated via direct organogenesis in leaf explants of *Paulownia elongate,* forest species of commercial interest. For inducing mutagenesis were used different concentrations of the mutagenic agent and variable exposure times, the explants were immediately placed in culture medium *in vitro* following the protocol for regeneration via organogenesis. The genetic variability was evaluated by RAPD marker.

133.

Characterization of a chloroplast targeting DNA vector in *Nicotiana tabacum* with potential use as recombinant vaccine against toxA from S. aureus

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The use of recombinant vaccines against bovine mastitis has been studied for many years, but has not been successfully developed in order to prevent the infection in dairy cattle. Mastitis represents a great issue in milk production since it is the main cause of economic loss for the dairy industry worldwide and it is most frequently associated to the enterotoxin A (toxA) produced by *Staphylococcus aureus*. This enterotoxin is regarded as a superantigen because of its ability to bind to class II MHC molecules on antigen presenting cells. The way this protein binds stimulates the activation of large populations of T cells, generating a massive immune response that is not specific to any particular epitope of the superantigen; this results in a massive cytokine release that leads to an acute toxic shock. It is well known that the exposure to certain epitopes from a protein may be used to generate immunization in the host of the vaccine; therefore, the epitopes of toxA can be used as immunogens to induce a better response in order to prevent an infection. An important matter in the development of a plant-based vaccine is the expression level that can be obtained through transformation of the plant's genome. Initial developments have been limited by low-level expression of immunogens from nuclear transformation, which is why it is mostly chosen to transform the plastid genome. Protein expression from plastid transformation offers major advantages over nuclear gene expression since there can be hundreds of chloroplasts per cell, and hundreds of copies of circular DNA molecules per chloroplast. For this reason, chloroplasts are used as main target for the transformation of plants where high levels of protein expression are needed, turning the transformed plant into a bioreactor of the antigen wanted. The objective of this study was to characterize plasmid pCNTTOXA in order to verify the proper insertion of the toxA gene from *S. aureus* in expression vector pCNT. This vector was designed as follows: the homologous recombination site *rrn16,* followed by the *Prrn* promoter, then the toxA gene with the *aadA* gene (Streptomycin/Spectinomycin Adenylyltransferase Gene) and the *trnV* terminator, ending with the homologous recombination site *rps12/7*. The characterization was made through enzymatic digestion, PCR amplification, and selection of antibiotic resistant bacterial cultures. The enzymatic digestion was done using *BamHI* and *XbaI* restriction enzymes which flank the toxA gene inside the expression vector, after which a fragment of about 1077 bp was obtained; said fragment is not found in the negative control digestion. During the PCR, specific primers were used in order to amplify the toxA gene, producing an amplicon about 1000 bp long. The PCR product was then compared to that of the positive control, containing the toxA gene; the fact that both fragments matched in length is consistent with the initial assumption. With the purpose of complementing the previous results and assuring the right insertion of the gene in the plasmid, a bacterial culture was performed with the transformed bacteria in presence of

134.

Expresión de epitopes vacunales en cloroplasto

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The development of strategies for the creation of a recombinant vaccine is of great importance, especially for recurrent infections such as those caused by Staphylococcus aureus, causing mastitis in animals and food poisoning in humans, where the toxin is responsible for 80% cases, toxicity is caused by their superantigenicity feature, the ability to cause poisoning is determined by separate parts of the protein (epitopes). It has been observed that the epitopes of a protein can be used as antigens to generate immunogenicity in a host, so the epitope of this toxin can be used to induce protection in the host. The chloroplast protein expression has great advantages, high expression levels, the opportunity to express genes in operons among others. These features makes it highly recommended for genetic transformation. The objective of this study was to construct an expression vector called pcNtTOXA subcloning the A toxin gene using the enzymes XbaI and SmaI adjacent aadA gene in the expression vector pcnt said vector has two homologous recombination sites, the first corresponding to ribosomal rrn16 gene and a second gene-3'rps12, TOXA gene insertion was checked by PCR. Once confirmed the vector was used to bombard pcNtTOXA explants of N. tabacum using gold particles coated with the expression vector, using the following conditions: distance of 15 cm and 100 shot Lb/p2, explants were cultured in vitro for four months RMOP medium with concentration of 500 mg / L streptomycin. 20 clones were analyzed by enzymatic digestion using BamHI and XbaI enzymes (enzymes TOXA flanking the gene) and Southern blot (using a probe specific for the 16S ribosomal gene.) 3 clones were tested transformants when performing enzymatic digestion of the total DNA with the restriction enzyme BamHI, to make the comparison with wild snuff DNA (digested with BamHI) was observed that there is a difference of ~ 1000 bp which is attributed to the aadA gene weight, this confirms that there are sequences within the homologous recombination site are not chloroplast DNA in the wild.

135.

Stress marker in *Vallisneria americana* by enrichment of nitrogen

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The feasibility of biochemical tools was evaluated through an *in vitro* experimental approach to recognize multiple markers associated with the stress produced by enrichment of N in the submerged macrophyte *Vallisneria americana*. *In vitro* regenerated plants came from seeds placed on pH 7.5 in biphasic culture without N and autotrophic condition. The content biomass, protein and chlorophyll a in leaves as well as the NH_4, NO_2 and NO_3 in tissue of leaves and roots lyophilized was experimentally measured. The experiment design included two factors: concentrations of total N (four treatments from 500 to 2000 $\mu g\ L^{-1}$), and three ion sources of N (NH_4, NO_3 and NH_4: NO_3) in the aqueous medium. Each treatment was replied five times. Most of the multiple correlations among markers were statistically significant ($0.71 \leq r \geq 0.95$; $p < 0.05$). However, 68% of the maximum R^2 values (68 –92) were recorded with N concentrations. They were 18% of the significant correlations for the ion sources of N and 66% for the N concentration. Correlations were highlighted with NO_2. The use of multiple markers to quantify the differential effect of N enrichment was feasible for this aquatic macrophyte with ample distribution in North America.

136.

On in vitro growth and morphology and pathogenicity in common beans of *Macrophomina phaseolina* UV-mutants

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The fungus *Macrophomina phaseolina* (Tassi) Goid causes charcoal rot disease in more than 500 plant species worldwide. Charcoal rot is a major disease of common beans (*Phaseolus vulgaris* L.) in Mexico due progress under water deficits and most of beans in the country grow under rainfed conditions. Pathogenicity mechanisms are closely linked to envorinmental conditions during its growth. Thus, fungus triggers its growth and development from single cell to mycelium or vice versa as well as the production of secondary metabolites as pigments and toxins. The importance of these metabolites on disease development was evaluated using nine UV-mutants of two *M. phaseolina* strains (HMP5-highly virulent, HMP46-lowly virulent). The UV-mutagenesis affected capability for pigment and toxin production as well as *in vitro* growth of strains. Pathogenicity was assessed in plantlets (greenhouse) and seeds (*in vitro*) of two common bean cultivars (Pinto Saltillo and Negro Jamapa). UV-mutagenesis reduced virulence of strains compared with wild strains on the basis of growth parameters as plant height, charcoal rot severity, pre and post-emergence death percentages and biomass production of bean germplasm. Mutant D8 from strain HMP46 produced white mycelium, not produced sclerotia and was avirulent in bean seeds and seedlings. We found direct correlation between pigment production and ability for both spore formation and pathogenicity in *M. phaseolina*.

137.

Physical-chemical composition of *Scenedesmus dimorphus* for biodiesel production

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Global warming is a consequence of the increase in global energy demand that has been going on in the planet in this century. In addition of the increase in oil prices due to the depletion of fossil fuels. For years it has been considered to use biodiesel as a promising energy alternative that has proved to been very attractive. it is an alternative source of energy with lower levels of pollution than current fuels. Using a process called transterification we can produce biodiesel from microalgae species for their lipids are produced in abundance, of which triglycerides are the best substrate for the production of biodiesel. It is also possible to increase the synthesis and accumulation of large amounts lipid by optimizing growth factors, which can be achieved by inducing stress conditions in the cell through physical or chemical stimuli. This will manipulate the culture conditions to improve the production and concentration of lipids for use as feedstock for biodiesel production. The nutrient limitation is certainly a viable strategy to increase lipid content in cells. This aims to induce lipid synthesis in green microalgae species of the genus Chlorophyceae; microalgae Scenedesmus dimorphus commercial origin. Cells were cultured for 15 days in static or batch systems under different concentrations of nitrogen and phosphorous to promote nutrition stress and induce lipid synthesis. Cells were harvested at different stages of growth to assess lipid accumulation during the development of crop. In turn, the behavior was observed for the content of protein, carbohydrate, and biomass production. In the investigation it was found that the biochemical composition (lipids, proteins and carbohydrates) of the microalga was affected by the growth phase, as well as initial nitrogen concentration. No significant effect was observed by the initial concentration of phosphorus. The results indicate that Scenedesmus dimorphus responded to induction by limitation of lipids and / or nitrogen deficiency, recording of high lipid content in the stationary phase in those treatments containing the lower levels of nitrogen (S1, S2, SC) with the mean values of 42.11 ± 1.24%, 38.74 ± 0.85% and 44.03 ± 1.11% lipid, respectively, while the treatments containing a higher concentration of nitrogen (S3, S4 and SN), recorded a lipid content of 25.37 ± 0.76 %, 19.60 ± 1.84% and 20.29 ± 0.84%, respectively and obtained 0.60 g l-1 and 1.06 g l-1 for S4 and SN treatments, respectively. The lowest carbohydrate values were recorded during the exponential phase, while the highest values are presented during the stationary phase, showing a higher carbohydrate content in those treatments containing lower concentrations of nitrogen. Higher values of carbohydrates were 40.14 ± 2.58%, 38.79 ± 0.50% and 38.02 ± 2.02% for treatments S1, S2, SC, respectively.

138.

The plastid AtHXK3: new insights in sugar sensing in *Arabidopsis thaliana*

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Hexokinases (HXK) are dual function enzymes that, in addition of its catalytic activity, act as sugar sensors modulating gene expression involved in photosynthetic activity in response to sugar levels. In most plant HXKs are encoded by a small gene family, for example the Arabidopsis genome encodes for 6 related HXK genes. The participation of HXK in the sugar sensing mechanism has been mainly inferred from studies with the *HXK1* gene. Mutants in this HXK (*gin2-1*) are insensitive to sugars and display developmental defect. Recent analyses have shown the AtHXK2 appears to have similar functions than the AtHXK1. However, the role of the plastid-localized AtHXK3 in the sugar perception is not clear. Some studies suggested that AtHXK3 act as a node of convergence between sugar signaling and plastid to nucleus communication mediated by GUN1. In this analysis it is reported that inhibition of AtHXK3 expression increases cellular sensitivity to sugar. To analyze in more detail the function of AtHXK3 in sugar signaling in this work we analyzed the sugar sensitivity in plants that overexpressed this gene. We analyzed the seed germination of four transgenic lines that overexpress the *AtHXK3* gene in the presence of different glucose concentrations. We observed that these lines display a hypersensitive sugar phenotype compared to the wild type. We also analyzed seed germination in a mutant for this gene (*hxk3)* using similar experimental conditions. However the analysis of the *hxk3* mutant did not displayed an obvious phenotype in the glucose treatments in contrast to the wild type. We conclude that the HXK3 acts sugar sensor in plastids. However, is a complex process involving other Hexokinases and other sugar sensors that may be redundant. We would have to analyze in detail its role in sink tissues because the *HXK3* expression is increased. These results open new possibilities for the study of the HXK3 role in the plastids.

139.

Synthetic sucrose isomerase gene for isomaltulose accumulation

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Incorrect eating habits which highlights the excessive consumption of carbohydrates has led health problems such as increased diseases as diabetes mellitus type 2, obesity, metabolic syndrome and dyslipidemia. Sucrose is the primary sweetener or food additive used today, and perhaps contributes to metabolic disorders. It is advisable to substitute this sweetener for other with lower caloric income. However, the candidate should be soluble in food, stable at different intervals of temperature and pH, and tolerate various conditions and types of processes that are employed, should not have any adverse effect on the consumer, and particularly having a sweetness which is similar or superior to that of sucrose. Isomaltulose is a natural sweetener that has half value in calories than as sucrose, yet their physiological effects varying the sucrose. Sacarosa isomarase gene from *Pantoea dispersa* was recovered from NCBI**.** Restriction sites were removed and codon optimization for monocots was achieved by DNA 2.0. Synthetic gene was inserted in *E.coli,* and analyzed sucrose conversion to isomalulose. To assay eukaryotic expression, synthetic SI gene was transferred to a plant vector and inserted to sugarcane genome by *Agrobacterium tumefaciens. T*he infection of sugar cane callus, was developed in 3 days, placing them in filter paper to decrease bacterial growth, then a disinfection was performed by washing the callus with different solutions, finally they were planted on induction medium containing hygromicin. Putative transgenic shoots were visible 5 weeks of selection. Developed plantlets will be analyzed by PCR and Sohtern blot analysis.

140.

Molecular cloning and functional characterization of ERF transcription factor genes in papaya

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Papaya is a tropical fruit of high nutritional value, and Mexico is the world's largest exporter of this important commodity. As a tropical crop, is highly susceptible to attack by pathogens such as *Colletotrichum gloeosporioide*s, causal agent of anthracnose and cause of postharvest losses. The publication of the genome sequence of papaya has opened the possibility to accelerate the discovery of genes involved in pathogen resistance to genetically improve this crop. Transcription factors (TF) are proteins that regulate gene expression. Among these proteins, we have identified several members of the Ethylene Response Factors family, (ERF-TF) which activate the expression of pathogenesis -related genes (PR) in plants. Have been reported an increase in its expression in response to pathogens and hormones involved in biotic stress as salicylic acid, jasmonic acid and ethylene. Overexpression of these TFs has shown an increase in tolerance to pathogens in model plants such as Arabidopsis and Tobacco. In a previous study from our laboratory, we were characterized five genes homologous to ERF transcription factors (TF) in papaya, named CpERF1 - CpERF5 . These genes showed similar structural characteristics to ERF-TF family whose function is related to the resistance of plants to pathogens. The aims of the present study are to: a) assess the expression of these genes in papaya in response to biotrophic pathogens as well as phytohormones involved in the response to biotic stress as salicylic acid (SA) and ethylene (ET) by induction experiments in papaya seedlings using BTH and ethephon as inducers , b) show the characteristics of transcription factors of the proteins encoded by these genes , by subcellular localization assays in onion cells , the transcriptional activity assays in yeast and electrophoretic mobility shift assays to observe their interaction with DNA and c) assess the effect of its overexpression in resistance against pathogens by genetic transformation of tobacco as a model plant . The results of this work to increase awareness of the FT in the resistance against pathogens biotrophes appearing in papaya and develop strategies for genetic improvement of this crop.

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141.

Novel strains of a begomovirus encode replication proteins with different DNA-binding specificity.

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The begomoviruses are pathogens of plants transmitted by the whitefly *Bemisia tabaci*, a cosmopolitan agricultural pest. These viruses have genomes composed by one or two circular molecules of ssDNA (~2,6 kb) which are replicated by a rolling-circle (RC) mechanism in the nucleus of the plant cell. The virus-encoded Rep protein is essential for this process. In 2006 we isolated from a leguminous weed (*Rhynchosia minima*) collected in Sinaloa a new begomoviral species named *Rhynchosia mosaic Sinaloa virus* (RhMSinV). More recently, two different begomoviruses were isolated from a single *R. minima* plant collected in Colima, and their sequencing revealed that they are new strains of RhMSinV, according to the ICTV taxonomic criteria. One of those strains exhibited four Rep-binding sites (iterons) similar to those of the original isolate of Sinaloa (i.e., TGGAGGA), whereas the second strain displayed iterons with a TGGAGTC core sequence. Alignments of the Rep proteins encoded by those viruses showed a difference in the aa residue at position 71 (N/ D), which is one of the five aa residues which were predicted to function as major determinants of geminivirus Rep DNA-binding specificity by Londoño et al. (2010. Arch.Virol.155:1033-46). Modeling of the 3-D structure of the proteins encoded by those RhMSinV strains showed that residues 9, 11, 71 and 73, point their side chains toward the exposed surface of the small beta-sheet (s1-s5) element, that is apparently critical for high-affinity DNA-binding of Rep proteins. Therefore, the distinct chemical nature of the aa residue at position 71 could to explain the differences in the cognate DNA sequences of the proteins of both RhMSinV strains. Experiments to verify this hypothesis are in progress.

142.

Massive micropropagation of elite organisms (*Saccharum officinarum*) variety mex 69-290

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Sugarcane (*Saccharum officinarum*) is a tropical grass, a giant grass related to sorghum and maize whose stem is formed and accumulated a rich juice of sucrose, compound to be extracted and crystallized is sugar. Today it is required an increase in food production. Therefore the plant tissue culture is an alternative to this concern, since this technique allows an accelerated spread on a large scale production; in reduced space and time for obtain plantlets at any time of year, so ensuring the quality and health sugarcane materials. It is therefore developed a massive micropropagation of procedure for elite sugarcane (*Saccharum officinarum*) variety MEX 69-290. To evaluate the ability of *in vitro* propagation of sugar cane variety MEX 69-290 it was used MS medium. Immature leaves rolls were used as explants, disinfected explants were placed on medium for callus formation therefore MS medium was supplemented with 2,4-D (0,1 and 3 mg/L). Once formed calli were transferred them to a germination medium placing them on MS medium without hormones. Already with these plantlets were transferred to MS medium rooting supplemented with NAA (2 y 7 mg/L), and finally transferred to soil. The callus induction stage took 4 weeks obtaining calli from immature leaves rolls, the best response was observed when added 3 mg/L 2,4-D for the production of calli. Once formed calli were transferred to media without hormones which started after two weeks it was observed green calli, after 6 weeks in the same medium was achieved seedlings. Root plantlets obtained when were used 2 mg/L NAA obtaining a whole plant. These plants were transferred to soil obtaining 80% of acclimatized plants after 4 months. Therefore it was established a micropropagation procedure for sugarcane for the development for massive plantlet production. With an excellent acclimatization ratio, therefore this methodology could be used successfully in field.

143.

Proteomic analysis of the response to water stress in mycorrhizal and non-mychorrhizal *Sorghum vulgare* roots

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Water stress is one of the most important abiotic factors limiting crop productivity worldwide. For this reason, there is a need to study the response of plants to the lack of water, in order to understand the mechanisms of survival and in the future to apply this knowledge to the genetic improvement of susceptible cereals. Sorghum (*Sorghum vulgare*) is a drought tolerant cereal and survives well in arid climates that are usually less suitable for other grain crops. In addition to its socio-economic importance, sorghum is considered a model to study the molecular mechanisms of drought tolerance in cereals. When the plant is under water stress conditions it uses different strategies to cope with it. The root is the first organ of the plant that perceives and responds to the stress conditions of water deficiency. It has been reported that plants associated with mycorrhizal fungi improve the assimilation of nutrients and water absorption. There are also several reports that prove at physiological and biochemical level that mycorrhizal fungi induce a greater tolerance to drought. The objective of this study is to determine changes in the expression of root proteins in mychorrizal and non-mychorrizal sorghum plants, under water stress conditions, by a comparative proteomic approach using two-dimensional electrophoresis as a tool. The identification of differentially expressed proteins will allow us to determine the possible processes involved in the response to the limited tolerance of waterThe plant material came from sorghum roots (*Sorghum vulgare* var. BJ 83 Caloro) grown under greenhouse conditions. Water stress was applied at the 56th day after sowing for seven days. In order to obtain the two-dimensional gels total soluble proteins were extracted using the technique of extraction with phenol, from well watered plants (control) and stressed plants as well as mycorrhizal plants (mycorrhizal control) and stressed mycorrhizal plants. The proteins were separated in the first dimension (IEF) using 18cm gels with a pH gradient 4-7 (GE Healthcare). For the second dimension 12% acrylamide-SDS gels were used. Protein profiles were analyzed with a image analysis software, Melanie ® 7.0 (Genebio.) The results obtained so far have identified changes in the expression of stressed mychorrizal and non-mychorrizal plants. The changes were greater in the case of non-mychorrizal plants, which suggests that the association with mycorrhizal fungi contribute to drought resistance. Besides, three proteins were detected responding to water stress only regardless of mycorrhizae. These proteins could be essential for the response to this type of stress. Currently the identification of differential expression proteins by mass spectrometry is being carried out.

144.

Proteomic analysis of chloroplast biogenesis-affected *Arabidopsis thaliana* mutants uncovers proteins potentially involved in chloroplast development: the GrpE chapter

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Among eukaryotes, plant cells are most remarkable by their ability to generate biomass from air and sunlight, this phenomenon takes place only on the many membranes and compartments of an outstanding organelle, the chloroplast. In the last few decades the chloroplast enzymatic machinery and developmental processes have been subject of intense research and the many efforts (including omic technologies) of the scientific community have led to the identification of a plethora of proteins whose functions are important for the making of a functional chloroplast. Plant mutants in the genes that code for those proteins often display pigment-accumulation defects showing pale-green, yellow or albino phenotypes. Proteomics has been used to explore the proteome of whole chloroplasts as well as suborganellar compartments such as the stroma or thylakoids of wild-type and single chloroplast-biogenesis affected plants. Furthermore, only one study has been reported to analyze and compare the proteomes of several chloroplast-biogenesis affected mutants. Here we present a comparative proteomic analysis of four different chloroplast-biogenesis affected mutants. The analysis consisted in the 2D-PAGE separation of protein samples and computational processing of the resulting images to select spots with abundance shifts of at least 2-fold, the selected spots were subjected to MALDI-TOF mass-spectrometry for protein identification. This work-flow led to the discovery of three novel proteins potentially involved in the development of *Arabidopsis thaliana* chloroplasts. Mutation of the genes coding for the identified proteins was found to yield albino plants that present low levels of mRNA accumulation for several chloroplast-development marker genes. One of such genes is *EMB1241*, it codes for a GrpE domain-containing protein that is most likely involved in the proper folding of chloroplast proteins. Experimental data addressing the existence of interactions between EMB1241 protein and proteins key to chloroplast biogenesis will be presented at the meeting.

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The Biotechnology Of The Microalgae

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In this century humanity is facing huge problems due to the increasing energy demand worldwide, depletion of fossil fuels, increasing price of oil and environmental difficulties caused by greenhouse gases and their consequence: global warming. This situation urgently demands alternative energy sources with lower pollution indexes, among which there's a viable option; this is the development of biofuels from microorganisms. The biodiesel can be obtained from a wide variety of sources, where the microalgae can be found. They have had a very particular interest as one of the most promising source for the biofuels production due to their high content in lipids (1-70% in dry weight), especially the triglycerides that are the best substrate for the biodiesel production, and it is possible to increase the synthesis and storage of great amounts of lipids optimizing growing factors. The lipids can be used for biodiesel production using a simple process called transesterification. Thanks to their short growing cycles, high lipids content and their easy-to-modify genetically feature have been proposed as potential candidates for the biofuels production.This writing has the objective of inducing the lipids synthesis on the microalgae species of the green genre *Chlorophyceae*; *Chlorella sorokiniana* that was isolated from a secondary settler in the residual water treatment plant of Chihuahua City. The cells were cultivated during 15 days in static systems or batch under different nitrogen and phosphorus concentrations in order to create a nutritional stress and therefore induce the lipids synthesis. The cells were harvested during the different growing phases to evaluate the lipids content over the development of the microalgae culture. Inducing lipids through limiting their nutrients seems to be a viable strategy to increase the lipids content on the cells; nevertheless, from a technical point of view, cultivating cells in nutrimental limiting conditions and lacking adequate concentration for their development limits the cellular growing affecting in a negative way lipids production. On the other hand, the behavior of protein content, carbohydrates, as well as the biomass production was observed in order to consider this species as a possible nutritional source for animals, biofertilizers or as a promising biofuels production alternative. In this study it has been found that the biochemical composition (lipids, proteins and carbohydrates) of the microalgae was affected by the growing phase, as well as the initial nitrogen concentration, without observing significant effects due to the initial phosphorus concentration. The *Chlorella sorokiniana* didn't respond to inducing lipids registering an average of lipids approximated to 18.39 ± 0.57 % for each one of the treatments (average all over the experimental period). However, a higher biomass concentration was registered reaching values of 1.62 g l^{-1} y 2.22 g l^{-1} in the treatments C_2 and CN respectively. Also a higher lipids production was registered due to the high growing rates that it presented. Results show that the carbohydrates were the main reserve component registering at any moment a high concentration. The lowest values of this component were registered during the exponential phase, while the highest values were found during the stationary phase observing higher carbohydrates content in those treatments containing the lowest nitrogen concentration. In this species the highest protein content took place on the

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Antioxidant capacity and anti-inflammatory effect of *Hamelia patens* extracts

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Arnica sp., *Azadirachta indica* and *Hamelia patens* are medicinal plants used in some Mexican areas to treat different diseases. In this study, we determined the content of polyphenols, flavonoids, and antioxidant activity in aqueous and ethanolic extracts of leaves, flowers, stems and seeds of *Arnica sp., A. indica* and *H. patens*. Also we tested the anti-inflammatory effect by the *H. patens* extract in carrageenan-induced model of local inflammation. The total phenol and flavonoid content was estimated using the Folin-Ciocalteau method and colorimetric assay, respectively. Antioxidant activity was determinate by reduction of DPPH radical and by redox potential. The anti-inflflammatory effect was tested only with the *H. patens* extract. The results indicate that the leaves of three plants have a significant amount of flavonoids. The leaves of *H. patens* showed high antioxidant activity, probably due to the high amount of flavonoids founded in this plant. This is the reason because we analyzed anti-inflammatory activity. In this respect, we found a significant anti-inflammatory effect in the model used. *Arnica sp., A. indica* and *H. patens* could improve the treatment of diseases associated with oxidative stress by their high antioxidants content. Especially the leaves of *H. patens* could have an important application in the treatment of inflammatory diseases.

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Biochemical and molecular identification of a plant natriuretic peptide in garlic (*Allium sativum* L.)

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Natriuretic peptides (NP) are vertebrate hormone, involved in the regulation of ion and water homeostasis. In plants have been identified homologous known as plant natriuretic peptides (PNP) some of which have been identified by immunoblotting with antibodies of human origin and with molecular and biochemical methods. The PNPs are peptides with a molecular weight between 10 and 20 kDa, sharing similarity with the N-terminal domain of expansins as a result of convergent evolution. The PNPs function seems to be involved in stomatal opening, opening and closing of Na^+ K^+ channels stimulating the synthesis of second messengers such as cGMP and ion transport. In this study, a PNP gene in garlic (*AsPNP*) was identified by PCR, the amplified nucleotide sequence was similar to a part of the first exon of *AtPNPA* gene. The deduced amino acid sequence displayed a conserved sequence among PNPs from other plant species, including a sequence of human origin. The phylogenetic tree showed greater sequence similarity of AsPNP with *Erucastrum strigosum*, *Hedera helix* and *Arabidopsis thaliana*. The analysis of real-time PCR showed that there are two copies of the gene, similar to those found in *Arabidopsis thaliana*: *AtPNPA* y *AtPNPB*. Immunoblotting with PNs antibodies of human origin (anti-c-terminal ANPA), verified the presence of PNP in protein extracts from garlic leafs and in a protein fraction partially purified by ion exchange chromatography. The immunoblotting positive fraction was also able to stimulate stomatal opening in cuticles of *Opuntia ficus-indica*, showing an increase in stomatal opening compared with control samples.

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In silico mining of the *Candidatus Liberibacter* asiaticus genome and its interaction with the host: a model for genomic transportome

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The Huanglongbing or yellow dragon disease is the most destructive citrus illness. The disease originated in Africa, then it dispersed through Asia and it is present in America since 2004. The disease has caused great damage to citrus agriculture in Brazil, Cuba and North America. In México this disease has a quarantine pest category, and to date it is not possible to control or treat it. The causal agent related to the disease in Mexico is *Candidatus* Liberibacter *asiaticus*, an alpha proteobacteria, which compared to the American and African varieties, is the most widespread and destructive of the three. One of the main obstacles for disease control is that the bacteria is limited to the phloem and cannot be cultivated. The complete genome is already available due to culture free techniques or metagenomic techniques. In this work, a throughout genome analyses of *Ca.* Liberibacter *asiaticus* has allowed the transport potential identified in the genome and development of an *in silico* model of bacteria interaction in its microenvironment. The analyses was performed with two reported genomes of Ca. L. asiaticus (psy62 and gxpsy), and shows than 37 genes coding to transport proteins and 30 of this genes are ABC transporters and 3 genes coding to thiamine importing. The importers in the genome of *Ca.* Liberibacter *asiaticus* are glucose/galactose and prolyne/glycine transporters, but the incomplete pathway of *Ca.* Liberibacter *asiaticus* propose than the bacteria feeds with amino acids or partially processed carbohydrates like Glycerate.

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Analysis of expression of genes involved in carbohydrate metabolism and distribution of these in plants of *Agave tequilana*

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Fructans are complex carbohydrates found in *Agave* species, where they are synthesized and stored in the stem structure known as the 'piña'. Fructans are fructose polymers that are synthesized by two or more enzymes with fructosyltransferase activity. In contrast invertases are responsible for the hydrolisis of sucrose to glucose and fructose. Currently we are determining the expression pattern of FFT's and invertase genes *in situ* in different tissues, using *Agave tequilana* plants cultured *in vitro*. Subsequently, to determine the distribution of these carbohydrates we will employ a range of histological techniques on the same tissues.

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