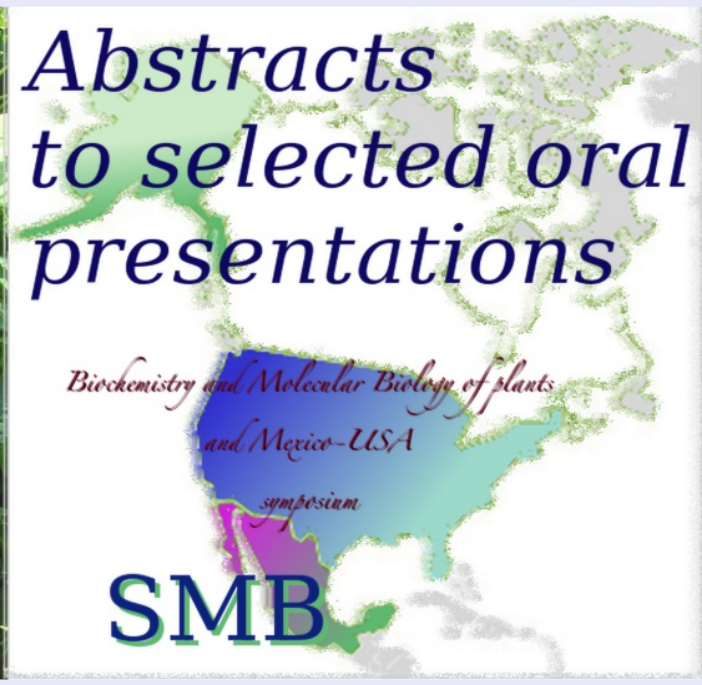
Number 3 Issue 3 October 2013

International Biotechnology Color Journal

A Scientific Peer Reviewed Journal with Focus on BIOTECHNOLOGY
and Covering Its Many Hues, Tints, Tones & Shades



Special issue:
Abstracts to selected oral presentations from the XV Biochemistry and Molecular biology of plantas meeting and 8th symposium Mexico-USA

October 21 to 25, 2013 Xcaret, Q.Roo, Mexico

Produced and hosted by *Centro de Investigación Científica de Yucatán, A.C.*,
in collaboration with *Universidad Nacional Autónoma de México*,
and the *International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society*

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The International Biotechnology Color Journal appears every 4 months in the last week of February, June and October of each year, starting in October 2011. It is a non-profitable Electronic Publication. In its initial phase it does not requires neither publication nor access fees. Its goal is the publication of Original Scientific Information, previously unpublished, and provided by the contributing authors out of its own will. *Int. biotechnol. color j., ISSN 2226-0404.*

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(IBCJ)

is the official quarterly publication of the **International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C.** (IFBR&ESCHNSAST&S).

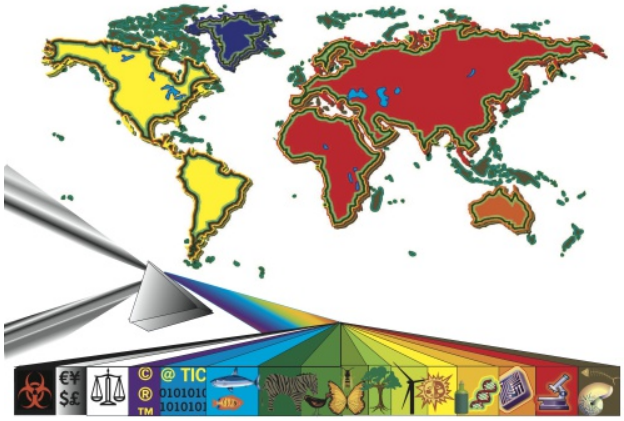
The IFBR&ESCHNSAST&S is a civil association and nonprofit organization.

Its main goal is to help bringing together efficient biotechnological solutions for many human problems, with the need for environmentally friendly and sustainable processes.

As means to such goal, the Foundation counts on education to create social awareness to biotechnology's benefits and risks, and to promote the formation of highly qualified professionals and research scientists.

The constitutive act of the foundation was signed in the Heroic City of *Huajuapan de León, Oaxaca*, on September 14th, 2009, at the *NOTARÍA PÚBLICA No. 61 de los ESTADOS UNIDOS MEXICANOS*. As .

Susana Lozano Muñiz
President of the Foundation



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Editorial section

International Biotechnology Color Journal (IBCJ) is an electronic Open Access journal, devoted to rapidly publishing full peer-reviewed articles covering all the fields of biotechnology. Though the central focus of IBCJ is to publish scientific papers, it provides a forum for reviews of special interest, notes presenting relevant findings in a short format, essays with new technical advances or relevant updates of reported protocols, book reviews, scientific meetings, and letters to the editor. Instructions for every type of contribution are presented in the journal's Homepage and in PDF format in the last issue of each year.

The Editorial Board of IBCJ is fully committed to publish articles innovating in all areas of biotechnology. Contributions are reviewed from a rigorous optic of scientific criticism; thus, any original contribution that fits within the scope of the journal and promotes the advancement of biotechnology are particularly welcome.

Editorial comments to the contents of this issue

By José Juan Zúñiga-Aguilar, Chief editor.

In this special issue of the International Biotechnology Color Journal, we are particularly glad to present the selected abstracts to the oral sessions from the XV Meeting of Biochemistry and Molecular Biology of Plants, and the 8th symposium Mexico-USA, held in Xcaret, Q. Roo, Mexico, from October 21th to 25th, 2013.

Xcaret, a beautiful and peaceful Resort, hosted this meeting of the Plant Biochemistry and Molecular Biology branch of the "Sociedad Mexicana de Bioquímica" (Mexican Society of Biochemistry), organized by an international committee with the support of the Society and the "Centro de Investigación Científica de Yucatan". The meeting offered an international forum for scientists working in diverse plant research areas.

To enhance the visibility of the works presented at the meeting, IBCJ now publiches selected abstracts to those oral presentations were the authors gave their consent.

Plant research is a dynamic area with high impact in biotechnolgy. Scientific meetings of high standards, such as this one, are relevant to the advancement of Biotechnology and the International Biotechnology Color Journal is fully committed to disseminate the science shared during these activities.

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ABSTRACTS TO SELECTED PRESENTATIONS[1]

SMB PLANT MEETING 2013

The Meeting was organized by Sociedad Mexicna de Bioquímica A.C. and Centro de Investigación Científica de Yucatán, A.C.

The XV Biochemistry and Molecular Biology of Plants Meeting and the 8th symplsium Mexico-USA was held in Xcaret, Q. Roo., Mexico, on October 21 to 25, 2013.

Organizing comittee:

Manuel Martínez Estévez, Laura Hernández Terrones, and José Juan Zúñiga Aguilar

from Centro de Investigación Científica de Yucatán, A.C.

Sarah Hake

from Department of Plant & Microbial Biology

University of California, Berkeley

Donald P. Ort

from Department of Plant Biology

University of Illinois at Urbana-Champaign

(1) The abstracts presented here were not peer reviewed

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Enzymological approaches to understand plant responses to the environment

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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.

Green: Wildlife, Ecology & Environment Biotechnology

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

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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.

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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.

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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. **This work has been funded by Comercializadora Naturista del Bajío.**

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Nothing like we imagined – uncovering the terpenome of liverworts

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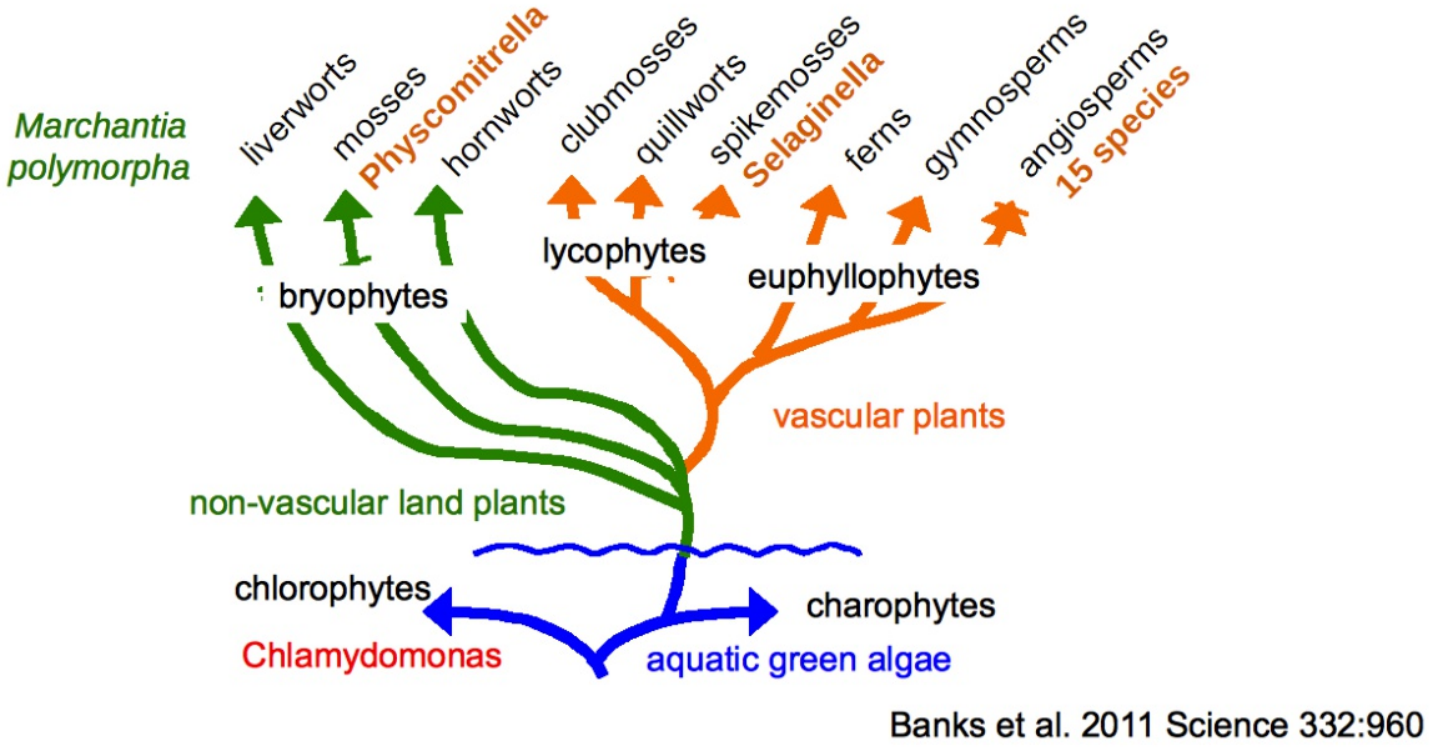
Plants produce a wealth of terpenes that serve physiological and ecological roles, and the basic biosynthetic pathways for many classes of terpenes have been elucidated. These studies have logically led to sophisticated structure-function studies of terpene synthase enzyme families using a variety of 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.

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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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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.

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.

The work was funded by the SEP-CONACYT project 82884 and a postdoc fellowship from CONACYT to HAA.

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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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* roots treated with NF were purified by IMACFe^{+}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.

International Biotechnology Color Journal

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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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.

Green: Wildlife, Ecology & Environment Biotechnology

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* plants 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 °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 °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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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.

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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).

Hekonnen 2012. The water footprint of humanity. Proceedings of the National Academy of Sciences, USA 109: 3232-3237

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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 µM 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.

International Biotechnology Color Journal

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*.

Green: Wildlife, Ecology & Environment Biotechnology

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.

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.

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 ζ-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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International Biotechnology Color Journal

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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.

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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International Biotechnology Color Journal

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 involvement 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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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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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. ***DGAPA IN210312, PAIP-FQ 429015***

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.

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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.

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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 localized. 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.

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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.

(1) Duke et al., 2002. Weed science 50: 138-151.

(2) Srinivasan K, 2007, Crit. Rev. Food Sci. Nutr. 47: 735-48.

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 este metabolito secundario. Se encontraron las siguientes características: la *K*m es 44 mM y la *V*max 303 µmol mg de proteína^{-1}min^{-1}; 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 cuantificó la producción de afinina y la actividad de la valina descarboxilasa durante el primer mes de desarrollo de la planta. Se observó una mayor actividad de la enzima un día antes del incremento en la producción de afinina.

***English translation**:** To improve the knowledge of affinin synthesis in *Acmella radicans*, experiments were carried out to examine the behavior of Valine decarboxylase (ValDC). This enzyme is involved in the biosynthesis of that secondary metabolite. The enzyme was found to have the following properties: *K*m was 44 mM and *V*max was 303 µmol mg. protein^{-1} min^{-1}, an optimum pH from 8 to 10, it has a wide temperature working range from 25 to 42 ºC, without important variations inside it. The reaction was quantifiable from 10 minutes onwards. It is PLP-dependent and has activity against the L and D-valine enantiomers. The next findings relate to the pre-purification steps: there is no need for PLP addition during desalting, and quantifiable activity halves upon its addition, indicating the absence of inhibitors in the crude extract. The recommended $(NH_4)_2SO_4$ concentration is 75% and the performance of a postprecipitation dialysis is required to prevent the loss of activity. Finally, the affinin production was measured along with valine decarboxylase activity along the first developmental month of the plant. An activity peak preceded the increase in affinin production by one day.

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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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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.

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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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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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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.

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.

June Simpson[1,6], Corina Hayano Kanashiro[2], Octavio Martínez de la Vega[3] , Humberto Reyes Valdés[4], José Luis Pons Hernández[5], Rocío Aguilar Rangel[1,3] and Ruairidh Sawers[3].

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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.

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Traditional and genetic improvement of sugar cane

Quintín Rascón-Cruz

Universidad Autónoma de Chihuahua

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 (p = 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.

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.

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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.

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

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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.

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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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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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: Sattler et al., 2010. Plant Science 178:229-238. - 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.

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.

English translation:* The D-cyclins are fundamental proteins, as they allow proper interpretation of environment conditions to be interpreted by the cell in terms of the proliferation capacity. All pluricellular organisms present D-type cyclins, though their number varies. While all mammal cells have three different types (with alternative splicing variants), plants can have multiple types, reaching 17 different genes in the case of maize. Our studies have shown the expression of most of those genes in different plant tissues with distinct patterns. The preparation of antibodies against some of these proteins has allowed the study of their ontogeny, their associations with cyclin dependent kinases (CDKs), essential for their regulation and cell cycle progression, as well as the differential kinase activation, or repression analysis os these complexes. Such activation state may depend on the CDKs phosphorylation state, both on activity stimulatory or inhibitory residues. Preliminary work indicated a CDK phosphorylation requirement for kinase activity, and variations in the phosphorylation state of several residues along germination, which may depend on the type of D-cyclin bound. In these context, the different D-cyclin do not appear to have redundant roles.

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