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LIBRO DE ABSTRACTS

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We have developed industrial scale biotechnological production systems for wild and rare Nordic berry species as cell cultures with consistent quality and defined chemical composition. Moreover, through our novel bioprocessing technologies, such as biotransformation, the berries and their side streams can be modified to obtain ingredients with new or improved activities, colours and flavours. The example for the sustainable production of raspberry ketone, one of the most expensive natural flavours, using engineered plant cells fed with betuligenol will be presented.

Modern biotechnology i.e using plant cell and tissue cultures, offers many advantages to develop new type of ingredients for industrial applications in an environmental friendly and sustainable way. We have shown that phenolic compounds in berries very efficiently inhibit the growth of many human pathogens including skin pathogens without affecting the growth of beneficial bacteria, such as lactic acid bacteria. Several mechanisms of action, including the weakening of the outer membrane of Gram-negative bacteria, are involved in the growth inhibition. More interestingly, various berry phenolics are shown to specifically block cell-to-cell signalling (quorum sensing) in a bacterial community. The unusual phenolic profile of the cultured berry cells as well as their fatty acid composition with a high proportion of α-linolenic acid and high protein content makes them a unique and interesting alternative e.g. for the cosmetic industry.
Bioprocessing including enzyme treatment and fermentation combined with special dry fractionation technologies of the berry material has resulted in increased bioactivities, such as antimicrobial, anti-inflammatory and antioxidant activities. Efficient utilization of the biomolecules requires controlled delivery systems into the skin, such as microcurrent patches developed at VTT, which are based on renewable enzyme catalysts and materials. In this presentation, the possibilities of using natural plant cell-derived ingredients combined with a new delivery system will be discussed.
Tuesday 27th June
Room Gran Salon Catalunya
SESSION 3. SYSTEMS BIOLOGY
15:30 to 17:15

Keynote lecture: A systems biology perspective to the evolution of virus-plant interactions
Santiago Elena

A SYSTEMS BIOLOGY PERSPECTIVE TO THE EVOLUTION OF VIRUS-PLANT INTERACTIONS
Santiago F. Elena¹,²,³

¹Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Campus UPV CPI 8E, Ingeniero Fausto Elio s/n, 46022 València, Spain
²Instituto de Biología Integrativa de Sistemas (CSIC-UV), Parc Científic UV, Catedrático Agustín Escardino 9, 46980 Paterna, València, Spain
³The Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA

Understanding the mechanisms by which viruses overcome host defenses in order to proliferate has been a challenging problem owing to the multiplicity of factors and complexity of interactions involved. The advent of genomic techniques has opened the possibility to grasp a global and dynamic picture of the interaction. We are tackling this problem combining two approaches. Firstly, we have been experimentally evolving potyviruses in a number of ecotypes of Arabidopsis thaliana that differ in their susceptibility to infection, followed by the characterization of the transcriptomic responses of plants to infection with ancestral and evolved viruses. Secondly, we have performed meta-analyses of available transcriptomic data from a collection of viruses all infecting A. thaliana. Evolution experiments resulted in the emergence of a gene-for-gene relationship by which more restrictive ecotypes selected for generalist viruses, whilst ecotype-specialized viruses evolved in the more susceptible ecotypes. Generalist viruses perturb the transcriptomes of different plant ecotypes in a similar manner; by contrast, specialist viruses tend to differ in the way they interact with each ecotype. Comparative meta-analyses revealed that phylogenetically-related viruses significantly alter the expression of similar genes and that viruses that naturally infect plants from the Brassicaceae display a greater overlap in the lists of altered genes. Furthermore, when virus-manipulated genes were contextualized into A. thaliana transcriptional and protein-protein interaction networks, we uncovered a general mode of action of plant viruses, in which perturbations preferentially affect genes that are highly connected, central and organized into modules.
Wednesday 28th June
Room Gran Salon Catalunya

PLENARY CONFERENCE:

CarolightR: A plant biotechnology product portfolio for human and animal health and nutrition
Paul Christou
08:30 to 9:30

High carotenoid corn: A plant biotechnology product portfolio for human and animal health and nutrition

We recreated the carotenoid and ketocarotenoid pathways in elite South African white maize inbred lines. One of the resulting high carotenoid transgenic lines was registered in Spain under the name of Carolight®. We describe the process used to generate Carolight® and also a breeding program and experimental field trials to assess the performance of elite transgenic hybrids using locally adapted commercial inbred lines. We present data on the interactions of Carolight® with pests and diseases in the field. The use of Carolight® in poultry and swine production in a commercial setting will be discussed. Experiments demonstrating the beneficial effects of a high carotenoid corn diet specifically delivered through Carolight® in human health, using an experimental animal model will be described. The performance of a high ketocarotenoid line in fish production will be used as a case study to illustrate transition from the laboratory to a commercial setting. IP and FTO analysis as well as regulatory aspects will also be discussed to address remaining barriers to commercialization.
Wednesday 28th June  
Room Gran Salon Catalunya  
SESSION 5. BIOTIC STRESS, PLANT-PATHOGEN INTERACTIONS  
09:30 to 11:15

Keynote lecture:  
Plant responses to spider mite feeding: step by step  
Isabel Díaz

PLANT RESPONSES TO SPIDER MITE FEEDING: STEP BY STEP

M. Estrella Santamaría, Ana Arnaiz, Pablo González-Melendi, Vojislava Grbic,  
Manuel Martinez, Isabel Díaz*

Centro de Biotecnología y Genómica de Plantas. Universidad Politécnica de Madrid (UPM), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA).  
Campus Montegancedo, 28223 Pozuelo de Alarcón, Madrid, Spain

Plants rely on a battery of mechanisms to detect pathogens and pests in order to mount appropriate defence reactions. In the case of pests, the induction of plant defences is initiated when specific receptors detect either the presence of herbivore through the recognition of Herbivore-Associated Molecular Patterns (HAMPs), or the damage incurred by plant tissues as consequences of herbivore feeding, or even the presence of volatiles emitted as plant-plant cues. Among pests, the two spotted spider mite *Tetranychus urticae* is the most harmful phytophagous acari, since it is a species that feeds on more than 1,100 host plants causing more than $1 billion in damage annually. We focused our research on Arabidopsis and tomato responses to spider mite infestation to identify and characterize new molecules involved in the defence as potential alternatives to chemical control. We followed step by step the plant defence pathways, since the perception that begins with the spider mite contact that triggers signal transduction pathways, to the generation of final defence products. We checked the variation in susceptibility to spider mite-induced damage in tomato and Arabidopsis accessions. We assayed transcriptional responses in the most susceptible and resistant accessions after spider mite feeding and examined reciprocal genome-wide responses of mites and its host Arabidopsis. As expected, a large set of data was obtained with a robust list of differentially expressed genes. We selected genes with higher expression levels in the resistant vs the susceptible accessions. The genes were validated and molecularly characterized. In parallel, other studies were developed to identify the pattern of mite feeding, to decipher the spider mite digestive proteases, to develop biotechnological strategies to enhance resistance to spider mites, and to identify differential spider mite-induced responses between Arabidopsis and tomato. In conclusion, we address questions like how plant responses are elicited by mite feeding, what are the molecular bases of mite-induced plant responses, which hormones are involved in intra plant transmission of information about mite infestation, and which physiological changes are induced to create defences.
Thursday 29th June  
ROOM SALON CATALUNYA  
SESSION 9 ABIOTIC STRESS  
10:00 to 11:45  
Keynote lecture: Effects of abiotic stress on source-sink relations in crop plants  
Uwe Sonnewald

Impact of abiotic stress on source-to-sink relations in crop plants  
Sophia Sonnewald, Bernadetta Hastilestari, Max Kraner, Günter Lehretz, Uwe Sonnewald

Potato is the third most important food crop in the world after rice and wheat. Because of its widely-distributed cultivation and high yields, it is considered a critical species in terms of food security in face of a growing world population. However, potato is particularly vulnerable to high temperature during various stages of its life cycle. Elevated temperatures strongly suppress tuberization, negatively affect storage and shelf life of tubers and reduce fitness of seed potatoes. Breeding new heat-stress tolerant cultivars is therefore an urgent need for sustainable increases in potato production. To achieve this goal an integrated approach combining physiology, biochemistry and molecular genetics is followed to analyze the impact of elevated temperatures on source-sink relations of potato plants, potato tuber development, starch accumulation and tuber quality and tuber dormancy. First results indicate that heat effects source-sink relations by altered expression of the tuber inducing signal FT, by stimulating shade-avoidance responses of the shoot and by decreasing sink-strength of developing tubers. Sink strength of growing potato tubers is mainly regulated by the activity of sucrose synthase. Measuring sucrose synthase expression and activity of heat grown potato tubers revealed a significant down-regulation of the enzyme which is consistent with reduced tuber growth. Molecular studies suggest that FT is not only transcriptionally regulated but also post-transcriptionally by siRNAs. This assumption is based on a comparative miRNA analysis of potato plants grown under control and heat conditions. Several known miRNAs are significantly regulated during heat treatment, in addition one siRNA potentially targeting SP6a could be identified. The importance of this siRNA is currently under investigation. By ectopic overexpression of an artificial siRNA-resistant FT gene, tuberization could strongly be accelerated. This premature tuberization negatively influenced shoot growth and the ability of leaves to accumulate transitory starch, indicating that FT is a master regulator of source-to-sink relations. Although FT seems to play a major role in regulating tuberization, sink-derived signals are likely to be involved in orchestrating the heat-induced shift in assimilate allocation. This assumption is based on experiments in which soil and air temperatures of pot grown potato plants were independently controlled in growth chambers and transcript as well as sugar and enzyme profiles were recorded. Based on the results discussed above it is evident that cell-to-cell movement of source and sink signals play essential roles in balancing source-sink relations. This transport is mediated by plasmodesmata (PD). Up to now little is known about the protein composition and regulation of PD function. Proteomics studies on Arabidopsis mutants, unable to develop complex PD in mature leaves, revealed a first insight into proteins specifically associated with complex PD. Functional analysis of these proteins will allow to get a better understanding of the molecular composition of PD and their role in source-to-sink communication.
Thursday 29th June  
ROOM CAPRI + MEDES  
SESSION 10. APPLIED PLANT PHYSIOLOGY AND MOLECULAR BREEDING  
10:00 to 11:45

Keynote lecture:  
The role of chromatin dynamics on plant phenotype - stories of two different plant species  
Margarida Oliveira

THE ROLE OF CHROMATIN DYNAMICS ON PLANT PHENOTYPE - STORIES OF TWO DIFFERENT PLANT SPECIES  
Oliveira MM, Santos AP, Ferreira L, Saibo NJM, Barros P.  
ITQB NOVA, Av. da República, 2780-157 Oeiras, Portugal

We have been trying to link genome regulation and phenotypic plasticity by uncovering different levels of regulation. Here we will focus on the functional role of epigenetic mechanisms in two case studies: rice and cork oak (Quercus suber). We investigated the role of epigenetic regulation in rice response to salt stress. We found that varieties with contrasting salt tolerance showed differential methylome flexibility (global DNA methylation), with the tolerant ‘Pokkali’ being able to quickly relax DNA methylation in salt stress response. Phenotypic traits related to salinity tolerance (root length and biomass) revealed better performance under stress of a DNA-methyltransferase-deficient mutant (osdrm2), suggesting that higher methylome flexibility is important for tolerance.

A deeper analysis into the leaf methylome profile of ‘Pokkali’ (using MeDIP-seq), comparing control vs. salt stress conditions, allowed identifying salt stress-specific Differentially Methylated Regions (sDMRs). Under salinity, these sDMRs became hypomethylated and occasionally their position correlated with salt stress-gene induction, suggesting a role as expression regulators. When investigating specific histone modifications in the regulation of rice ROOT MEANDER CURLING gene (OsRMC, highly induced by salt stress and very conserved among rice varieties) we found, by Chromatin ImmunoPrecipitation, differential enrichment of euchromatic marks relating to the promoter region. Under salinity, the region of TFs-binding was highly enriched in histone modification marks related to euchromatin structure, indicating a nucleosome repositioning pattern underlying OsRMC salt stress activation.

In cork oak, we used MSAPs (Methylation-Sensitive Amplified Polymorphism) to investigate putative correlations between DNA methylation and cork quality. We found four markers statistically associated with quality traits (wood inclusions and porosity), thus supporting a potential role of DNA methylation in modulation of phellogen activity, and putative new tools to assess cork quality.
Oral Presentations

TUESDAY 27th JUNE
ROOM: GRAN SALÓN CATALUNYA
9:30-11:15

Session 1. Growth and Development

C0065 UV-B INDUCED PHOTOTROPISM IN ARABIDOPSIS

Lucas Vanhaelewyn¹, Alejandro Serrano², Carlos Ballaré³, Dominique Van Der Straeten¹, Maria Veronica Arana², Filip Vandenbussche¹

¹Ghent University (Ghent) Belgium
²CONICET (Mendoza) Argentina
³CONICET (Buenos Aires) Argentina

1 Resumen

To efficiently capture light for photosynthesis or to regulate floral display, plants orient their organs towards the light in a process called phototropism. Phototropism has been mainly associated with blue light, perceived by specific photoreceptors called phototropins. Recently we found that in Arabidopsis, monochromatic unilateral ultraviolet (UV)-B (280-315nm) induces phototropism in etiolated phot1phot2 seedlings devoid of phototropins. This response is mediated by the specific UV-B receptor UV RESISTANCE LOCUS 8 (UVR8) and its downstream component ELONGATED HYOCOTYL 5 (HY5). However, wild type seedlings respond faster and are more sensitive to UV-B than phot1phot2 seedlings, indicating participation of phototropins to UV-B perception. Moreover, since uvr8 mutant seedlings behave as wild type in unilateral UV-B, phototropins appear important UV-B receptors and mask any UVR8 effect. The photosignaling mechanism of phototropins in UV-B is similar to that in blue light, and has differential auxin signaling downstream. In addition, we found that in Arabidopsis inflorescence stems, UV-B appears more effective than blue light to stimulate phototropism. Contrasting to the situation in etiolated seedlings, the main photoreceptor for UV-B phototropism in inflorescence stems is UVR8, with only a minor role for phototropins. In these conditions, based on reporter genes, a light signal gradient can be observed. Downstream, based on pharmacological assays, mutant analysis and reporter lines, again an auxin signal gradient is associated with the differential growth in the inflorescence stem. Thus, UV-B phototropism in etiolated seedlings and inflorescence stems relies on similar components, yet balanced in a different way.
C0101 NOVEL INSIGHTS IN THE BRASSINOSTEROID-MEDIATED STEM CELL DIVISION MECHANISM

Nadja Bosch Moreno¹, Isabel Betegón-Putze¹, Cristina Martínez², Sacco de Vries³, Salomé Prat², Ana I Caño-Delgad³

¹Department of Molecular Genetics, Centre for Research in Agricultural Genomics (CRAG) Cerdanyola del Vallés (Barcelona) España
²Department of Plant Molecular Genetics, Centro Nacional de Biotecnología (CNB) (Madrid) España
³Department of Agrotechnology and Food Sciences, Wageningen University and Research (Wageningen) Netherland

1 Resumen

Brassinosteroids (BRs) are essential polyhydroxylated steroid hormones involved in plant growth and development. In the primary root apex BRs control cell cycle progression and transition to differentiation in the meristem¹. In the stem cell niche BRs promote quiescent centre (QC) renewal and differentiation of distal stem cells, suggesting that counteracting BR signalling is a mechanism to preserve the low rates of cell division in the QC. A proper plant architecture requires a balance between cell division and differentiation.

Our laboratory identified BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER), the first QC-specific repressor of quiescence in plants. We proposed a mechanism for this transcription factor in controlling quiescent cells in response to BRs². When the BR pathway is activated, BRAVO is recruited by BR-specific transcription factor BES1, thus leading to stem cell division and renewal². Despite the importance of the stem cell niche in meristem maintenance and plant growth and productivity, the molecular mechanisms that control stem cell division and fate has only started to be elucidated².

Recent studies indicate that the transcriptional co-repressor TOPLESS (TPL) binds to BRAVO promoter through the formation of a complex with BES1, promoting QC cell division by repressing BRAVO. These results reveal the role of these components together in BR-regulated expression in the root meristem³.

We are currently working in the identification of the native BRAVO protein complex in the stem cell niche using in vivo immunoprecipitation assays. By genetic and biochemical approaches, we aim to characterize the functional BRAVO interactors and its relevance for stem cell development in roots. That will lead us to understand the molecular mechanisms involved in stem cell homeostasis.
C0102 DOF AFFECTING GERMINATION 1 PROTEIN CONTROLS THE SEED-TO-SEEDLING TRANSITION IN ARABIDOPSIS

Riccardo Lorrai¹, Veronica Ruta¹, Alessandra Boccaccini¹, Francesco Gandolfi¹, Rosalba Lepore¹, Paolo Costantino¹, Paola Vittorioso²

¹Sapienza University of Rome, (Rome) Italy
²Vittorioso Rome, Italy

1 Resumen

The transition from a growth-arrested seed to a germinating seed represents a crucial developmental switch in the life cycle of a plant. Arabidopsis seeds develop dormancy during the late stages of their development; although mature, these seeds are not capable of germinating even under favourable environmental conditions. Abscisic acid (ABA) produced during seed maturation is necessary to induce seed dormancy; gibberellins (GAs) release dormancy and promote germination, thus counteracting the effects of ABA, whereas the role of GAs during seed development is less clear. Inactivation of the gene DAG1 (DOF AFFECTING GERMINATION1) reduces seed dormancy. DAG1 is a repressor of the seed germination process in Arabidopsis: dag1 null mutant seeds require lower GAs and red light fluence rates than wild-type seeds to germinate. DAG1 acts in the phytochromeB (phyB)-mediated pathway, downstream of PIF1 (PHYTOCHROME INTERACTING FACTOR1). Indeed, DAG1 controls the level of GAs and ABA during seed maturation and dormancy by repressing GA3ox1 and CYP707A2 through direct binding to their promoters.

We have also previously shown that dag1 mutant seedlings show significant shorter hypocotyls compared to the wild-type, suggesting that DAG1 is a negative component of the light-mediated inhibition of hypocotyl elongation. To further analyse the role of DAG1 in the seed-to-seedling transition phase, a transcriptome analysis has been performed on dag1 and wild-type hypocotyls and seedlings. Results from this study further confirm that DAG1 as a key player in the control of the developmental switch between seed dormancy and germination.
C0112 A FAMILY OF ENZYMES PRODUCES ISOPRENOID PRECURSORS FOR CAROTENOID BIOSYNTHESIS IN TOMATO

Victoria Barja Afonso, Manuel Rodríguez Concepción
Centre for Research in Agricultural Genomics (CRAG) Bellaterra (Barcelona) España

1 Resumen
Carotenoids represent a group of plastidial isoprenoids highly demanded as natural pigments and health-promoting nutrients (e.g. beta-carotene as pro-vitamin A). In plants, carotenoids are essential for photoprotection in leaves and contribute to animal-driven pollination and seed dispersal by coloring flowers and fruits. They are also precursors for the production of apocarotenoids with roles as hormones (such as abscisic acid and strigolactones), pigments and aromas. As most plastidial isoprenoids, carotenoids derive from geranylgeranyldiphosphate (GGPP) synthesized by GGPP synthase (GGPPS) enzymes encoded by gene families in plants. In Arabidopsis thaliana, 5 GGPPS isoforms produce GGPP in plastids (2), endoplasmic reticulum (2), and mitochondria (1). However, the production of carotenoids depends solely on the activity of GGPPS11, the only essential GGPPS in Arabidopsis. Only fragmented information is available for GGPPS families in other plants, including those with interest as a source of dietary carotenoids. We are currently analyzing the GGPPS family in tomato (Solanum lycopersicum). Tomato fruit ripening involves a dramatic increase in the production of carotenoids, which provide orange and red colors to ripe tomatoes. We speculate that carotenoid production in tomato fruit most likely involves the activity of specific GGPPS paralogs which may be different from those producing GGPP for carotenoids involved in photoprotection (in leaves) or mycorrhizal associations (in roots). Based on stringent homology criteria, we found 9 putative GGPPS paralogs in the tomato genome. We experimentally confirmed that 4 of them are primarily localized in plastids. From these, all 4 are expressed at similar levels in leaves but only 1 is primarily expressed in roots and the other 3 are up-regulated during fruit ripening. Identifying which of the GGPP-producing plastidial isoform(s) is responsible for carotenoid biosynthesis in particular organs will allow us to specifically improve the nutritional quality of fruits without interfering with photoprotection or tolerance to stress.
Unusual fatty acids, such as epoxy and hydroxylated ones, have many uses as industrial feedstock for polymers, lubricants, and synthetic chemistry. Plants that accumulate these unusual fatty acids in their seed oils typically have poor agronomic performance.

Castor plant (Ricinus communis) is an important non-edible oilseed crop widely cultivated in tropical-subtropical and temperate countries. Moreover, castor oil compromises up to 50-60% of the seed weight of this plant, which reaches productivities up to 3000 kg oil/ha. Furthermore, this castor seed oil accumulates high amount of ricinoleic acid in TAG because it has special enzymatic machinery that channel this fatty acid into TAGs. Also, recent studies demonstrate castor plant accumulates unusual fatty acids like vernolic acid to TAG, features that are not present in other oil seeds. Therefore, castor plant looks like an ideal platform to produce and accumulate unusual fatty acids via genetic engineering. Unfortunately, in addition to large amounts of oil, castor seed also contain concentrated amounts of the citotoxic lectins, ricin and agglutinin, two potent ribosome inactivating proteins that makes castor seeds and extracted meal highly toxic.

In the present work we have designed genetic structures for metabolism edition and gene silencing in castor seed. In order to test genetic structures we developed a method for transient expression in castor seed that consists in agroinfiltration directly in the fruit. pCambia vector with GUS as a marker was tested through this method and results reveals good levels of in vivo transformation in seed. Right now we are using genetic construction that encodes epoxidases or elongases in order to change fatty acids composition in mature castor seed and use castor like unusual fatty acids producer.
C0186 MASS PRODUCTION OF ROSMARINIC ACID BASED ON METHYL JASMONATE-ELICITED CELL CULTURES OF SATUREJA KHUZISTANICA

Abbas Khojasteh1, Regine Eibl2, Rosa M. Cusido1, Javier Palazon Barandela3

1Universitat de Barcelona (Barcelona) Barcelona
2Zurich University of Applied Sciences (Zurich) Suiza
3Facultat de Farmacia. Universitat de Barcelona Barcelona (Barcelona) España

1 Resumen
Rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid, is widely distributed in the plant kingdom, including Satureja khuzistanica, a native Iranian plant species. Interest in RA as a pharmaceutical or dietary supplement is growing with increasing awareness of its potential benefits for human health. Among its most promising biological activities are cognitive-enhancing and cardioprotective effects, cancer chemoprevention properties and a potential use in the treatment of Alzheimer’s disease. The current possibilities that plant biotechnology offers for the production of plant secondary metabolites and the important bioactive properties of RA have prompted many researchers to establish RA-producing biotechnological platforms. In order to meet the growing demand for RA and to preserve its natural sources, the aim of this work was to develop a new biotechnological platform for RA production based on methyl jasmonate-elicited cell suspension cultures of S. khuzistanica scaled up to a 2 L single-use wave-mixed bioreactor. The results obtained in shake flasks show that S. khuzistanica cell suspensions synthesized high amounts of RA, which accumulated mainly inside the cells. MeJA increased RA productivity more than 3-fold, reaching an RA production of 3.9 g L⁻¹, without significantly affecting the system’s biomass productivity. When the process was scaled up to a wave-mixed BioSTAT CultiBag RM of 2 L (WV of 1 L) working in batch mode, a maximum RA production of 3.6 g L⁻¹ and a biomass productivity (CDW) of 22.4 g d⁻¹ was achieved, demonstrating the suitability of S. khuzistanica elicited-cell suspensions for the biotechnological production of this bioactive plant secondary metabolite.
RESUMEN

La temperatura baja es uno de los factores más importantes afectando el crecimiento vegetal, directamente alterando el proceso fotosintético y provocando foto-inhibición. Con el fin de abordar la respuesta aclimatativa fotosintética de *L. japonicus* bajo estrés frío, se estudiaron dos ecotipos (MG-1 y MG-20). Datos previos mostraron que la foto-inhibición ocurre en el estrés y diferencialmente entre ecotipos, siendo MG-1 más afectado que MG-20. Además, se generó un desequilibrio redox en el cloroplasto debido a baja temperatura, pero sólo en el ecotipo sensible.

En este estudio, se utilizó una abordaje proteómico para evaluar cambios en el proteoma cloroplástico de ambos ecotipos MG-1 y MG-20 tras 7 días de tratamiento con temperatura baja y control. Se identificaron 724 proteínas, de las cuales 12 presentaron interacción significativa entre ecotipos y tratamientos de temperatura, 66 proteínas diferencialmente abundantes entre ecotipos y 64 entre tratamientos de temperatura.

La función de la proteína se clasificó en términos de KEGG, Uniprot y LegumeI databases, así como con el software STRING. Se identificaron más proteínas relacionadas con la fotosíntesis "reacciones de luz" (en particular, fosforilación oxidativa y proteínas de antena) en el ecotipo MG-1 en comparación con el MG-20. Al contrario, en MG-20, proteínas relacionadas con el metabolismo del carbono, el acné oxidativo y la plegada proteínas fueron más abundantes.

Los cambios en el fotosintético y en el metabolismo del carbono sugirieron dos estrategias aclimativas diferentes en los cloroplastos de *L. japonicus*. Mientras que en el ecotipo sensible MG-1, esos mecanismos parecen implicarse en la dissipación energética en los fotosistemas. La regulación del carbono fuente/sumidero parece ser preponderante en el tolerante MG-20. Datos mostraron que la disociación diferencial de ambas *L. japonicus* ecotipos a estrés frío podría ser, en lo menos parcialmente, explicado por estas diferentes estrategias de aclimatación. En conclusión, nuestros resultados resaltaron la relevancia del metabolismo del carbono en la aclimatación al estrés frío del modelo leguminoso *L. japonicus*.
C0241 MULTIOMICS CHARACTERIZATION OF ADAPTIVE MUTATIONS SUPPRESSING IMMUNE-RELATED HYBRID INCOMPATIBILITY

Kostadin E. Atanasov, Changxin Liu, Rubén Alcázar

Section of Plant Physiology. Department of Biology, Healthcare and Environment. Faculty of Pharmacy. University of Barcelona. Barcelona (Barcelona) España

1 Resumen
According to the Bateson-Dobzhansky-Muller (BDM) model, hybrid incompatibilities (HI) in plant accessions or either varieties, can represent an incipient mechanism for speciation. In Arabidopsis thaliana, about of 3% of the crosses between natural accessions produce incompatible hybrids, which involve temperature-dependent immune activation of defense, cell death, dwarfism and reduced fitness.

The hybrid incompatibility in the Lansdberg (Ler)/Kashmir-2 cross is due to an epistatic interaction between a derived NB-LRR locus in Ler (accession originally from Górzow, Poland), and allelic variants of a SRF3 receptor like-kinase highly frequent in Central Asia. The occurrence of adaptive mutations that reduce or suppress fitness costs in nature would take long evolutionary time scales and, as such, incompatible hybrids are—in theory—purged by selection. Through a suppressor screen of the Ler/Kas-2 HI, we have identified artificially-induced adaptive mutations that suppress fitness costs on growth. We’re using these adapted mutants to characterize the molecular bases of RPP1-like triggered defense response and the global metabolic costs derived from maintaining an autoactivated immune system.

We’re also interested in comparing the microbiota associated to these genotypes with their parental lineages in order to determine potential feedback modulation between the allelic composition of Resistance genes in nature and the composition of microbial communities. This, together with the reconstruction of plant microbiota through synthetic microbial communities (SynCom) experiments in gnotobiotic plant systems will enable the determination of fitness benefits or costs in plant-microbe interactions and how these may impact immune receptor variability.
C0317 EVOLUTIONARY ANALYSIS OF DELLA FUNCTION

Jorge Hernández García¹, Asier Briones-Moreno¹, Carlos Vargas-Chávez², David Alabadi¹, Miguel A. Blázquez¹

¹Instituto de Biología Molecular y Celular de Plantas, (IBMCP, CSIC-UPV) Valencia (Valencia) Spain
²Institute for Integrative Systems Biology, (Universidad de Valencia) (Valencia) Spain

1 Resumen

DELLA proteins are transcriptional regulators which have been shown to modulate the activity of over 100 transcription factors in Arabidopsis that are involved in multiple physiological and developmental processes. It has been proposed that DELLAs transduce environmental information to pre-wired transcriptional circuits because their stability is regulated by gibberellins (GAs), whose homeostasis largely depends on environmental cues. The ability of GAs to promote DELLA degradation has been associated to the emergence of vascular plants. However, DELLA proteins are present also in non-vascular land plants, raising at least two questions: what regulatory properties have DELLAs provided to the transcriptional networks that govern development in land plants, and how has the recruitment of DELLAs by GA signaling affected this regulation. We have addressed these issues with a combination of phylogenetic analyses, in silico comparison of gene co-expression networks in different plant species, and a preliminary analysis of DELLA functions in non-vascular plants using an emerging model, the liverwort Marchantia polymorpha. Our results indicate that DELLAs have provided the capacity to coordinate independent transcriptional responses, and suggest that the ancestral DELLA may have already developed important roles in the control of development and stress responses in the common ancestor of all land plants.
ROOM: SALA CAPRI+ MEDES

Session 4. Mineral Nutrition and Water Relations

C0184 NITROGEN SOURCE AFFECTS SULFUR METABOLISM IN BRASSICACEAE

Inmaculada Coleto¹, Iraide Bejarano¹, Marlon de la Peña¹, Pedro M. Aparicio-Tejo², Carmen González-Murua³, M. Begoña González-Moro³, Daniel Marino Bilbao³

¹Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU) (Bizkaia) Spain
²Departamento de Ciencias del Medio Natural, Universidad Pública de Navarra (Navarra) Spain
³Department of Plant Biology and Ecology, University of the Basque Country (UPV/EHU) (Bizkaia) Spain
⁴Daniel Marino Leioa (Bizkaia) España

1 Resumen

The coordination between nitrogen (N) and sulfur (S) metabolism is required to suitably provide plants with organic compounds essential for their development and growth. The N source induces the adaptation of many metabolic processes in plants; however, there is scarce information about the influence it may exert on the interaction between N and S. In this sense, we have studied how the exclusive access to the two most common N sources, nitrate (NO₃⁻) and ammonium (NH₄⁺), affects plant S metabolism in different species of the Brassicaceae family: the model plant Arabidopsis thaliana and two Brassica crops, broccoli (Brassica oleracea) and oilseed rape (Brassica napus). The content of glucosinolates, which are one of the most abundant S-containing compounds in Brassicaceae, accumulated in leaves of the three species when grown with ammonium. Moreover, a proteomic and transcriptomic approach performed in Arabidopsis revealed an important regulation of glucosinolate metabolic process by the N source. The increase of glucosinolate content could be a consequence of the stimulation of S assimilation under ammonium nutrition. Besides, given that glucosinolates are synthesized from amino acids and ammonium-fed plants accure large quantities of amino acids to avoid cytosolic NH₄⁺ accumulation, glucosinolate content increase could be also a way to store N. Besides, a putative glucosinolate role in the signaling processes associated to ammonium nutrition cannot be discarded, since glucosinolates have been suggested as signaling molecules during abiotic stresses. In this context, on-going work includes the use of Arabidopsis mutants in different steps of the glucosinolate pathway. Taken together, the results highlight the impact of the N source on different steps of N and S metabolism and put forward the potential of N source management to modulate the synthesis of compounds with biotechnological interest, such as glucosinolates.
C0243 IDENTIFICATION AND CHARACTERIZATION OF ARABIDOPSIS MUTANTS IN GENES USEFUL FOR PHYTOREMEDIATION

Maria Sanz Fernandez\(^1\), Maria C Romero Puertas\(^1\), Luisa M Sandalio Gonzalez\(^1\), Maria Rodriguez Serrano\(^1\), Ana Sevilla Perez\(^2\), M Dolores Mingorance\(^2\), Liliana Pena\(^3\)

\(^1\)Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC (Granada) España
\(^2\)Instituto Andaluz de Ciencias de la Tierra (UGR_CSIC (Granada) España
\(^3\)Department of Biological Chemistry, University of Buenos Aires and IQUIFIB, CONICET (Buenos Aires) Argentina

1 Resumen

Emissions of heavy metals have risen largely over the past 200 years and significantly exceed those from natural sources for practically all metals. Soil represents the most important sink for anthropogenic and natural heavy metal. Thus soil contaminated with metals pose a major environmental and human health problem that is still in need of an effective technological solution. Phytoremediation is a cost-effective 'green' technology based on the use of metal-accumulating plants to remove pollutants from the environment. Phytoremediation strategies may be able to recover soil productivity in self-sustaining ecosystems; however, there are few descriptions of the molecular mechanisms involved in plant heavy metal perception and signalling.

The aim of this work is to assemble a "molecular tool box" of genes useful for phytoremediation. To identify mutants with different heavy metal-tolerance, we first selected a medium from mixtures containing three metals based on their presence in two Spanish mining areas: Riotinto, highly contaminated and Alquife, moderately contaminated. We have screened about 7,000 lines of Arabidopsis T-DNA mutants and we found 74 lines more resistant and 56 more susceptible than wild type (WT). We have identified all these genes and classified depending on their metabolic function, showing that they were mainly linked to transport, protein modification and signalling, with RNA metabolism being the most representative category in the resistant phenotypes and protein metabolism in the sensitive ones. We have characterized some resistant mutants and one sensitive. These mutants showed differences in growth, oxidative metabolism and metal translocation. Additionally, we found that these mutants keep their phenotype in amended former soils, suggesting that these genes may be useful for phytoremediation and the recovery of contaminated soils.

This study was supported by the Fundación Ramón Areces (http://www.fundacionareces.es) Spain, by ERDF co-financed grant BIO2015-67657-P from MICINN and the Junta de Andalucía (BIO-337).
C0264 MTMOT1.3 IS A PLASMA MEMBRANE MOLYBDENUM TRANSPORTER REQUIRED FOR NITROGENASE ACTIVITY IN MEDICAGO TRUNCATULA ROOT NODULES

Patricia Gil-Diez, Manuel Tejada-Jimenez, Javier Leon-Mediavilla, Jiangqi Wen, Kirankuma S. Mysore, Juan Imperial, Manuel Gonzalez-Guerrero

1Centro de Bioteconologia y Genomica de Plantas (UPM-INIA) Pozuelo de Alarcon (Madrid) España
2The Samuel Roberts Noble Foundation (Oklahoma) USA
3Consejo Superior de Investigaciones Cientificas (Madrid) España

1 Resumen
Nitrogenase, a key enzyme in Symbiotic Nitrogen Fixation (SNF), requires the presence of molybdenum in its active center, as part of the Iron-Molybdenum cofactor. The host plant has to deliver this metal to the bacteroids, inside the root nodules, in order to synthesize the cofactor. In legumes, transporters mediating molybdenum transport have not been identified so far. In model legume Medicago truncatula we have identified a nodule-specific member of molybdate transporter family MOT1(1), MtMOT1.3. Heterologous expression in Saccharomyces cerevisiae reveals that MtMOT1.3 transports molybdate into the cytosol. Immunolocalization studies show that MtMOT1.3 is expressed in the plasma membrane of nodule cells. A MtMOT1.3 knockout mutant exhibited reduced nitrogenase activity and impaired growth, compared to the wild type under symbiotic conditions. The mutant phenotype was restored by reintroducing a functional copy of MtMOT1.3 or increasing molybdate concentrations in the nutritive solution. No differences in phenotype were observed between WT and mot1.3-1 plants when adding an assimilable nitrogen source. These results indicate the important role of MtMOT1.3 in molybdenum homeostasis in M. truncatula nodules.


This work was supported by ERC Starting Grant (ERC-2013-StG-335284).
Session 5. Biotic Stress, Plant-Pathogen Interactions

C0060 G PROTEINS AS CENTRAL COMPONENTS OF THE PLANT IMMUNE SYSTEM

Yuri Trusov, Natsumi Maruta, Maria Nieves Aranda-Sicilia, Urvi Parekh, Jimmy Botella

University of Queensland, (Queensland) Australia

1 Resumen

Heterotrimeric G-proteins (G-proteins), consisting of α, β and γ subunits, are universal signal transducing proteins that, in animals, mediate signaling from G-protein coupled receptors (GPCRs). G-proteins have been known for a long time and most research has been concentrated in humans where they play crucial roles in a multitude of cellular and developmental pathways. It is well established that G-proteins mediate multiple processes in plants, including an important role in immune responses but unlike defence related hormones (e.g. salicylic acid, jasmonic acid) which are involved in specific defence signalling pathways, heterotrimeric GTP-binding proteins provide defence against bacterial, fungal and viral pathogens making them ‘universal’ defence mediators. Until very recently, G protein signalling has been a ‘black box’ as little was known about the members of the signalling pathway or how the pathway was activated. Over the last two years, we and our collaborators have accumulated evidence that plant G-proteins mediate defence transmitting signals from receptor-like kinases instead of the classic GPCRs, as is the case in animal systems. We have now identified a set of defence related receptor like kinases that interact physically and functionally with G proteins. Additionally, we have recently identified a new central regulator within this black box: extra-large G-protein subunits, XLGs, that form unconventional XLG/β/γ heterotrimers and are critical components of the plant immune response.

We will provide data supporting the crucial role for G proteins in plant defense and discuss the role of the individual subunits in the defense response. Finally we will present a mechanistic model for plant G-proteins that does not rely on the accepted animal paradigm using GTP/GDP exchange for activation.
C0110 JAZ2 CONTROLS STOMATA DYNAMICS DURING BACTERIAL INVASION

Selena Gimenez Ibanez1, Marta Boter1, Andres Ortigosa1, Gloria Garcia Casado1, Andrea Chini1, Mathew Lewsey2, Joseph Ecker3, Vardis Ntoukakis4, Roberto Solano1

1Centro Nacional de Biotecnologia CSIC Madrid (Madrid) España
2La Trobe University (Bundoora) Australia
3The Salk Institute for Biological Studies (La Jolla) USA
4University of Warwick (Warwick) UK

1 Resumen

Coronatine (COR) facilita la entrada de bacterias al interior del apoplasto al estimular la apertura de los estomas. Las señales COR-Inducidas en los estomas de guardia permanecen en el aire. Encontramos que el COR y jasmonato isoléucina (JA-Ile) co-receptor JAZ2 está expresado de forma constitutiva en las células de guardia y modula la dinámica de los estomas durante la invasión bacteriana. Analizamos los patrones de expresión de los genes AtJAZ y medimos la apertura de estomas en mutantes de pérdida y ganancia de función. Mutantes jaz2 de Arabidopsis están parcialmente disminuidos en la apertura de estomas inducida por el patógeno y más susceptibles a Pseudomonas. Las mutaciones de ganancia de función en JAZ2 previenen el reabrir los estomas por COR y son altamente resistentes a la penetración bacteriana. El JAZ2 targeta a MYC2, MYC3 y MYC4 para regular la expresión de ANAC19, ANAC55 y ANAC72 para modulación de la apertura de los estomas. Dado que las interacciones antagonistas entre el ácido salicílico (SA) y la defensa de JA, los esfuerzos para aumentar la resistencia a biotróficos resultan en una mayor susceptibilidad a necrotróficos, y vice versa. Notablemente, los mutantes jaz2Δjas son resistentes a P.syringae y mantienen la resistencia no alterada a los necrotróficos. Nuestros resultados demuestran la existencia de un COI1-JAZ2-MYC2,3,4-ANAC19,55,72 módulo responsable por el control de la apertura de los estomas que es jalecado por el COR bacteriano. También proporcionan estrategias novedosas para la protección de la agricultura contra biotróficos sin comprometer la resistencia a necrotróficos.
Symbiosis with arbuscular mycorrhizal fungi (AMF) triggers changes in the plant’s metabolism that allow mycorrhizal plants to defend better against an infection so-called mycorrhizal induced resistance (MIR). Callose (a β-1,3-glucan polysaccharide) is one of the first physical barriers of plant defense against pathogens together with other components of the papillae. We have studied the effect of MIR in tomato plants upon a necrotrophic fungus (*Botrytis cinerea*) infection. Mycorrhizal plants showed higher levels of callose compared with non-mycorrhizal plants following fungal attack. We hypothesize that starch degradation provides sugars available for a faster callose deposition, so we investigated the mechanisms underlying sugar homeostasis by profiling the gene expression of sugar transporters (**SUT**s, **SWEET**s), sucrose synthases (**SUS**s), invertases (**LIN6**), amylases (**BAM1**) and the callose synthase (**GLS5**). Mycorrhizal plants showed an enhanced gene expression of all of them. Thus, the symbiosis may cause changes in the plant sugar metabolism which have an impact in defense by activating starch hydrolysis that generates free monosaccharides that can be used in the callose deposition by the callose synthase **PMR4**. Vesicular trafficking was suggested to participate in the callose deposition in Arabidopsis. The callose synthase and monosaccharides are transported by actin-dependent vesicle trafficking, then the SNARE complex mediates the fusion of these vesicles to the plasma membrane. In our study, we tested whether this process can be stimulated by symbiosis. The gene expression of **SYP121** and **ATL31**, encoding two Q-SNARE proteins relevant in callose deposition, have been investigated during MIR. Concluding, our goal is to decipher the mechanism of callose deposition during MIR.
C0092 PHOTOSYNTHESIS AND PHENYLALANINE AMMONIA LYASE ACTIVITY IN GRAPE BERRY (VITIS VINIFERA)

Andreia Garrido¹, Diana Pimentel², João Serôdio², Ana Cunha⁴

¹University of Minho, Department of Biology, School of Sciences Braga Portugal
²Centre for the Research and Technology of Agro-Environmental and Biological Sciences – CITAB, Department of Biology, University of Minho, Campus de Gualtar, Braga Portugal
³Centre for Environmental and Marine Studies - CESAM, Department of Biology, University of Aveiro Campus de Santiago, Aveiro Portugal
⁴Centre for the Research and Technology of Agro-Environmental and Biological Sciences – CITAB, Department of Biology and Centre of Biological Engineering – CEB, University of Minho, Campus de Gualtar, Braga Portugal

1 Resumen

Grape berries (Vitis vinifera L.) exhibit photosynthetic activity during its developmental phase. This activity is particularly relevant in exocarp, perivascular tissues and seed teguments and up to véraison. The putative effect(s) of fruit photosynthesis are not yet clear, but studies suggest that it may contribute to the production of organic compounds, important in the metabolism and development of the fruit and seed. The climatic conditions of the growing region, such as light, radiation, temperature and soil water availability, have an impact on foliar photosynthesis. In addition, the structure of the vine canopy influences the area and time of light exposure of the clusters affecting overall fruit photosynthetic activity. Both disturbances (climatic and canopy management) have an effect on fruit physiology, namely on the synthesis and accumulation of various compounds of primary and secondary metabolism, and consequently on grape berry development and quality. The main objective of this work was to study the influence of light intensity of the fruit growing microenvironment in the photosynthetic activity tissues (exocarp and seed teguments) throughout its development and how this function is related with the secondary metabolism of the berry. In the vineyard, three canopy microenvironments, from low (LL), medium (ML) and high light (HL) were selected and the clusters were sampled from young green to mature stages. The photosynthesis of the exocarp and seed teguments was analyzed by pulse amplitude modulated (PAM) imaging fluorometry. Optimization assays allowed defining a protocol for the determination of phenylalanine ammonia lyase (PAL) enzyme activity of grape berry isolated tissues. The results revealed a relationship between photosynthetic and PAL activities consistent with a role of fruit photosynthesis in seed secondary metabolism.
C0252 RESPONSES OF REPRODUCTIVE TISSUES TO VEGETATION PROXIMITY IN ARABIDOPSIS THALIANA.

Irma Roig-Villanova¹, Estefanía López-Ortiz¹, Jaume Martínez-Garcia Villanova²

¹Centre for Research in Agricultural Genomics, CRAG Cerdanyola del Vallès (Barcelona) España
²CRAG e ICREA (Barcelona) España

1 Resumen
The yield and quality of fruits, the edible part of many crops, are of key importance in agriculture. In the model plant Arabidopsis thaliana we define yield as the final amount of seeds per plant, a parameter determined by environmental and genetic factors that affect both plant architecture and ovule and seed development. One of these affecting environmental factors is vegetation proximity.

In high plant density, such as in orchards, plants sense the proximity of competing vegetation as a change in light quality, i.e., a reduced red (R) to far-red (FR) light ratio (R:FR). In many plant species this signal triggers a set of responses (known as the “shade avoidance responses”), aimed to “escape” from shade. Shade responses in seedlings, such as the induction of the hypocotyl elongation, have been extensively studied. In the stage of adult rosettes, plants also display a broad range of responses to shade, including decreased branching, accelerated flowering, and thus a general altered seed set and production. However, little is known about how vegetation proximity affects and regulates seed production once flowering is induced and the architecture of the adult plant is established. We have characterized the morphological changes in the reproductive tissues of Arabidopsis plants in response to shade related to ovule and seed development. To identify genetic components regulating these responses, we looked for (i) genes differentially regulated by shade in these tissues, analysing candidate genes by real-time and using whole genome transcriptome approaches; and (ii) factors that participate in this regulatory network by using genetics. Up to now, we have identified some factors controlling the shade avoidance responses in both seedlings and reproductive tissues, and others that are specific of this later developmental phase. Our lastest advances will be presented.
C0322 MAIZE BHLH128 TRANSCRIPTION FACTOR ACTIVATES ZMC4-NADP-ME GENE EXPRESSION

Ana Rita Borba1, Paulo Gouveia1, Alicja Górska1, Tânia Serra1, Isabel A. Abreu1, M. Margarida Oliveira1, Julian M. Hibberd2, Nelson J. M. Saibo1

1Instituto de Tecnologia Química e Biológica – Universidade Nova de Lisboa Oeiras Portugal
2Department of Plant Sciences, University of Cambridge (Cambridge) UK

1 Resumen

Many of the world’s most productive crops are C4 plants. The key innovation in C4 photosynthesis (compared with the ancestral C3) is the carbon concentrating mechanism, which pumps CO2 from the mesophyll cells into bundle sheath where RuBisCO is located. C4 metabolism is accomplished through cell-type specific accumulation of enzymes, transporters and regulators. However, the cis-elements and transcription factor regulatory networks controlling C4 photosynthesis remain largely unknown. Despite more than one thousand transcription factors have been predicted in silico to be related to C4 in some respect, all of them lack experimental validation. In order to identify maize transcription factors regulating the gene encoding the maize C4 NADP-ME enzyme and the cis-elements to which they bind, we used the Yeast One-Hybrid system and Electrophoretic Mobility Shift Assays. We identified and functionally characterized two paralog transcription factors, ZmbHLH128 and ZmbHLH129, that bind to the ZmC4-NADP-ME core promoter. Among several bHLH cis-element motifs present in this promoter, we identified two that facilitate the binding of these transcription factors. In addition, when these two cis-elements were analysed in NADP-ME gene promoters of C3 and C4 representative species, we observed a clear evolutionary pattern, indicating that these bHLH cis-elements might have been involved in the C3 to C4 evolution. We also showed that the ZmbHLH128 act as a transcriptional activator of the target gene ZmC4-NADP-ME and that its transcriptional activity is changed upon heterodimerization with its paralog ZmbHLH129. This work contributes for the characterization of the C4 photosynthetic pathway. As far as we are aware, ZmbHLH128 and ZmbHLH129 are the first transcription factors experimentally validated.
Room: GRAN SALÓN CATALUNYA
15:00-16:45

Session 7. Metabolism and Biochemistry

C0177 Study of the Phosphorylated Pathway of Serine Biosynthesis Unveils Its Connection to Sulfate and Ammonium Assimilation Pathways

Armand D. Anoman¹, María Flores Tornero², Sara Rosa Téllez¹, Jesús Muñoz Bertomeu², Stephan Krueger³, Saleh Alseekh⁴, Alisdair Fernie⁴, Juan Segura¹, Roc Ros¹

¹ERI de Biotecnologia i Biomedicina, Departament de Biologia Vegetal, Facultat de Farmàcia, Universitat de València, Burjassot, (Valencia) Spain
²Departament de Biologia Vegetal, Facultat de Farmàcia, Universitat de València, Burjassot, (Valencia) Spain
³Botanical Institute II, Cologne Biocenter, University of Cologne, (Cologne) Germany
⁴Max Planck Institut für Molekulare Pflanzenphysiologie, (Potsdam-Golm) Germany

1 Resumen

In plants, L-serine (Ser) is synthesized through at least three pathways: the glycolate pathway, which is associated with photorespiration, the glycerate pathway, and the phosphorylated pathway (PPSB). Plant PPSB synthesizes L-Ser from 3-phosphoglycerate (3-PGA) in the plastids in a three-sequential reactions catalyzed by 3-PGA dehydrogenase (PGDH), 3-phosphoserine aminotransferase (PSAT), and 3-phosphoserine phosphatase (PSP). The precursor of PPSB, 3-PGA, is oxidized by PGDH utilizing NAD⁺ as cofactor to form 3-phosphohydroxypyruvate, which is converted to 3-phosphoserine by PSAT in a transamination reaction using L-glutamate as an amino donor and liberating 2-oxoglutarate. The last step is the conversion of 3-phosphoserine into Ser in a reaction catalyzed by PSP. In order to study the PPSB, we targeted genes coding for the first and last enzymes of the pathway. PSP mutants were lethal, as were mutant of PGDH, the most important isoform of PGDH. A transcriptomic analysis of PSP conditional mutants revealed alteration of genes associated with sulfate and ammonium assimilation under non-inducing conditions. Metabolites associated with ammonia assimilation like asparagine and glutamine were more abundant in mutants than in control plants. Besides PSP mutants showed elevated ammonia levels in roots. Since plastids are the site of the primary ammonium assimilation, these results led to the hypothesis that 2-oxoglutarate, produced by PSAT and required by GOGAT to generate two molecules of glutamate from glutamine, was necessary for a proper functioning of the GS/GOGAT pathway. We also hypothesized that Ser deficiency in the plastid, as the precursor of O-acetyl-serine required for sulfur assimilation, affects sulfur metabolism. PSP mutants displayed lower level of sulfur-containing metabolites than controls, which corroborated our hypothesis.

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PGI1 is an important determinant of seed yield in Arabidopsis

Javier Pozueta Romero1, Goizeder Almagro1, Francisco José Muñoz1, Ángela María Sánchez López1, Kinia Ameztoy1, Marouane Baslam1, Ignacio Ezquer1, M. Carmen Sampedro1, Ramón José Barrio2, Nuria De Diego5, Edurne Baroja Fernández1, Abdellatif Bahaji1

1Instituto de Agrobiotecnologia (CSIC, UPNA, Gobierno de Navarra) Mutilva (Navarra) España
2Dipartimento di BioScienze, Università degli Studi di Milano (Milan) Italia
3Central Service of Analysis of Alava, SGIker, University of the Basque Country, UPV/EHU, Vitoria (Álava) España
4Department of Analytical Chemistry, Faculty of Pharmacy, University of the Basque Country, UPV/EHU, Vitoria (Álava) España
5Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University (Olomouc) Czech Republic

1 Resumen
Phosphoglucose isomerase (PGI) catalyzes the reversible isomerization of fructose-6-phosphate and glucose-6-phosphate (G6P). It is involved in glycolysis and in the regeneration of G6P molecules in the oxidative pentose phosphate pathway (OPPP). The plastid-localized phosphoglucose isomerase (PGI) isoform, PGI1, is an important determinant of growth and photosynthesis in Arabidopsis likely as a consequence of its involvement in the synthesis of plastid-localized isoprenoids and derived compounds (Bahaji et al. 2015, PLoS ONE. DOI:10.1371/journal.pone.0119641; Sánchez-López et al. 2016, Plant Physiol. 172: 1989-2001). In this work we investigated the contribution made by PGI1 to seed yield and oil synthesis. Gene expression analyses showed that PGI1 is strongly expressed in vascular tissues of roots, leaves, cotyledons and shoots, and maturing seed embryos. The seed yield of PGI1-null pgi1-2 plants was only ca. 30% that of wild type (WT) plants. Silique number per plant in pgi1-2 was strongly reduced when compared with WT plants as a consequence of a diminution in the number of inflorescences, a phenotype that could be rescued by exogenous gibberellin (GA) application. pgi1-2 seeds showed reduced seed weight and accumulated only ca. 50% of the WT fatty acid (FA) content, causing a wrinkled seed phenotype that could be restored to WT by ectopic expression of PGI1. Moreover, pgi1-2 seedlings showed reduced frequency of establishment, which could be rescued by provision of exogenous sucrose. Seeds from crosses between pgi1-2 and WT plants accumulated WT levels of FA, strongly indicating that the low seed oil content phenotype of pgi1-2 is zygotically determined. Our results provide evidence that PGI1 is an important determinant of Arabidopsis seed yield as a consequence of its involvement in two independent processes: theformation of inflorescences through GA action, and the metabolic conversion of plastidic glucose-6-phosphate into FA.
Caffeoyl coenzyme A 3-O-methyltransferase (CCoAOMT) and caffeic acid-O-methyltransferase (COMT) are key enzymes in the biosynthesis of coniferyl and sinapyl alcohols, the precursors of guaiacyl (G) and syringyl (S) lignin subunits. The function of these enzymes was characterized in single and double mutant maize plants.

In this work, we determined that the comt (brown-midrib 3) mutant plants display a reduction of the flavonolignin unit derived from tricin (a dimethylated flavone), demonstrating that COMT is a key enzyme involved in the synthesis of this compound. In contrast, the ccoaomt1 mutants display a wild-type amount of tricin, suggesting that CCoAOMT1 is not essential for the synthesis of this compound. Based on our data, we suggest that CCoAOMT1 is involved in lignin biosynthesis at least in midribs. The phenotype of ccoaomt1 mutant plants displays no alterations, and their lignin content and composition remain unchanged. On the other hand, the ccoaomt1 comt mutant displays phenotypic and lignin alterations similar to those already described for the comt mutant. Although stems from the three mutants display a similar increase of hemicelluloses, the effect on cell wall degradability varies, the cell walls of ccoaomt1 being the most degradable. This suggests that the “positive” effect of lignin reduction on cell wall degradability of comt and ccoaomt1 comt mutants is counteracted by “negative” changes occurring in lignin composition, such as the decreased S/G ratio. Finally, the role of the flavonolignin unit derived from tricin in cell wall degradability is also discussed.
C0117 A MECHANISTIC APPROACH TOWARDS THE RESTORATION OF HEAVY METAL POLLUTED ANDALUSIAN SALT MARSHES: SPARTINA MARITIMA ECOPHYSIOLOGICAL RESPONSE TO RHIZOBACTERIAL INOCULATION

Jennifer Mesa Marín¹, Ignacio David Rodríguez Llorente², Eloisa Pajuelo Dominguez², Miguel Angel Caviedes Formento², Susana Redondo Gomez¹, Enrique Mateos Naranjo¹

¹Dpto. Biología Vegetal y Ecología, Facultad de Biología, Campus de Reina Mercedes Sevilla (Sevilla) España
²Dpto. Microbiología y Parasitología, Facultad de Farmacia (Sevilla) España

Heavy metal pollution is a major environmental problem. The joint estuary of Tinto and Odiel rivers in Spain is one of the most polluted areas by heavy metals in the world. It drains the region of the world’s oldest continuously operating mine and the area is strongly industrialized. This Mediterranean salt marsh – type ecosystem is protected by provincial and state policy plans and needs restorative intervention urgently. In this regard, the indigenous C₄ halophyte Spartina maritima grows in these estuaries showing a natural metal bioaccumulation capacity, making it useful for phytostabilization of estuarine sediments. Within this scenario, plant – soil bacteria interactions come under the spotlight, since inoculation with autochthonous plant growth promoting rhizobacteria (PGPR) may improve S. maritima growth and metal uptake.

Cultivable bacteria were isolated from the rhizosphere of S. maritima in polluted estuaries and a bacterial inoculant was designed using the best-performing strains. The efficiency of the proposed strategy was tested under greenhouse conditions with natural plants and soils. The ecophysiological response of S. maritima after PGPR inoculation was analyzed.

Inoculated S. maritima plants growing in heavy metal polluted sediments increased their belowground biomass and root metal uptake was stimulated up to 20% for As, 25% for Zn or 50% for Cu. Bacterial inoculation lowered in vivo root respiration, mainly via alternative oxidase pathway (AOP), and reduced the activity of antioxidant enzymes, whereas favouring photosynthetic metabolism, which explained the increased root biomass formation and heavy metal rhizoaccumulation.

This plant-microbe mechanistic approach may be highly relevant in heavy metal hyperaccumulator plants like S. maritima, given their biotechnological potential in environmental decontamination. On the basis of these results, the inoculation of S. maritima with indigenous metal-resistant PGPR may be used as an efficient method to face current abiotic stress challenges and preserve endangered ecosystems.
Resumen

In the recent decades the lands subjected to drought has been increased. Thereby, forest trees not adapted to drought are facing most commonly drought periods. In this sense, *Populus tremuloides* is a drought sensitive tree. However the symbiosis establishment with ectomycorrhizal fungi (EMF) like *Laccaria bicolor* may enhance *P. tremuloides* drought tolerance. At the same time, drought tolerance may be affected by different regulation of aquaporins expression and root hydraulic properties, which both can be regulated by plant hormones. Moreover, it is not known if the responses of trees to EMF are local or systemic. Therefore, the objective of the present study was to analyze if the response of *P. tremuloides* to *L. bicolor* in terms of root hydraulic properties, and hormone content were local or systemic under well watered, drought or partial drought conditions by means of divided root system. Results showed that root hydraulic conductivity increased under drought conditions only when both root sides were inoculated, similar effects were observed for stomatal conductance and leaf water potential. Root cytokines decreased locally by drought stress independently of EMF inoculation, while the contrary (an increase caused by drought) was observed for abscisic acid. Curiously, salicylic acid levels increased by drought, but mostly when EMF was presented. Finally, the expressions of several plant aquaporins were up-regulated by drought and others down regulated by EM symbiosis. It is concluded that EM symbiosis regulated systematically root hydraulic properties of *P. tremuloides* trees, especially aquaporin expression, but hormones content are not involved in such process.
A STABLE EPIGENETIC MEMORY OF TEMPERATURE DURING EMBRYOGENESIS INFLUENCES TIMING OF BUD BURST TIMING IN CLONAL EPITYPE TREES OF PICEA ABIES THROUGH MODULATION OF THE TRANSCRIPTOME

Marcos Viejo Somoano¹, Elena Carneros³, Hugh Cross¹, Igor Yakovlev², Carl Gunnar Fossdal², Jorunn E. Olsen³

¹Marcos Viejo Ás (Akershus) Noruega
²NIBIO (Akershus) Noruega
³NMBU (Akershus) Noruega

1 Resumen

Bud burst timing in P. abies is a trait affected by the temperature conditions during zygotic embryogenesis through the establishment of a long-lasting epigenetic memory. Similarly, during somatic embryo production different temperatures lay down an epigenetic memory generating warm (WE) and cold epitypes (CE) which display different timing of autumnal bud set in the regenerated seedlings. In spite of the accumulated knowledge, little is known about the persistence of the epigenetic memory from a molecular perspective and its reflection in the later phenology of the epitype trees. To shed light on this, detailed spring bud burst stages were monitored in 15-year old clonal WE and CE individuals growing in field in Ås, Norway (69°N latitude). Furthermore, a targeted transcriptomic analysis of microdissected mother cells and leaf primordia in addition to whole bud sections from apical buds from the first whorl was conducted on materials harvested in early spring several weeks before bud burst. The analysis of phenology not only showed differential timing of bud swelling and bud burst in the epitypes with the CE being advanced development relative to the WE, but also confirmed a long-lasting effect of the epigenetic memory. The transcriptomic data and Gene Ontology terms showed distinct cell-type upregulation of transcripts related to light and temperature signalling in the CE. Besides, integration of external and internal cues through modulation of chromatin and thus transcription is likely cell-type specific for genes involved in DNA methylation and histone modifications (H3K9me, H3K27me or H3S10p). Consistent with advanced preparation for bud burst, the CE showed upregulation of cell cycle-related genes whereas hormone signalling pathways showed cell type-specific upregulation in the WE, probably reflecting its delayed bud burst-preparation. These results demonstrates that the epigenetic memory established during embryogenesis leads to differential bud burst timing in the epitypes through cell type-specific transcriptomes.
THURSDAY, 29TH JUNE
ROOM: GRAN SALÓN DE CATALUNYA
10:00-11:45

Session 9. Abiotic Stress

C0129 SILENCING BARLEY CYSTATINS HVCPI-2 AND HVCPI-4 SPECIFICALLY MODIFIES PLANT RESPONSES TO DROUGHT

Blanca Velasco Arroyo¹, Mercedes Díaz Mendoza¹, Andrea Gómez Sánchez¹, Beatriz Moreno García¹, Maria Estrella Santamaría¹, Miguel Torija Bonilla¹, Goetz Hensel², Jochen Kumlehn², Manuel Martínez¹, Isabel Díaž²

¹Centro de Biotecnología y Genómica de Plantas (CBGP) Pozuelo de Alarcon (Madrid) España
²IPK (Stadt Seeland) Alemania

1 Resumen
The cooperative role of cystatins and their functional relationship with cysteine proteases along stress episodes has been highlighted by the enhanced/reduced tolerance to drought of barley plants silencing phytocystatins. The altered sensitivity seems to be a consequence, at least in part, of adjustments over endogenous developmental leaf senescence programs. Proteolysis of plastidial components as a main source of N and other relevant nutrients, and the subsequent transport of the hydrolyzed fragments from the source leaves to the developing grains, represent the key hallmarks associated with leaf senescence and abiotic stresses in cereals. Therefore, the greater the efficiency along the process, the better the yield and plant survival. Two barley phytocystatins, HvCPI-2 and HvCPI-4, were specifically induced after water deprivation. These two members are responsible for the inhibition of cysteine proteases from the families C1A and/or C1A/C13. Cysteine proteases are largely involved in protein degradation along leaf senescence and stress, among other processes, as it has been displayed in several plant species. To assess the roles of the abovementioned cystatins in planta, artificial microRNA lines independently silencing the two members were generated. Molecular, biochemical and metabolomics data showed up specific modifications regarding protein and pigment accumulation as well as differential metabolite quantity and composition. Interestingly, detected alterations in the proteolytic patterns were concomitant with modifications in the expression of some target proteases. Strikingly, an accelerated leaf senescence or a “stay green”-like phenotype, depending on the silenced cystatin, was exhibited either under natural or drought-induced leaf senescence. Reported data and observations support a promising use of these plants to specifically modulate cereal responses facing abiotic stress. The main goal points towards the maintenance of the stability in the crop production or even the improvement under the evinced climate change framework and the imminent growth of the world population.
Recent work has revealed that ABA receptor proteins, e.g. PYR1, PYL4 and PYL8, can be degraded via an ubiquitination-dependent mechanism through single subunit and CUL4-based E3 ligases (Bueso et al., 2014; Irigoyen et al., 2014; Belda-Palazón et al., 2016). In particular, we have identified a 10-member family of single-subunit E3 ubiquitin ligases, named RFA for RING finger ABA-related, acting as E3 ligases of the PYR/PYL/RCAR ABA receptors and therefore controlling their ubiquitination and half-life. The RFA family is different from the CULLIN4-RING E3 ubiquitin ligase (CRL4) complex that interacts with ABA receptors through the substrate adapter DDB1-ASSOCIATED1 (Irigoyen et al., 2014). RFAs are structurally characterized by the presence of three putative RING domains in tandem (Bueso et al., 2014), i.e. a canonical RING domain containing a C6HC zinc finger flanked by two RING finger-like domains and according to this structure they belong to RBR (RING between RING fingers) E3 ligases (Marin et al., 2010). We provide evidence that RFA4 interacts with ABA receptors in the nucleus and promotes their ubiquitination in vitro and their degradation in vivo. Additionally, we have identified UBC26 as the cognate nuclear E2 interacting with the RFA4 E3 ligase. Therefore, the concerted action of UBC26/RFA4 regulates half-life of ABA receptors in the nucleus. Altogether, our results reveal a sophisticated targeting of ABA receptors at different subcellular locations, which involves at least the single-subunit RBR E3 ligases and the multiple-subunit CRL4 complex.
C0143 EFFECTS OF MANGANESE TOXICITY ON THE PROTEIN PROFILES OF TOMATO (SOLANUM LYCOPERSICUM) ROOTS USING TWO PROTEOMIC APPROACHES

Laura Ceballos Laita1, Hiroyuki Imai2, Matsuo Uemura3, Anunciación Abadía Bayona1, Javier Abadía Bayona1, Ana Flor López Millán1

1Estación Experimental Aula Dei (CSIC) Zaragoza (Zaragoza) Spain
2United Graduate School of Agricultural Sciences (Iwate University) (Morioka) Japan
3Cryobiofrontier Research Center (Iwate University) (Morioka) Japan

1 Resumen
Manganese (Mn) is a cofactor in several enzymes involved in many physiological and biochemical processes, and is necessary for plant growth and development. However, an excess of this metal, usually occurring in acidic soils, becomes toxic for plants and animals. Proteomic approaches can be useful to elucidate the effects of Mn-toxicity on plant metabolism. The aim of this work was to assess the effects of Mn toxicity on the tomato root protein profile using two proteomic approaches: mass spectrometry-based shotgun analyses and two-dimensional electrophoresis (2-DE). Roots were harvested eight days after treatment onset and frozen in liquid N₂. Proteins were precipitated with 0.1 M ammonium acetate and analyzed by label-free nanoLC-ESI-MS/MS (2.5 µg) using a Michrom Advance UHPLC with a Thermo LTQ Orbitrap XL and by 2-DE (80 µg) using a miniprotean and a nHPLC system 1200 series connected to a HCT Ultra high-capacity ion trap. To assess the effects of Mn toxicity, the ratio of normalized abundance between Mn-toxicity and control samples was calculated. Proteins were identified (at least two peptides matched) using the NCBI database. Only proteins showing statistically significant (p ≤ 0.05; ANOVA) and biologically relevant (fold ratios ≥ 2 or ≤ 0.5) changes were considered. The thresholds described for protein identification were met by 136 proteins and 87 spots in shotgun analysis and 2-DE, respectively. More than 60% of the root proteins changing in relative abundance as a result of Mn excess decreased when compared to the control plants. The functional category most affected by Mn excess was stress followed by protein metabolism. Manganese toxicity led to decreases in all root proteins involved in protein metabolism and signaling. The comprehensive qualitative and quantitative analyses of the relative changes induced by Mn toxicity in the root proteome will help elucidating the mechanisms involved in the response to this nutrient stress.
C0077 HORMONAL HOMEOSTASIS REGULATES ADVENTITIOUS ROOT FORMATION IN CARNATION CUTTINGS

José Manuel Pérez-Pérez¹, Antonio Cano², Ana Belén Sánchez-García¹, Sergio Ibáñez¹, Alfonso Albacete³, Rebeca González-Bayón¹, Manuel Acosta²

¹Instituto de Bioingeniería, Universidad Miguel Hernández de Elche (Alicante) Spain
²Departamento de Biología Vegetal (Fisiología Vegetal), Universidad de Murcia (Murcia) Spain
³Departamento de Nutrición Vegetal, CEBAS-CSIC (Murcia) Spain

1 Resumen

Carnation is, after rose, the most important species on the worldwide market of cut flowers. The production of young plantlets is frequently hampered by minimal adventitious root (AR) formation from stem cuttings, which has a strong genetic dependency and which leads to production losses in certain carnation cultivars.¹,²

By whole transcriptome sequencing and metabolite profiling, we identified the genes and hormonal pathways involved in AR formation in two carnation cultivars with contrasting rooting efficiencies. Our results indicate that active polar auxin transport through the stem leads to a localized auxin gradient in the stem cutting base required for AR formation. Quantitative histology allowed us to define the cellular dynamics during the early stages of AR initiation. Differences in biosynthesis, transport and conjugation of auxin levels in the stem cutting base during the first hours after excision accounts for the differences observed in AR formation between cultivars with contrasting rooting efficiencies.

Our findings will provide tools to monitor adventitious rooting in a wide collection of carnation germplasm and to select the best-rooting cultivars for breeding purposes.

References:

¹Agulló-Antón et al. (2014) Phisiol. Plant. 150: 442
²Birlanga et al. (2015) PLOS ONE 10: e0133123

Work funded by MINECO/FEDER (AGL2012-33610 and BIO2015-64255-R)
C0195 IMPROVEMENT OF MICROSPORE EMBRYOGENESIS INDUCTION AND REDUCTION OF CELL DEATH BY AUTOPHAGY AND CYSTEIN PROTEASES INHIBITORS IN BARLEY

Ivett Bárány1, María Teresa Solís1, Eduardo Berenguer1, Yolanda Pérez-Pérez1, Estrella Santamaría2, Isabel Díaz2, María-Carmen Risueño1, Pilar Sanchez Testillano1

1Biological Research Center, CIB-CSIC (Madrid) Spain
2Center of Plant Biotechnology and Genomics, CBGP, UPM-INIA (Madrid) Spain

1 Resumen

The microspore, at the vacuolated stage, can be reprogrammed in vitro by stress treatments, becoming a totipotent cell and producing doubled-haploid embryos and plants, very useful in plant breeding, via microspore embryogenesis. The efficiency of the process is limited by the occurrence of cell death after the inductive stress.

In plants, papain-like C1A Cys-proteases or cathepsins are the most abundant enzymes with proteolytic activity, with a prominent role in plant senescence and PCD events. Autophagy is a degradation pathway that recycles cell materials upon stress conditions or during specific developmental processes. Together with a survival role, autophagy can play critical roles as cell death initiator and/or executioner.

In this work we have studied the activation and possible involvement of cathepsins and autophagy in cell death occurrence during stress-induced microspore embryogenesis of Hordeum vulgare (barley). In isolated microspore cultures, application of a stress treatment to induce embryogenesis lead to high levels of cell death, ROS production and increased activities of cystein proteases like cathepsins L, B, H and caspase 3-like proteases, as well as up-regulation of several autophagy (Atgs) and cathepsin genes. Concomitantly, autophagosomes, revealed by MDCV, also increased. Treatments with ROS scavengers, inhibitors of autophagy, caspase-3 and cathepsin proteases activities reduced cell death levels and increased embryogenesis induction rate in microspore cultures. Taken together, results indicate that autophagy is activated in stress-treated microspores suggesting autophagy plays a role in cell death at early stages of stress-induced microspore embryogenesis.

Work supported by project grant AGL2014-52028-R of the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER).
C0311 LONG DURATION DRYING CYCLES DURING PARTIAL ROOTZONE IRRIGATION IMPROVES PLANT-WATER TRANSPORT BY INCREASING ROOT BIOMASS OF LEMON TREES

Juan Gabriel Pérez-Pérez1, Juan Miguel Robles García1, Ana Belén Mira García1, Pablo Botía Ordaz1, Ian C Dodd2, Carlos de Ollas3, Aurelio Gómez-Cadenas3

1Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) La Alberca (Murcia) España
2Lancaster University (Lancashire) United Kingdom
3Universitat Jaume I (Castellón) España

1 Resumen

Partial rootzone irrigation (PRI) is an irrigation technique that spatially discriminates distinct drying and wetting parts of the rootzone, which periodically alternates irrigation placement to induce several plant physiological changes. This study evaluated the impacts of applying long drying cycles (28 days) of PRI compared to conventional deficit irrigated (RDI) on field-grown adult lemon (Citrus limon (L.) Burm. fil.) trees grown in a semiarid climate. Three irrigation treatments were established: Control (100% ETc), PRI and RDI. In both deficit irrigated treatments, the irrigation water volume applied was 75% ETc (from middle of April to the beginning of August) and 100% ETc for the rest of the season. In PRI trees, the wet and dry parts of the rootzone were alternated every 28 days. During the application of the PRI cycles, fewer roots actively took water, decreasing trunk sap flow in PRI trees compared with conventional drip irrigation (Control and RDI). In PRI plants, roots from the wet side grew faster than those on the dry side in PRI trees, with the net effect being greater root growth in PRI than in Control and RDI trees after a complete wetting/drying cycle. Roots from the dry part of the soil showed higher ABA concentration, whereas IAA concentrations increased in roots exposed to wet soil. These changes in root growth dynamics and phytohormone concentration increased overall root density of PRI trees compared with Control and RDI trees. In turn, this improved the capacity of the root system to take up water from the soil, increasing trunk sap flow after the full irrigation dose was re-established on both sides of the root system in PRI trees.
**FLASH TALKS**

**TUESDAY 27TH JUNE**  
**ROOM: GRAN SALÓN CATALUNYA**  
**11:45-12:30**

**SESSION 1. GROWTH AND DEVELOPMENT**

**C0068 BETA-(1,4)-GALACTAN REMODELLING ALTERS THE CELLULOSE/XYLOGLUCAN NETWORK IN ARABIDOPSIS CELL WALLS**

María Moneo-Sánchez¹, Alejandro Alonso¹, Josefina Hernández-Nistal², Emilia Labrador¹, Berta Dopico Rivela¹, José Ignacio Martín¹

¹Dpto. Botánica y Fisiología Vegetal. CIALE. Universidad de Salamanca (Salamanca) España  
²Dpto. Biología Funcional. Universidad de Santiago de Compostela (Lugo) España

1 Resumen

β-(1,4)-galactan is one of the main side chains of rhamnogalacturonan I. Although the specific function of this polymer has not been completely established, it has been related to different processes, such as cell elongation, fiber differentiation or fruit ripening, and seems to regulate the mechanical properties of cell walls [1].

The main objective of this work is to deepen in the function of this pectic polysaccharide by studying the alterations caused in Arabidopsis cell walls when β-(1,4)-galactan content is reduced or in excess. For this purpose, we have used two different approaches. We have obtained a double Arabidopsis loss-of-function mutant for BGAL1 and BGAL3 β-galactosidases (bgal1/bgal3) to increase galactan levels. Also, transgenic Arabidopsis plants overexpressing chickpea CarBGal1 (coding for βI-Gal β-galactosidase) under 35S CaMV promoter (35S::βI-Gal) have been generated, expecting a reduction of galactan side chains.

The studies on the substrate specificity of these three β-galactosidases has confirmed their action on β-(1,4)-galactan in vitro, a fact that is also observed in muro after a detailed study of bgal1/bgal3 and 35S::βI-Gal cell walls, both by immunolocation assays and the analysis of the different fractions (water, CDTA and KOH) extracted from the alcohol insoluble residue by epitope detection chromatography.

The role of galactan in cell elongation has been confirmed in bgal1/bgal3 plants, which show reduced growth in elongating organs, such as apical internodes or young hypocotyls. Furthermore, our results point to a crucial role of galactan in the maintenance of the cell wall architecture, since alterations of this polymer vary the levels of KOH-extracted xyloglucan(decreased in 35S::βI-Gal plants and enhanced in bgal1/bgal3), and are conditioning the interactions not only between pectins and other polysaccharides, but also between hemicelluloses and cellulose.

C0164 FUNCTIONAL ANALYSIS OF TGA GROUP OF BASIC LEUCINE ZIPPER TRANSCRIPTION FACTORS IN NITRIC OXIDE SENSING

Maria Guadalupe Fernandez Espinosa, Oscar Lorenzo
University Of Salamanca (Salamanca) Spain

1 Resumen
Corresponding author: O Lorenzo (oslo@usal.es)

Nitric oxide (NO) is a ubiquitous signaling molecule involved in many plant developmental processes\textsuperscript{1,2}. One way of NO sensing is the post-translational modification of key proteins affecting their activity and function through S-nitrosylation. The identification of targets is crucial for understanding cellular redox-regulation and the physiological role of NO in plants\textsuperscript{3}. Among them, the Arabidopsis TGA family of basic leucine zipper transcription factors regulates the expression of pathogenesis-related genes and is required for disease resistance\textsuperscript{4}. Redox regulation of TGA1 has been proposed to involve S-nitrosylation\textsuperscript{5}.

Our previous research highlighted an important role of NO in regulating stem cell decisions\textsuperscript{2}. However, a specific role for the TGA group in root stem cells has not been described. Here, we show the putative involvement of some members of the TGA family in root development and stem cell maintenance and their link with NO. To investigate the mechanism by which NO modulates these processes, firstly, we first evaluated the phenotypes of loss- and gain-of-function TGA lines in the presence and absence of NO. Secondly, sequences of corresponding proteins were subjected to \textit{in silico} analysis for S-nitrosylation site prediction. Finally, \textit{in vitro} S-nitrosylation of a specific Cys residue in a member of TGA group after GSNO treatment, followed by analysis with mass spectrometry (LC-MS/MS), will be presented.

References
\textsuperscript{1} Fernández-Marcos et al. (2011) \textit{PNAS}, 108: 18506.
\textsuperscript{2} Sanz et al. (2014) \textit{Plant physiology}, 166: 1972.
\textsuperscript{4} Kesarwani et al. (2007) \textit{Plant physiology}, 144: 336.

C0176 VOLATILE COMPOUNDS EMITTED BY THE FUNGAL PHYTOPATHOGEN PENICILLIUM AURANTIOGRISEUM PROMOTE CHANGES IN THE ROOT ARCHITECTURE OF ARABIDOPSIS THALIANA THROUGH AUXIN ACTION

Pablo Garcia Gomez¹, Marouane Baslam², Francisco Jose Muñoz³, Angela Maria Sanchez Lopez⁴, Abdellatif Bahaji³, Goizeder Almagro³, Nuria De Diego⁴, Lukáš Spíchal⁴, Karel Doležal⁴, Edurne Baroja Fernandez³, Javier Pozueta Romero³

¹Instituto de Agrobiotecnologia Mutitva (Navarra) España
²Graduate School of Science and Technology and Department of Applied Biological Chemistry (Niigata) Japan
³Instituto de Agrobiotecnología (Navarra) España
⁴Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science (Olomouc) Republica Checa

1 Resumen

It is well known that volatile compounds (VCs) emitted by beneficial rhizosphere bacteria and fungi can promote plant growth. We recently showed that this action is not only restricted to beneficial microorganisms, but extends to pathogens and microbes that do not normally interact mutualistically with plants (Sánchez-López et al. 2016, Plant Cell Environ. 39: 2592-2608; Sánchez-López et al. 2016, Plant Physiol. 172: 1989-2001). In this work we show that VCs emitted by the fungal phytopathogen Penicillium aurantiogriseum not only promote growth and changes in the metabolome of Arabidopsis plants, but also changes in the root architecture that are caused by two main factors: (i) an increase of the length and a burst in the number of root hairs, especially in root tips, and (ii) an increase of the number and an arrest in the growth of lateral roots. These changes suggested that auxins could be involved in the response of Arabidopsis to P. aurantiogriseum VCs. To test this hypothesis we characterized the root architecture of aux1-T, eir1 and tir1 auxin mutants cultured in the absence or presence of VCs emitted by P. aurantiogriseum. These analyses revealed that, unlike the tir1 auxin signaling mutant, the aux1 and eir1 auxin transport mutants do not respond to VCs. Auxin content analyses showed that rosettes of VCs-treated plants accumulate lower levels of auxins than those of VCs non-treated plants. No difference could be found between auxin levels in roots of VCs-treated and non-treated plants. The overall data indicate that changes in the root architecture of Arabidopsis promoted by P. aurantiogriseum VCs could be the consequence of altered distribution of auxins.
C0183 FROM ARABIDOPSIS TO CROPS: FUNCTIONAL CHARACTERIZATION OF STOMATAL DEVELOPMENT GENES FROM SOLANUM AND VITIS

Alfonso Ortega Garrido, Alfonso Ortega Garrido Garrido, Alberto de Marcos Serrano, Jonatan Illescas, Carmen Fenoll Comes, Montaña Mena Marugán

UCLM Campus de Toledo (Toledo) España

1 Resumen
The gene networks controlling the development of stomata from protodermal cells have been described in Arabidopsis, but the information for crops is still scarce. In Arabidopsis, the process is regulated by the interplay of positive, stomata-promoting factors and negative regulators that inhibit stomatal fate. Three of the positive regulators are closely related basic helix-loop-helix (bHLH) transcription factors (SPEECHLESS (SPCH), MUTE and FAMA), which control consecutive stages in stomatal lineage progression. Their activity determine stomatal abundance in mature organs, a parameter known to influence the maximum stomatal pore area available for gas exchange and, therefore, plant performance under different growth conditions. For instance, higher stomatal abundance relates to higher transpiration and photosynthesis, which improves cooling and productivity under heat conditions in irrigated crops. In contrast, lower stomatal abundance optimizes water use efficiency during water shortage.

By phylogenetic analysis we identified the putative orthologues of the three Arabidopsis bHLH-coding stomatal regulator genes in Solanum lycopersicum (SlSPCH, SlMUTE and SlFAMA), and cloned their full-length cDNAs from developing Moneymaker tomato cotyledons. We used these coding regions and their GFP fusions to test whether they complement the corresponding Arabidopsis loss-of-function mutants when expressed with their respective Arabidopsis promoters or with a constitutive, β-estradiol inducible promoter. GFP fusions to the S. lycopersicum promoters were also introduced in wild-type Arabidopsis plants to explore the conservation of cis-regulatory mechanisms. A similar approach has been in Vitis vinifera. Identifying alleles for these genes (by genomic sequencing in different cultivars, by TILLING in mutagenized collections, and by eco-TILLING), or designing specific variants with altered properties conferring beneficial physiological traits will contribute to tomato and grapevine breeding for future climate scenarios.
C0185 POSSIBLE ROLE FOR THE PREFOLDIN COMPLEX IN CHROMATIN REMODELING IN ARABIDOPSIS.

Cristina Marí Carmona, David Esteve Bruna, Noel Blanco Touriñán, Miguel A. Blázquez, David Alabadí

Insto. Biología Molecular y Celular de Plantas. CSIC/IBMCP Valencia (Valencia) España

1 Resumen

Prefoldin (PFD) is an evolutionarily conserved heterohexameric chaperonin that delivers unfolded tubulin to the main cytosolic chaperone CCT (Siebert et al., 2000). This function takes place in the cytosol, but it has been shown that when DELLA proteins are accumulated, PFD changes its localization and moves to the nucleus, impairing tubulin folding and microtubules arrangement (Locascio et al., 2013). This DELLA-dependent localization also opens the possibility –not considered until now- that PFD may also perform a role in the nucleus.

An in silico analysis of the public interactomes from yeast and flies has allowed us to identify numerous putative nuclear interactors for PFD subunits in Arabidopsis. Remarkably, we have demonstrated that some of these interactions are conserved, suggesting new roles for the PFD complex in plants. In particular, the interaction with different subunits of SWR1 complex (a chromatin remodeling complex that exchanges histone 2A with the variant H2A.Z; March-Diaz and Reyes, 2009) suggests that PFD might control gene expression by modulating the deposition of this histone variant, a hypothesis that is being challenged by genomic approaches. Interestingly, the similar early flowering phenotype of mutants in both complexes supports the physiological relevance of this interaction.


C0198 INDUCTION OF AUTOPHAGY, METACASPASE AND CATHEPSIN ACTIVITIES DURING DEVELOPMENTAL PCD OF TAPETUM

Ivette Bárány1, María Teresa Solís1, Eduardo Berenguer1, Elena Minina2, Estrella Santamaria1, José-Luis Crespo4, Isabel Díaz1, Peter Bozhkov2, María-Carmen Risueño1, Pilar Sanchez Testillano1

1Biological Research Center, CIB-CSIC (Madrid) Spain
2Uppsala BioCenter, Swedish University of Agricultural Sciences and Linnean Center for Plant Biology, (Uppsala) Sweden
3Center of Plant Biotechnology and Genomics, CBGP, UPM-INIA (Madrid) Spain
4Inst.Plant Biochemistry and Photosynthesis, IBVF, CSIC (SEVILLA) Spain

1 Resumen
The innermost cell layer of the anther, tapetum, has an important nutritive function and a critical role during pollen development, being involved in the biosynthesis and secretion of pollen wall components. Developmental program of tapetum culminates in programmed cell death (PCD) whose alterations in most cases lead to male sterility. Recent works have revealed significant roles of autophagy in the regulation of plant developmental PCD.

In this work we have studied the activation of autophagy in tapetal PCD in Brassica napus using multidisciplinary approach that involved protease activity assays (for metacaspases, cathepsins and enzymes with caspase 3-like activity), gene expression analysis and protein localization by immunofluorescence and immunogold labeling of Atg5, Atg8 and cathepsins, as well as ultrastructural analysis.

We have found that during early tapetal PCD, ATG5 and ATG8 autophagy-related genes were up-regulated, Atg5 and Atg8 proteins localized to autophagosome-like structures and vacuoles. Interestingly, metacaspase activity was significantly enhanced at early PCD. Electron microscopy analysis revealed formation of autophagosomes and vacuoles. Cathepsin L, B, H and caspase 3-like enzymatic activities were likewise correlated with PCD. Taken together, these results indicate that autophagy and metacaspases could be involved in the initiation of tapetal PCD.

Funding:
Work supported by project grant AGL2014-52028-R of the Spanish Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF/FEDER), and STSM grant to EB of Transautophagy COST action CA15138.
C0210 A ROLE OF THE CIRCADIAN CLOCK REGULATING THE DNA DAMAGE RESPONSE IN ARABIDOPSIS THALIANA

Sergio Gil Rodríguez¹, Paloma Más Martínez²

¹Centre for Research in Agricultural Genomics (CRAG) Cerdanyola del Vallès (Barcelona) España
²Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, 08193-Cerdanyola del Vallès (Barcelona) España

1 Resumen

The circadian clock is responsible for sustaining biological rhythms in many organisms. Specifically in plants, it plays a fundamental role connecting plant physiology and metabolism with predictable environmental changes that occur during the day/night cycles. The circadian clock also plays a key role timing plant responses to stress conditions. Among those, stresses that affect genome integrity are particularly cytotoxic for cell viability, inhibiting cell growth as a result of cell cycle arrest. In our studies, we aimed to uncover the connection between the circadian clock and the DNA Damage Response. To that end, we examined transcriptional changes of plants in which DNA Double Strand Breaks (DSB) were induced by the use of the drug bleomycin. Gene expression analyses by RT-QPCR at different times during the circadian cycle showed that the induction of DNA Damage Response (DDR) genes was gated by the clock. Consistently, analyses of DNA damage using Comet Assays also showed a gated induction of DNA damage. The results were also confirmed by in vivo luminescence assays using promoters of DDR genes fused to the luciferase as a reporter. Altogether, our results suggest an important role of the circadian clock regulating the DNA Damage Response in Arabidopsis thaliana.
C0253 HFR1 AND SHADE TOLERANCE IN CARDAMINE HIRSUTA

Sandi Paulisic¹, Christiane Then¹, Jaume Martínez-García²

¹Centre for Research in Agricultural Genomics (CRAG) Bellaterra (Barcelona) Spain
²CRAG and ICREA (Barcelona) Spain

1 Resumen
Proximity of neighbouring vegetation results in a reduction of red to far-red light ratio, a strong signal for a variety of developmental and growth adaptations of plants. Two basic strategies of response to this shade signal have evolved in plants: avoidance or tolerance. The shade-avoider species Arabidopsis thaliana usually responds by increasing hypocotyl, stem and petiole elongation, and inducing flowering. This is known as the Shade Avoidance Syndrome (SAS). In contrast, the shade-tolerant species Cardamine hirsuta, an Arabidopsis relative, do not elongate hypocotyls in response to shade signal. We are using Cardamine to study the mechanism of shade tolerance.

Genetic screenings in Arabidopsis have found mutants defective in negative SAS regulators because of their exaggerated response to simulated shade compared to the wild-type controls. One of such negative regulators is LONG HYPOCOTYL IN FAR-RED 1 (HFR1), a transcriptional cofactor shown to dimerize with PHYTOCHROME INTERACTING FACTORs (PIFs) and inhibit their DNA-binding activity, thus modulating shade signalling in Arabidopsis. Therefore, we suggested that HFR1 might also repress hypocotyl elongation in Cardamine. To test this hypothesis, we generated and characterized RNAi-HFR1 transgenic Cardamine plants. Hypocotyl assays revealed a significant elongation response of RNAi-HFR1 Cardamine seedlings to shade, associated with an increase in shade marker genes expression. Under this framework, shade tolerance in Cardamine can be achieved with 1) higher intrinsic HFR1 protein activity, 2) higher HFR1 promoter activity, and/or 3) altered shade signalling components connected to HFR1 action (e.g. lower PIF activity). Our latest results to discriminate between these various possibilities will be presented.
C0257 SUPERCENTIPEDE FUNCTION IS EPISTATIC TO β-CYANOALANINE SYNTHASE-MEDIATED CYANIDE DETOXIFICATION IN ARABIDOPSIS ROOT HAIR ELONGATION

Lucía Arenas Alfonseca, Cecilia Gotor, Luis C Romero, Irene García
Instituto de Bioquímica Vegetal y Fotosíntesis Sevilla (Sevilla) España

1 Resumen
In Arabidopsis thaliana, cyanide is produced concomitantly with ethylene biosynthesis and is mainly detoxified by the β-cyanoalanine synthase CAS-C1. In Arabidopsis roots, CAS-C1 activity is essential to maintain a low level of cyanide for proper root hair development. Root hair elongation relies on polarized cell expansion at the growing tip. CAS-C1 locates in mitochondria and accumulates in root hair tips during root hair elongation, as shown by observing the fluorescence in plants transformed with a ProC1:CASC1-GFP construct. On the other hand, the supercentipede (SCN1) protein participates in the pathway aiming to regulate the NADPH oxidase RHD2/AtrbohC, which catalyzes the generation of ROS and the Ca²⁺ gradient at the tip of the root hair that drives root hair elongation. Mutants in the SCN1 gene are affected at the very early steps of the root hair development, producing supernumerary root hair primordia that do not elongate, while the root hairs of the cas-c1 mutant correctly start to grow out but they do not elongate either. Genetic crosses between the cas-c1 mutant and a scn1 mutant demonstrate that scn1 mutation is epistatic to cas-c1, without affecting the CAS-C1 biochemical function. Moreover, our results show that the cyanide effect in root hair elongation is independent of ethylene, H₂O₂ production or direct NADPH oxidase inhibition.
C0047 AGROBACTERIUM-MEDIATED TRANSFORMATION OF EUROPEAN CHESTNUT SOMATIC EMBRYOS WITH A CASTANEA SATIVA (MILL.) ENDOCHITINASE GENE

Laura María Bouza Morcillo, Mª Carmen San-José Capilla, Antonio Ballester Álvarez-Pardiñas, Elena Corredoira Castro

Instituto de Investigaciones Agrobiológicas de Galicia Santiago de Compostela (A Coruña) España

1 Resumen

Chestnut blight, caused by Cryphonectria parasitica, is a severe disease that has devastated chestnut stands in North America and Europe. The use of different classes of pathogenesis-related proteins is a very promising alternative to obtain blight tolerant chestnuts. Chitinases belonging to the PR-3 family of proteins hydrolyze the β-1, 4 glycosidic bonds that link the N-acetylglucosamine residues of chitin and play a direct role in plant defence by hydrolyzing chitin of fungal wall. This report describes a reliable and efficient procedure for the Agrobacterium-mediated transformation of somatic embryos of European chestnut with the endogenous CsCh3 gene that codes for a chitinase. Explants consisting of small clumps of two to three somatic embryos in globular or early-torpedo stages isolated from three chestnut embryogenic lines were co-cultured for 5 days with Agrobacterium tumefaciens strain EHA105 harbouring with pK7WG2-CsCh3 binary vector. Both plasmids contain the neomycin phosphotransferase (nptII) selective gene and the green fluorescent protein (egfp) reporter gene. The transformation efficiency, determined on the basis of the fluorescence of surviving explants, was genotype-dependent. Although somatic embryos of all three lines evaluated were transformed, the best results were obtained with somatic embryos derived from line Cl-9(20%). A total of 88 independent transformed lines were obtained. The presence of transgenes was confirmed by green fluorescent protein (GFP) expression and PCR analysis. The transgenic plants obtained after maturation and germination of somatic embryos were acclimatized in the greenhouse. Fluorescence indicating overexpression of the transgenes was also observed in shoots and leaves. No phenotypic differences were found relative to control plants, suggesting a lack of any cytotoxic effects of the GFP.

Acknowledgements

The authors thank Dr I. Allona and Dr C. Aragoncillo for providing the CsCh3 and CsTL1 genes. This research was partially funded by Ministerio de Economía y Competitividad (Spain) through the project AGL2013-47400-C4-3-R and AGL2016-76143-C4-4-R.
C0069 EPIDEMIC REGULATION OF FLOWERING TIME BY HISTONE DEMETHYLASES

Laura Poza, Jenifer Pozas, Pedro Crevillén Lomas

Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM) - Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) (Madrid) España

1 Resumen

Flowering time is an important agronomic trait with a direct impact on crop yield. Plants control when to flower in response to a number of physiological and environmental cues. A fascinating environmentally induced developmental responses is vernalization: the acceleration of flowering time in response to winter cold. This is an important crop trait for cereals and Brassica cultivars. In all studied species vernalization involves the epigenetic regulation of the local chromatin environment of key floral regulator genes. In Arabidopsis thaliana, vernalization induces epigenetic silencing of the floral repressor FLC by repressive Polycomb histone H3 lysine 27 methylation (H3K27me). However, FLC locus is reactivated (reset) during embryo development to ensure a vernalization requirement every generation. To characterize this resetting process we undertook a genetic screening to identify trans-acting factors required for the epigenetic reprogramming of FLC. We found a resetting mutation that leads to transgenerational inheritance of a partially vernalized state. Mapping-by-sequencing revealed a hypomorphic mutation in the histone H3K27 demethylase ELF6 (1). Jumonji proteins ELF6 and REF6 counteract the histone methylation activities of repressive Polycomb complexes during plant development. In addition to our work on Arabidopsis, we are studying the role of these epigenetic factors in Brassica crops. We will present novel data regarding the characterization of Bra.elf6 and Bra.ref6 mutants and the epigenomic profiling of H3K27 in Brassica rapa. Our data show a key role for histone demethylation in the epigenetic regulation of flowering time and other developmental processes in plants.

(1) P. Crevillén et al., Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state Nature (2014)
C0105 NEW ELICITORS TO INCREASE TRANS-RESVERATROL PRODUCTION IN GRAPEVINE CELL CULTURES

Lorena Almagro Romero ROMERO1, Heriberto Vidal Limon2, Ana Belen Sabater Jara1, Sarai Belchi Navarro1, Javier Palazón1, Rosa María Cusido2, María Angeles Pedreño1

1Universidad de Murcia (Murcia) España
2Universidad de Barcelona (Barcelona) España

1 Resumen

The potential value of trans-resveratrol (trans-R) for human health has rapidly increased its demand, and so, new biotechnological strategies to obtain it from natural sources are being developed. In this sense, the combined use of cyclodextrins (CD) and methyl jasmonate on grapevine cell cultures enhances the production of trans-R (WO2009106662 A1). Recently, Syk?owska-Baranek et al. (2015) showed that the use of perfluorodecalins (PFD) increased taxane production in transformed root cultures of Taxus x media overexpressing the TXS gene. Moreover, (Z)-3-hexenol (Hex) is a green leaf C6-volatile able to induce secondary metabolite production as well as the expression of defense-related genes in several plant species (Matsui, 2006).

In this work, the effect of PFD both gassed and degassed (PDFgas and PDFdegas, respectively) or Hex, separately or in combination with CD and methyl jasmonate, on both trans-R production and the expression of genes encoding enzymes involved in its biosynthetic pathway was evaluated in grapevine cell cultures. The results showed the maximum level of trans-R produced by cells and secreted to the culture medium was reached with PDFdegas + CD and methyl jasmonate. In addition, all genes analyzed were induced by the elicitors used in this study. However, the gene expression of phenylalanine ammonia lyase, cinnamate 4 hydroxylase, 4-coumaroyl CoA ligase, and stilbene synthase was greatly enhanced by the presence of PDFgas+CD+ methyl jasmonate and PDFgas+CD+Hex although the levels of trans-R in these treatments were lower than that found in the treatment with PDFdegas + CD and methyl jasmonate. Therefore, despite the fact that trans-R production is related with the expression of genes involved in the biosynthetic process, other factors may be involved.

Acknowledgements

This work has been supported by the Ministerio de Economía y Competitividad (BIO2014-51861-R) and Fundación Seneca-Agencia de Ciencia y Tecnología de la Región de Murcia (19876/GERM/15).
C0136 POTENT REGULATES OSCILLATING GENE EXPRESSION TO ESTABLISH PERIODIC BRANCHING IN THE ARABIDOPSIS ROOT

Juan Periañez-Rodriguez, Alvaro Sanchez Corionero, Juan Carlos Del Pozo, Miguel A. Moreno Risueno
Centro de Biotecnología y Genómica de Plantas (UPM - INIA) Pozuelo de Alarcón (Madrid) España

1 Resumen

Plant postembryonic organogenesis requires new organs to be positioned and formed through the specification of organ founder cells. Organ founder cells have the developmental potential –pluripotency– of giving rise to all distinct tissues which make up an organ; and are, therefore at the basis of multicellularity. In Arabidopsis thaliana, (lateral) postembryonic root positioning is dependent on gene expression which oscillates in-phase and in antiphase; being these two sets of oscillating genes part of a developmental clock, the Lateral Root Clock. Maxima in the oscillations of the in-phase genes are followed by stable static expression to form a prebranch site. Prebranch sites subsequently develop to generate new lateral roots.

Out of an ethyl methanesulfonate mutagenesis screen, we identified a mutation with altered postembryonic organogenesis, which we named potent. RNA sequencing and marker expression data show that oscillations in potent are continuously activated resulting in the overproduction of prebranch sites and root founder cells. POTENT is an auxin signaling factor that is degraded in the presence of this hormone and interacts in roots with a FACTOR oscillating in antiphase. Our results show that this factor is required for correct positioning of prebranch sites and founder cells through regulation of in-phase gene expression, as shown by our RNA sequencing results. Our data indicates that POTENT signaling module functions as a transcriptional repressor of in-phase gene expression, and therefore we propose that a double repression mechanism is required to set the pace of organ positioning.
C0194 AAMYB1, AND ITS ORTHOLOGUE ATMYB61, AFFECT TERPENE METABOLISM AND TRICHOME DEVELOPMENT IN ARTEMISIA ANNUA AND ARABIDOPSIS THALIANA

Luis Matias Hernandez¹, Weimin Jiang², Ke Yang³, Kexuan Tang³, Peter E Brodelius³, Soraya Pelaz Matias⁵

¹Barcelona Science Park (Barcelona) Spain
²Plant Biotechnology Research Center, Shanghai, China (Shanghai) China
³Department of Chemistry and Biomedical Sciences, Linnaeus University, (Kalmar) Sueba
⁴Plant Biotechnology Research Center, Shanghai, China (Shanghai) China
⁵Centro en AgriGenomica (CRAG) (Barcelona) España

1 Resumen
The effective anti-malarial drug artemisinin (AN) isolated from Artemisia annua is relatively expensive due to the low content in the plant as it is only synthesized within the glandular trichomes. Therefore, genetic engineering of A. annua is one of the most promising approaches to improve AN yield. In this work, AaMYB1 transcription factor has been identified and characterized. When AaMYB1 is overexpressed in A. annua, either exclusively in trichomes or in the whole plant, essential AN biosynthetic genes are also overexpressed and consequently AN amount significantly increased. Artemisia AaMYB1 constitutively overexpressing plants displayed a higher number of trichomes. In order to study this role on trichome development and others possibly connected biological processes, AaMYB1 was overexpressed in Arabidopsis thaliana. For supporting our findings in A. thaliana, AaMYB1 orthologue from this model plant AtMYB61 was identified and atmyb61 mutants characterized. Both AaMYB1 and AtMYB61 affected trichome initiation, root development and stomatal aperture in A. thaliana. Molecular analyses indicated that two crucial trichome activator genes are misexpressed in atmyb61 mutant plants and in plants overexpressing AaMYB1. Furthermore, AaMYB1 and AtMYB61 are also essential for gibberellin (GA) biosynthesis and degradation in both species by positively affecting the expression of the enzymes that convert GA9 into the bioactive GA4 as well as the enzymes involved in the degradation of GA4. Overall, these results identify AaMYB1/AtMYB61 as a key component of the molecular network that connect important biosynthetic processes, and reveal its potential value for AN production through genetic engineering.
C0224 CRISPR/CAS9 MUTATION OF THE RICE STARCH BRANCHING ENZYME IIB GENE (OSBEIIIB) DOES NOT AFFECT THE CLOSELY RELATED POTENTIAL OFF-TARGET OSBEIIA AND RESULTS IN MONO AND BIALLELIC KNOCKOUTS IN THE T1 GENERATION

Can Baysal¹, Luisa Bortesi², Changfu Zhu, Gemma Farre¹, Stefan Schillberg³, Paul Christou⁴

¹Department of Plant Production and Forestry Science, School of Agrifood and Forestry Science and Engineering (ETSEA), University of Lleida-Agrotecnio Center (Lleida) Spain
²Institute for Molecular Biotechnology, RWTH Aachen University, Germany
³Fraunhofer Institute for Molecular Biology and Applied Ecology IME Germany
⁴ICREA, Catalan Institute for Research and Advanced Studies (Barcelona) Spain

1 Resumen

Genome editing with the CRISPR/Cas9 system allows mutations to be induced at any 20-bp target site in the genome preceded by the short protospacer adjacent motif (PAM) 5’-NGG-3’. The brevity and degeneracy of the PAM ensures that the motif occurs every 10 bp in plant genomes, and all plant genes therefore contain many targetable sites. However, the CRISPR/Cas9 system tolerates up to three mismatches in the target site, so the ability to target genes in a specific manner requires the design of synthetic guide RNAs that do not bind off-target sites anywhere else in the genome. This is straightforward for single-copy genes but more challenging if a target gene has one or more paralogs because the principles that balance targeting efficiency (the frequency of on-target mutations) and accuracy (the absence of off-target mutations) are not fully understood and may be partially species-dependent. To investigate this phenomenon in rice, we targeted the rice starch branching enzyme IIb gene (OsBEIIb) with two sgRNAs designed to differ at two and six positions, respectively, from corresponding sites in the close paralog OsBEIIa. In each case, half of the mismatches were in the essential seed region immediately upstream of the PAM and the other half were in the distal part of the target. We found that neither of the sgRNAs induced an off-target mutation in the OsBEIIa gene. The targeted mutation in the T0 line was stably transmitted to the next generation and we confirmed mono- as well as bi-allelic transgene-free T1 progenies. Our data indicate that a 1-bp mismatch in the seed region of a sgRNA is sufficient to avoid off-target effects even in closely related rice genes.
C0237 ABSCISIC ACID SIGNALLING MANIPULATION SUPPRESSES SENESCENCE OF A LEAFY VEGETABLE STORED AT ROOM TEMPERATURE.

Javier Alberto Miret Barrio1, Sergi Munné-Bosch1, Paul P Dijkwel2

1Facultat de Biologia. Fisiologia vegetal (Barcelona) Spain
2Institute of Fundamental Sciences, Massey University (Palmerston North) New Zealand

1 Resumen
Post-harvest senescence and associated stresses limit the shelf-life and nutritional value of vegetables. Improved understanding of these processes creates options for better management. After harvest, controlled exposure to abiotic stresses and/or exogenous phytohormones can enhance nutraceutical, organoleptic and commercial longevity traits. With leaf senescence, abscisic acid (ABA) contents progressively rise, but the actual biological functions of this hormone through senescence still needs to be clarified. Post-harvest senescence of detached green cabbage leaves (Brassica oleracea var. capitata) was characterised under cold (4ºC) and room temperature (25ºC) storage conditions. Hormonal profiling of regions of the leaf blade (apical, medial, basal) revealed a decrease in cytokinin contents during the first days under both conditions, while ABA only increased at 25ºC. Treatments with ABA and a partial agonist of ABA (pyrabactin) for 8 days did not lead to significant effects on water and pigment contents, but increased cell integrity, and altered 1-aminocyclopropane-1-carboxylic acid (ACC) contents and cytokinins signature. Transcriptome analysis showed transcriptional regulation of ABA, cytokinin and ethylene metabolism and signalling; ubiquitin protein-ligase proteasome components; senescence regulation; protection of chloroplast functionality and cell homeostasis; and suppression of defence responses (including glucosinolates and phenylpropanoids metabolism). It is concluded that increasing the concentration of ABA (and/or its partial agonist pyrabactin) from the start of post-harvest suppresses senescence of stored leaves, changes the transcriptional regulation of glucosinolates metabolism and down-regulates biotic stress defence mechanisms. These results suggest a potential for manipulating ABA signalling for improving post-harvest quality of leafy vegetables stored at ambient temperature.
C0296 RHAMNOGALACTURONASE LYASE GENE DOWNREGULATION IN STRAWBERRY AND ITS POTENTIAL ON MECHANICAL FRUIT PROPERTIES

Pablo Ric-Varas Varas, Sara Posé, Marta Barceló, Paul Knox, Rosario Blanco-Portales, Juan Muñoz-Blanco, Antonio Javier Matas, Miguel Ángel Quesada, José Ángel Mercado

1 Instituto de Hortofruticultura Subtropical y Mediterránea ‘La Mayora’, IHSM-UMA-CSIC, Departamento de Biología Vegetal, Universidad de Málaga Málaga, (Málaga) Spain
2 Instituto de Hortofruticultura Subtropical y Mediterránea ‘La Mayora’, IHSM-UMA-CSIC, Departamento de Biología Vegetal, Universidad de Málaga, Centre of Plant Sciences, University of Leeds, LS2 9JT, (Málaga) Spain
3 IFAPA, Centro de Churriana, Finca Cortijo de la Cruz, 29140 Churriana (Málaga) Spain
4 Centre of Plant Sciences, University of Leeds, LS2 9JT, (Leeds) United Kingdom
5 Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba, 14071, (Córdoba) Spain
6 Departamento de Biología Vegetal, Universidad de Málaga, 29071, (Málaga) Spain

1 Resumen

Strawberry softening is one of the main factors that reduces fruit quality and leads to economically important losses. Textural changes during fruit ripening are mainly due to the dissolution of middle lamellae, a reduction in cell-to-cell adhesion and the weakening of parenchyma cell walls as a result of the action of cell wall modifying enzymes. Functional studies of genes encoding pectinase enzymes (polygalacturonase, pectate lyase and b-galactosidase) support a key role of pectin disassembly in strawberry softening. Evidence that RG-I may play an important role in strawberry texture has been obtained from the transient silencing of a RG-lyase gene. Pectins are major components of fruit cell walls and highly dynamic polysaccharides, but due to their heterogeneity the precise relation between the structures and functions is incomplete. In this work, stable transgenic strawberry lines with a rhamnogalacturonate lyase gene (FaRGLyase1) down-regulated have been analyzed. Several transgenic lines showing more than 95% silencing of FaRGLyase1 displayed fruit firmness values higher than control. Cell walls from these lines were extracted and analyzed by ELISA and Epitope Detection Chromatography (EDC). This last technique is based on the detection of specific cell wall oligosaccharide epitopes and provides information on sub-populations of pectins containing homogalacturonan and RG-I domains, but also reveals potential links with other cell wall polysaccharides such as xyloglucan. The results obtained indicate that the silencing of FaRGLyase1 reduces degradation of RG-I backbones, but also homogalacturonan, in cell walls, especially in pectin fractions covalently bound to the cell wall. These changes contribute to the increased firmness of transgenic fruits.

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SESSION 3. SYSTEMS BIOLOGY

C0095 A SEED LONGEVITY GENOME-WIDE ASSOCIATION STUDY REVEALS NEW GENETIC COMPONENTS INVOLVED IN SEED DETERIORATION RESISTANCE

Joan Renard Meseguer, Eduardo Bueso Ródenas, Jose Gadea Vacas, Regina Niñoles Rodenes, Irene Martínez Almonacid, Ramón Serrano Salom

Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-Consejo Superior de Investigaciones Científicas Valencia (Valencia) España

1 Resumen

Seed life span, defined as the total time that seeds remain viable, from seed dispersal till germination, is a crucial trait to understand the ecological mechanisms of dispersion and also to preserve germplasm collection of endangered and cultivated species. Seed longevity is influenced by environment factors, being oxidation the one that claims to contribute most to seed deterioration. This trait is also conditioned by genetic factors, being protection structures, repair of nonfunctional molecules and detoxification of toxic compounds some of the processes identified so far. In the last years, our laboratory screened activation-tagging mutant collections of Arabidopsis thaliana in search of dominant contributors to seed longevity and revealed the important role of the seed coat in this process. We now extend the search of new actors contributing to seed longevity by exploring the natural variation of Arabidopsis. Up to 280 accessions have been phenotyped for seed viability using both accelerated aging and elevated pressure of oxygen assays, and a Genome-Wide Association Study (GWAS) has been performed in search of associated polymorphisms. The results points to a multigenic trait with a small effect of any associated region. Correlation between both experiments also allow us to discuss the relevance of oxidation events in the seed deterioration process. Finally, knock-out lines of candidate genes is revealing new genetic components involved in this important process of plant biology.
C0097 TRANSCRIPTIONAL ANALYSIS OF CORK SEASONAL GROWTH

Sandra Fernández Piñán, Pau Boher, Marçal Soler, Marissa Molinas, Mercé Figueras, Olga Serra

Universitat de Girona (Girona) España

1 Resumen

Cork tissue (phellem) is a water-resistant protective tissue made of suberized cell walls that forms the outer bark of tree. The cork oak (Quercus suber) produces a particularly thick and pure phellem, widely used for industrial applications. Hence, the factors controlling the cork growth and differentiation are of interest. To understand how cork formation is modulated during the seasonal growth we analysed the transcriptome by RNA-sequencing using Illumina platform at three different time points April, June and July and for a minimum of three replicates each. April, June and July were selected by their contrasted temperature and humidity conditions. Specifically, April corresponds to the cork meristem activation after winter pause, June to the maximum growth of the tissue, and July to a high tissue growth but under stressful conditions. The contigs identified by RNAseq were functionally annotated using Blast2GO platform and manual literature curation from TAIR database. Since cork is the paradigm for suberin studies, we first monitored the expression of genes related with the formation of this polyester. The trends of grouped genes in functional categories showed that in April the suberin biosynthesis is already activated but it is in June when there is a maximum activity. When comparing statistically the gene expression between time points, we observed that the transcriptomes of June/July were very similar (cork maximum activity), but they highly differed from April (cork growth starts). Differential expressed genes were grouped according to their expression pattern in five clusters and a functional enrichment analysis in each cluster was made. Our results will shed some light to the cork development and may allow the identification of new candidate genes to be used as markers of quality-cork to assist plant breeding.
C0122 METABOLOMIC OF OAT ROOTS REVEAL PREVALENT ROLES FOR BRASSINOSTEROIDS, BEHENIC ACID AND FATTY ACIDS IN DROUGHT TOLERANCE

Francisco J Canales Castilla¹, Luis A.J. Mur², Elena Prats Perez²

¹Institute for Sustainable Agriculture-CSIC (Cordoba) Spain
²Institute of Biological, Environmental and Rural Sciences, University of Aberystwyth (Wales) United Kingdom

1 Resumen

Drought is one of the major environmental factors determining plant yield. Despite drought sensing start at root system, our current understanding of the effects of drought on economically important crops is largely based on studies on above-ground traits. Given the importance of biochemical changes in the responses of plants to drought, metabolomic approaches would appear to be particularly appropriate in elucidating tolerance mechanisms. Thus, we have performed a metabolomic study to reveal metabolic changes occurring in the roots during the development of progressive drought. Samples from roots were taken from two well characterized oat genotypes for drought resistance. Metabolite profiles were obtained from different genotypes, time points and tissues using Direct Injection Electrospray Ionisation-Mass Spectrometry using a LTQ linear ion trap and a high resolution Orbitrap Fusion Tribrid Mass Spectrometer. Key metabolite differences were elucidated using multivariate statistical approaches, particularly discriminant Function Analysis (DFA). All metabolites were validated and the absolute concentration in samples calculated for comparison. Taken together the discriminatory metabolites suggested that brassinosteroids, behenic acid, and fatty acid biochemical pathways have key role within roots for inducing drought resistance. Independent targeted analyses are currently carrying out in order to substantiate the role of the differentially expressed pathways.
C0134 MOLECULAR CONVERGENCE OF PHOTORECEPTIVE AND CIRCADIAN CLOCK PATHWAYS IN THE REGULATION OF GROWTH

Guiomar Martín¹, Arnau Rovira¹, Judit Soy¹, Nil Veciana¹, Marc Boix¹, Eugenio G. Minguet², Gabriela Toledo-Ortiz², Rossana Hennques¹, David Alabadi², Karen Halliday⁴, Pablo Leivar⁵, ELENA MONTE⁶

¹Center for Research in Agricultural Genomics (CRAG) (Barcelona) Spain
²Instituto de Biología Molecular y Celular de Plantas (IBMCP) (Valencia) Spain
³Lancaster University (Lancaster) UK
⁴The University of Edinburgh (Edinburgh) UK
⁵IQS School of Engineering (Barcelona) Spain

1 Resumen
Seasonal differences in day length (photoperiod) provide an indicator of the progression of seasons, while diurnal changes are a measure the progression and time of the day. In the annual plant Arabidopsis, the long days (LD) of summer induce the transition to flowering, whereas daily hypocotyl growth increases during the short days (SD) of winter. Under SD, hypocotyl elongation rate increases specifically at dawn generating diurnal growth rhythms. Hypocotyl elongation in Arabidopsis requires integration of both light and clock signaling pathways. Phytochrome(phy)-mediated light signaling involves binding of the photoactivated phyS to the phy-interacting transcription factors PIFs, which are then targeted for rapid degradation causing diurnal oscillations in their abundance. PIFs act as promoters of growth directly inducing the expression of a cascade of growth-inducing factors at dawn. We have recently described that the central clock component TOC1 represses early PIF3 activity to prevent early growth during the night. However, whether similar regulatory mechanisms take place at other times during the photoperiod is not known. We will present novel data showing that TOC1 acts in concert with the related PSEUDO-RESPONSE REGULATORS PRR9/7/5 as circadian midmorning-to-midnight waves of PIF transcriptional repressors to prevent detrimental overgrowth.
C0141 MODULAR MODIFICATIONS IN THE CRISPR/CAS9 GUIDE RNA TO ENABLE TAG PURIFICATION AND THE COMBINATORIAL DESIGN OF PROGRAMMABLE TRANSCRIPTIONAL REGULATORS IN PLANTS.

Sara Selma García¹, Joan Bernabé Orts¹, Diego Vicente Orzáez Calatayud¹, Asunción Fernández Del Carmen¹, Marta Vásquez Vilar¹, Antonio Granell Richart¹

¹IBMCP Valencia España
²Wageningen University and Research (Wageningen) Países Bajos

1 Resumen

CRISPR/Cas9 is a programmable site-specific DNA nuclease with a wide variety of applications due to its versatility and efficiency. Modifications in the Cas9 protein or in its guide RNA (gRNA) enable the expansion of the range of Cas9 activities, from its original nuclease to other DNA binding-related activities such as transcriptional regulation. In plants, Cas9 has been used in its unmodified form for gene editing, or fused to transcriptional regulator domains as programmable transcriptional regulator; but to our knowledge no modifications in gRNA have been reported. The objective of this work is to design modular gRNA modifications that allow the attachment of regulatory domains to the CRISPR/Cas9 complex via its gRNA. To achieve this, an RNA aptamer that binds Ms2 phage coat protein will be added to free positions of the gRNA. This creates a structure comprising the chimeric RNA-guide and Cas9, which binds to Ms2 fused to a domain or protein of interest. In order to optimize the process, two chimeric RNA-guides have been designed that contain two copies of the Ms2 aptamer in two different positions. One of them adds the aptamer to the 3’end of the RNA-guide, whereas the second one incorporates it in a free loop of the RNA-guide hairpin. These structures will be used for the attachment of transcriptional activators and repressors to the gRNA/Cas9 complex (VP64, or SRDX) fused to the Ms2 protein. Taking advantage of the modular GB cloning system, this will facilitate the construction of a combinatorial array of programmable activators / repressors. The efficiency of this system will be compared with others where the activator / repressor domain was directly fused directly to the Cas9. Furthermore, the addition of a His-tag to Ms2 will be also performed, which should provide an efficient tool for purification of recombinant gRNA-Cas9 complexes in plants.
C0169 IDENTIFICATION AND CHARACTERIZATION OF KNOX GENE FAMILY IN PINUS PINASTER

Natalia Bueno Fernández¹, José M. Álvarez Díaz², Ricardo J. Ordás Fernández²

¹Laboratorio de Biotecnología Agroforestal Mieres (Asturias) España
²Laboratorio de Biotecnología Agroforestal Departamento Biología de Organismos y Sistemas, Universidad de Oviedo Mieres (Asturias) España

1 Resumen

KNOTTED1-LIKE homeobox genes (KNOX) constitute a family of plant-specific homeobox transcription factors present in all land plant groups and in some green algae. Basing on phylogenetic studies, sequence analyses and expression criteria, they are classified in two subfamilies, class I and class II, which might have originated after a process of gene duplication and neofunctionalization that took place at the base of the land plants lineage. Recently, KNOX genes lacking the homeodomain region have been described in some eudicot species, constituting the so-called class M. In angiosperms, class I KNOX genes are expressed mainly in meristematic regions and tissues with low degree of differentiation, and have been related to the maintenance of meristem homeostasis and leaf development. On the other side, class II KNOX genes are mainly expressed in differentiating tissues and mature organs, so it has been proposed that these genes promote differentiation of aerial organs. However, the knowledge about KNOX genes diversity and function in gymnosperms is still limited. In this work, we report the characterization of KNOX gene family in Pinus pinaster through the search in the transcriptome database obtained in the frame of the European projects ProCoGen and SustainPine. We identified four class I KNOX genes (KN1, KN2, KN3, KN4), in concordance with what was previously found in other conifer species, and two class II KNOX genes (KN5, KN6), being the first time that genes from both subfamilies were reported in conifers to our knowledge. No class M members were identified. Sequence alignments and phylogenetic analyses support the proposed high degree of conservation of KNOX gene family throughout seed plants. Expression analyses of these genes in different stages of development and plant tissues by RT-qPCR suggest that their roles might be partially conserved in gymnosperms, but not totally equivalent to those described for their angiosperm counterparts.
C0180 TRANSCRIPTOMIC ANALYSIS OF STORAGE LIPID MOBILIZATION IN THE OLIVE TREE POLLEN DURING GERMINATION

Rosario Carmona1, Maria José Jiménez-Quesada1, M Gonzalo Claros2, Juan de Dios Alché1, Antonio Jesús Castro López1

1Estación Experimental del Zaidín (CSIC) (Granada) España
2Plataforma Andaluza de Bioinformática, Departamento de Biología Molecular y Bioquímica, Universidad de Málaga (Málaga) España

1 Resumen

During its development, the pollen grain of oleaginous plants accumulates triacylglycerols (TAGs) in the cytoplasm. Yet, the information about the fate of these lipidic reserves and its physiological relevance for reproduction is still scarce. Here, we investigated this issue in the olive tree (Olea europaea L.) through a comparative transcriptional profiling of mature vs. germinated pollen. Overall, lipid metabolism accounted for about 1.8% of 1,283,143 mapped reads. We identified up to 288 orthologs of Arabidopsis genes involved in acyl lipid metabolism, including some regulatory proteins. All lipid-related metabolic pathways (as defined in the Aralip database), except those for suberin and cutin biosynthesis, were present in the pollen grain, with EC coverage values ranging from 43.8 to 92.3%. During pollen germination, global expression values gradually declined for all biochemical pathways analyzed. The mapped reads associated with glycerophospholipid metabolism represented as much as ~59% of total lipid metabolism. This fact might be explained by the major importance of this group of lipids in regulating pollen tube growth, as membrane and signaling compounds. Triacylglycerol and fatty acid (FA) mobilization counted for about 5.5% of lipid metabolism-related mapped reads. Interestingly, we found a significant rise in the transcriptional activity of TAG lipases, which is consistent with the mobilization of lipid bodies and the increase of free FAs observed. Our data also showed evidence for a functional glyoxylate cycle in the pollen tube, which suggest an active synthesis of oxaloacetate from acetyl-CoA for its further conversion to sugars. Together, our data provide a comprehensive list of enzymes and pathways involved in the utilization of storage lipids by the pollen tube during its post-germinative tip growth, and a resource for use in future comparative and functional genomic studies. This work was supported by co-funding from ERDF and MINECO (grants no. AGL2013-43042-P and BFU-2016-77243).
C0182 EXPLORING TRANSCRIPTOME AND REGULATORY NETWORKS INVOLVED IN SHORT TERM HIGH TEMPERATURE RESPONSE OF PINUS RADIATA

Mónica Escandón¹, Luis Valledor¹, José Manuel Álvarez¹, Gloria Pinto², Ricardo Ordás¹, Mónica Meijón¹, María Jesús Cañal Villanueva¹

¹Departamento de Biología de Organismos y Sistemas. Universidad de Oviedo (Asturias) España  
²Departamento de Biología y CESAM. Universidad de Aveiro (Aveiro) Portugal

1 Resumen
Studying the tolerance mechanisms of trees to heat stress is essential to preserve forest productivity and quality in the currently global warning. Pinus radiata is the most widely planted pine species in the world and studying thermotolerance processes would be the key to unravel conifer stress response and improve their breeding programs. Transcriptome of short-term heat-response was explored in P. radiata by using next generation sequencing (Illumina technology). More than 600 transcripts were identified as differentially expressed between the samples showing the most of them a decreased expression in heat-treatments. Additionally, transcriptome data were analyzed following an integrative multivariate approach that combined these data with omics and physiological data-sets obtained in previous works. Proteome identifications were significantly improved by adding transcriptome data base. Moreover, the integration of different data-sets and STRING function analysis of protein-protein interactions allowed to validate and redefine the complex pathways involved in acclimation to high-temperature in P. radiata as well as identify novel key elements, such as LIPOPOLYSACCHARIDE 1,2-N-ACETYLGLOUCOSAMINETRANSFERASE, CELLULOSE SYNTHASE and EUKARYOTIC INITIATION FACTOR 4A-1. Also, MITOCHONDRIAL SMALL HEAT-SHOCK PROTEIN was validated as a powerful tool to use in breeding programs. Through this work was determined that high temperature response involve a temporally ordered, orchestrated implementation of response elements at various system levels. The integration of transcriptomic, proteomic, metabolomics and physiological data allowed the description of complete pathways involved in the process of acclimation to heat-stress in P. radiata. Although heat-stress acclimation is still in its infancy in the forestry species, the obtained results are promising not only in defining the mechanisms behind high-temperature response in P. radiata and describing how the different stress responses are interconnected, but also in providing novel stress biomarkers with a direct potential use in breeding programs for early selection of thermotolerant trees.

C0088 ELECTROPHYSIOLOGICAL STUDY OF COPPER UPTAKE BY ARABIDOPSIS HIGH-AFFINITY CU+ TRANSPORTERS IN THE COPT FAMILY.

Amparo Sanz Grau1, Shanon Pike2, Àngela Carrió Seguí3, Lola Peñarrubia Blasco4, Walter Gassmann2

1Dpt. of Plant Biology, University of Valencia (València) Spain
2Division of Plant Sciences, CS Bond Life Sciences Center, University of Missouri-Columbia (Columbia) USA
3Universitat de València Burjassot (Valencia) España
4Dpt. of Biochemistry and Molecular Biology, University of Valencia (València) Spain

Resumen

Copper is an essential plant micronutrient. Under scarcity, Cu²⁺ is reduced to Cu⁺ and taken up through specific high affinity transporters (COPTs). In Arabidopsis thaliana the COPT family consists of 6 members, located at the plasma membrane (COPT1, COPT2 and COPT6) and at internal membranes (COPT5). Cu uptake by COPT proteins has been mainly assessed through complementation studies in yeast, but the mechanism of this transport has not been elucidated yet.

To test whether Cu is incorporated by an electrogenic mechanism, we studied putative electrophysiological changes induced in Arabidopsis thaliana by Cu addition to the medium. Wild type and mutant plants with altered expression of COPT transports (T-DNA insertion mutants, copt2 and copt5-2, and overexpressing lines, COPT1OE and COPT5OE) were used. No significant changes of Em were detected, regardless of genotype, nutritional status or Cu concentration supplied. In contrast, plants responded to iron with depolarization of the plasma membrane in both wild type and in mutant plants. Similar results were obtained when trans-plant-potentials (TPP) were measured. These results indicate that either the rate of Cu uptake is so low that its entry does not induce detectable electrical signals, or that uptake is electroneutral. We then expressed COPT2:GFP and COPT5:GFP in Xenopus laevis oocytes to potentially amplify Cu uptake signals. COPT2 and COPT5 cRNA-injected oocytes were tested for electrical currents upon metal addition using two-electrode voltage-clamp. Results with oocytes confirmed those obtained in plants. Additional uptake experiments were carried out with injected oocytes incubated in Cu solutions and their Cu contents measured by ICP-OES. A significant increase in Cu content in oocytes expressing COPT2:GFP with respect to control oocytes was detected. These results altogether suggest that Cu uptake could be electroneutral. This experimental approach seems suitable to further study other biochemical characteristics of PM-bound transport proteins.
1 Resumen
Mediterranean agriculture must cope with little water resources, therefore research on irrigation water use optimization is encouraged. In an attempt to evaluate the use of plant water status indicators to guide precise irrigation of fruit trees, a study was carried out in an early-maturing nectarine orchard at the CEBAS-CSIC experimental station in Santomera (Murcia, Spain). Canopy temperature, measured with infrared thermal camera, along with stem water potential ($\Psi_{stem}$) and gas exchange: net photosynthesis ($P_n$) and stomatal conductance ($g_s$) was measured at midday during the fruit growth period (March to May) in trees under different irrigation and crop load conditions. Irrigation treatments included well irrigated (one or two line of emitters for full water needs) and non-irrigated trees.
From the analysis of the thermal images, canopy temperature (Tc) and other indices were obtained. The results indicated that Tc taken from East orientation of the trees were about 2 ºC higher than from the other tree orientations, with West orientated images showing the lowest Tc values. Canopy to air temperature difference (Tc-Ta) values were slightly higher in non-irrigated than in the irrigated trees. A good relationship was observed between crop water stress index (CWSI) and the stomatal conductance which demonstrates that this thermal index provide a direct assessment of plant water status trough stomatal regulation of transpiration. Canopy temperature measured by thermal imaging has proved to be a good indicator of the water status of nectarine trees. However, the low evaporative demand of the atmosphere conditions during the experimental period resulted in light water deficits in non-irrigated treatment ($\Psi_{stem}$= -0.7 MPa) and more research are needed under severe water stress conditions.
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C0191 DISSECTING THE FOLIAR AND RADICULAR EFFECT OF HUMIC SUBSTANCES ON PLANT GROWTH.

David de Hita Mejía¹, Marta Fuentes¹, Angel María Zamarreño¹, Roberto Baigorri², Maite Olaetxea¹, María Garnica¹, Jose María García-Mina¹

¹Universidad de Navarra, Pamplona, España
²Timac Agro España (Grupo Rouiller) (Navarra) España

1 Resumen

Despite humic acid (HA) is widely used in agriculture, the mechanism of action of growth promotion on plants is still poorly understood. According to recent studies, the humic acid root application triggers changes in the concentration of the hormones (IAA, NO, ABA…), the enzymatic activity of PM H⁺ ATPase, the mineral nutrient and CK’s translocation and the ROS activity. Overall, these responses modify the gene regulation involved in the root development and architecture increasing plant’s growth.

In industrial crops and large-scale agriculture, HA is applied as a foliar spray rather than through drip or flood irrigation because they are more time and money consuming. It is proposed that foliar HA effects are the consequence of the interaction of the HA with soil and roots. However, this proposal is difficult to maintain since the concentration of HA applied on leaves is very low (3 L/ha in 300L of water solution). Our hypothesis is that the effects of foliar HA may result from an interaction with leaf surface, which might trigger a mild stress-related with beneficial effects on plant growth. In order to test this hypothesis, we have studied the effect of foliar application of HA on the growth and hormonal balance in cucumber.

We observed a 25-30% increase in dry weight (DW) in plants harvested eight days after the foliar treatment with the HA. Moreover, our results showed that the foliar application seemed to trigger a root to shoot translocation of CK’s and changes in IAA and ABA concentration in leaves. In summary, effects on plant growth caused by the foliar application of AH are expressed later than those caused by HA root application and the HA-mediated changes in hormonal balance are mainly located in the leaves, not in root as occurs in the case of HA root application.
Resumen

Ammonium and urea based nutrition may turn stressful to plant species, including important crops. One of the most dramatic plant adaptations to ensure adequate nitrogen acquisition is the modulation of root system architecture (RSA) in response to nitrogen supply. Ammonium has been described to change the shape of pea roots) and *Medicago truncatula*. Different signaling molecules as nitrate, reactive nitrogen species, or auxins have been suggested to be implicated. However, the effect of oximes, derived from the oxidation of aminoacids, in the development of this RSA is still unknown. Therefore, the objective of this work was to study the effects of oximes in RSA and the differences in oximes content in *Medicago truncatula* under different nitrogen-based nutritions (either nitrate, ammonium or urea) grown in axenic conditions to avoid the interference of microorganisms. *Medicago truncatula* seeds were germinated in agar plates containing Fahraeus medium with phytagel, as nutrient solution. During 14 days of growth a database was established with images from which information of primary and secondary roots was obtained (primary and secondary growth length, number of secondary roots...). The quantification of oximes was performed using High Pressure Liquid Chromatography. Our results indicate not only that oximes application have remarkable effects on *M. truncatula* roots, as a “superroot” phenotype, but also important changes occurred in the oximes contents, both in shoot and roots under different treatments. The better knowledge of the signaling mediated by oximes will increase the understanding of the mechanisms underpinning efficient N uptake and will help breeders to achieve the improvement of crop yield.
C0248 ROLE OF ENDODERMAL CASPARIAN STRIPS AND SUBERIN IN THE HYDROMINERAL STATUS OF PLANTS UNDER SALT STRESS

Monica Calvo Polanco¹, Zoe Ribeyre¹, Rochus Benni Franke², David Salt³, Thierry Simonneau¹, Bertrand Muller¹, Christophe Maurel⁴, Yann Boursiac⁴

¹UMR 759 INRA/SupAgro: Laboratory of 'Ecophysiologie des Plantes sous Stress Environnementaux' 2 Pierre Viala, (Montpellier) France
²Department of Ecophysiology of Plants, University of Bonn, Institute of Cellular and Molecular Botany (IZMB), (Bonn) Germany
³Faculty of Science, Sutton Bonington Campus, University of Nottingham, (Leicestershire) UK
⁴UMR 5004 CNRS/INRA/SupAgro/UM: Biochemistry and Plant Molecular Physiology, 2 Pierre Viala, (Montpellier) France

1 Resumen

Soil water uptake is limited by the radial transport across root tissues before being loaded into the xylem vessels, where water is exported to the shoot. Radial root transport involves the movement of water and dissolved solutes through the non-specific apoplastic pathway, or via the specific cell-to-cell pathway provided by aquaporins. Arabidopsis plants deposit hydrophobic barriers at the endodermis as Casparian Strips (CS) and suberin. CS have been recognized to be involved in controlling the movement of substances in and out of the vascular tissues, although it is difficult to generalize about the effects suberin may have on ion transport. The differentiation of the CS as well as the deposition of suberin may also alter the expression and localization of aquaporins, and this will have a remarkable impact on how water is transported in the plant.

The aim of the present research is to deliver an extensive understanding of the impact of the CS and suberin formation on whole plant water relations and growth in the context of salt stress. We have used a set of 25 Arabidopsis mutants (CS and suberin- ERA-CAPS Root Barriers Project) and tested them for their root hydraulic conductivity and aquaporin activity under control conditions and in the presence of NaCl. Even though alterations in CS and suberin in the mutants occur in roots, changes in water and solutes transport are expected to affect water potential gradients all along the transpiration stream. This will impact, in particular, cell turgor in expanding tissues or in the vicinity of the stomata, thereby altering growth and transpiration. We have used an automated phenotyping platform (Phenopsis, Montpellier Plant Phenotyping Platform) and exposed the plants to different levels of NaCl stress. The combined research will give new insights for the role of endodermal barriers to adverse soil conditions.
C0251 CHLORIDE AS MULTIFUNCTIONAL BENEFICIAL ION: BIOLOGICAL FUNCTIONS AND REGULATION

Juan de Dios Franco-Navarro¹, Paloma Cubero-Font¹, Miguel A. Rosales¹, Pablo Díaz-Rueda¹, Joaquín Espartero¹, Dietmar Geiger², Jose Manuel Colmenero-Flores¹

¹Instituto de Recursos Naturales y Agrobiología (CSIC) (Sevilla) Spain
²University of Würzburg, Institute for Molecular Plant Physiology and Biophysics, (Würzburg) Germany

1 Resumen

Chloride (Cl⁻) has been generally considered a toxic anion rather than a plant nutrient. However we have recently shown that, in addition to an essential micronutrient, Cl⁻ is a beneficial macronutrient (Franco-Navarro et al, 2016). Under non-saline conditions (1-5 mM), Cl⁻ specifically stimulates higher leaf cell size and leads to a moderate increase of plant fresh and dry biomass mainly due to higher shoot expansion. Chloride plays specific roles in regulating leaf osmotic potential and turgor, allowing plants to improve leaf water balance parameters. In addition, Cl⁻ regulates water relations at the whole plant level through reduction of plant transpiration. This is a consequence of a lower stomatal conductance, which results in lower water loss and greater photosynthetic and integrated water-use efficiency (Franco-Navarro et al, 2016).

We are currently studying the interaction of Cl⁻ homeostasis with: i) plant development; ii) plant tolerance to water deficit; and iii) carbon and nitrate (NO₃⁻) assimilation. These points will be addressed at the physiological level, and data concerning the molecular regulation of Cl⁻ nutrition and Cl⁻/NO₃⁻ interaction (Cubero-Font et al., 2016), will be presented.


We acknowledge MINECO-FEDER grants AGL2009-08339/AGR and AGL2015-71386-R.
C0310 NO$_3$-SELECTIVE MINI-ELECTRODES AS A TOOL TO INVESTIGATE THE NO$_3$-TRAFFIC IN CHLAMYDOMONAS REINHARDTII D.

Jordi Díaz García¹, Lourdes Rubio Valverdè², Aurora Galván Cejudo³, Emilio Fernández Reyes³, José A. Fernández García¹

¹Facultad de Ciencias. Universidad de Málaga (Málaga) España
²Dpto. Bioquímica y Biología Molecular. Universidad de Córdoba (Córdoba) España

1 Resumen

Ion selective NO$_3$ mini-electrodes were used to measure the external NO$_3$-concentration in C. reinhardtii liquid cultures. Electrodes were prepared using glass capillaries (1.5 mm external diameter). Capillaries were cut in 10 cm long pieces, dehydrated for 45 minutes in an oven and silanized by addition of dimethyldichlorosilane in bencene 0.1% (V/V). Once silanized, the capillaries were baked again for 30 minutes. Once cold the capillaries were backfilled with the NO$_3$-ionophore (Fluka: 72549), which contains PVC (5.75% w/w) dissolved in tetrahydrofurane. Then, the NO$_3$-mini-electrodes were stored in dark in a desiccator until tetrahydrofurane gets evaporated. Before use, NO$_3$-selective mini-electrodes were backfilled with 0.1 M NaNO$_3$ and 0.1 M KCl and connected to a high-impedance differential amplifier (WPI FD223). Mini-electrodes were calibrated in N-free Beijerinck medium, which contains 0.1 mM Cl$^-$. In those conditions, electrodes calibration slope was 54 mV/pNO$_3$ in the range 1 - 1000 µM NO$_3$. The mini-electrodes were used to continuous monitoring of the external NO$_3$-concentration in liquid culture of different C. reinhardtii strains, incubated in N-free Beijerinck medium supplemented with 100 µM NO$_3$Na. Previous to the assays, strains were N starved for 6 days. In the light, wild type strain uptakes NO$_3$ at a rate of 15 nmol NO$_3$·10$^8$ cells$^{-1}$·h$^{-1}$, in the dark this rate was one third of this figure. After 5 h, the external NO$_3$ levelled off at 10 µM in the light and around 30 µM in the dark. C. reinhardtii cells cultured in the presence of 2 mM NO$_3$NH$_4$ do not show significant NO$_3$-uptake nor a mutant strain, defective in nitrate transport and having an active nitrate reductase. However, a mutant strain lacking the nitrate reductase shows an enhanced NO$_3$-uptake rate, compared with the value obtained for the wild type in the light.

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C0313 USING CHLOROPHYLL FLUORESCENCE AND ARTIFICIAL INTELLIGENCE TO AUTOMATICALLY IDENTIFY MAIZE VARIETIES AND CLASSIFY LEAF RELATIVE WATER CONTENT

Jorge Marques da Silva¹, Tânia Tordo², Catarina Mendes², Hamilton Chiango¹, Pedro Correia¹, Carla Gameiro², Pedro Mariano¹

¹University of Lisbon, Faculty of Science and Biosystems and Integrative Sciences Institute (Lisbon) Portugal
²University of Lisbon, Faculty of Science (Lisbon) Portugal

1 Resumen
A combination of chlorophyll fluorescence and artificial intelligence was used to develop a non-invasive method for the automatic identification of maize varieties and classification of maize leaf water stress status. The rapid kinetics of PSII fluorescence emission in a dark-light transition was recorded in leaves from *Zea mays* var. Belgrano and *Zea mays* var. Lindsey, subjected to different levels of water stress, with a Handy PEA (Hansatech) chlorophyll fluorometer. The relative water content (RWC) of each leaf at the moment of the fluorescence measurement was determined. Both the entire data set of the fluorescence induction curves (Fluorescence Vs Time) and three calculated fluorescence indexes (Fv/Fm, PI and C-area) were used to construct classifiers based on decision trees, aimed to automatically assign each leaf to the corresponding RWC class. Four RWC (%) classes were considered: [100-80], [80-50], [50-30] and [30-0]. When the entire data set of the curves was used and only fully hydrated leaves (control, 100-80% RWC) were analyzed, the percentage of success in the automatic identification of the maize varieties reached 68%. Neither the decision criterion (Gini or Entropy) nor the maximum tree depth affected the percentage of success. Decision trees for the automatic classification of leaf relative water content are currently being tested.
C0046 CHARACTERIZATION OF APOPLAST-COLONIZING ENDOPHYTES FROM OILSEED RAPE LEAVES SHOWING ANTAGONISM TO CANOLA PHYTOPATHOGENS

Fernando Matias Romero Romero, Franco Rubén Rossi, Andrés Gárriz, Oscar Adolfo Ruiz

IIB-INTECH Chascomús (Buenos Aires) Argentina

1 Resumen
Many endophytic bacteria colonize host tissues internally without causing damage or eliciting disease symptoms and in some cases promote plant growth and protect them against pathogens. This work aimed to characterize bacterial members of the apoplast-colonizing community of oilseed rape plants grown under conditions of commercial production, and to analyze their antagonistic properties against phytopathogens. Several bacterial endophytes were isolated from the apoplast of field-grown oilseed rape leaves. These isolates were co-cultured with different canola pathogens, such as the fungal pathogens Leptosphaeria maculans and Sclerotinia sclerotiorum and the bacterial pathogen Xanthomonas campestris, in order to evaluate their antagonistic properties in vitro. In this way, three isolates (Apo8, Apo11 and Apo12) were selected based on their ability to inhibit all pathogens tested in vitro. These isolates were identified by sequencing the ARNr 16S gene as members of the genus Pseudomonas, specially related to the specie P. viridiflava. In order to test these isolates for their ability to diminish the infection provoked by X. campestris, oilseed rape seeds were inoculated with bacterial endophytes and four weeks after sowing these plants were challenged with X. campestris. Thus, plants inoculated with Apo11 showed a decreased pathogen propagation in comparison to mock-inoculated ones. Moreover, Apo11-inoculation induces the expression of defense genes involved in the salicylic acid and jasmonates signaling pathways. In addition to that, Apo11 inoculation produced a 50%-increment in fresh and dry weight of oilseed rape plants. This work allowed the identification of bacteria from apoplast of oilseed rape leaves able to inhibit growth of pathogens in vitro and to reduce disease symptoms caused by X. campestris when inoculated on canola plants. These features, along with their potential for plant growth promotion, render the above-mentioned isolate an interesting candidate for the development of biological formulations for growth promotion and control of canola diseases.
C0098 A CONSERVED PRO-SURVIVAL FUNCTION OF THE PLANT METACASPASE ATMC1 IN PROTEIN AGGREGATE CLEARANCE DURING STRESS

Liang Li1, Crina Popa, Sandra Malgrem-Hill2, Marc Valls, Thomas Nyström2, Núria Sánchez Coll1

1Centre for Research in Agricultural Genomics (CRAG) (Barcelona) Spain
2Department of Chemistry and Molecular Biology, University of Gothenburg (Gothenburg) Suecia

1 Resumen

In plants, there is a tremendous gap of knowledge concerning protein aggregate dynamics during biotic and abiotic stress. Recently we have found that the plant protease AtMC1 has a role in protein quality control, helping with the clearance of protein aggregates through an unknown mechanism that acts in parallel to autophagy. Metacaspases are a family of cysteine proteases present in plants, fungi and protozoa. Our previous studies established the plant metacaspase ATMC1 as a major positive regulator of pathogen-triggered programmed cell death (PCD), with a role analogous to mammalian caspase-1. The pro-death role of AtMC1 was shown to be conserved in the yeast single metacaspase MCA1.

Here, we present data showing that the also pro-survival function of metacaspase 1 is extensively conserved between yeast and plants. AtMC1 complements the growth defects of yeast mca1ydi1 double knock out mutants. In addition, we found the ATMC1 is localized into two protein quality control compartments, the insoluble protein deposit (IPOD) and juxtanuclear quality-control compartment (JUNQ), during aging and proteostatic stress. ATMC1 also has a function in removal of misfolded proteins in the yeast cell. This non-death, homeostatic activity of metacaspases might reflect an ancient function that represents the evolutionary origin of this family of proteins, later diversified to both pro- and anti-death functions.
C0119 IMAGING TECHNIQUES AND CLASSIFYING ALGORITHMS FOR BOTH DISEASE EVALUATION AND PREDICTION IN CUCURBITS CHALLENGED BY PATHOGENS

Mónica Pineda Dorado, María Luisa Pérez Bueno, Matilde Barón Ayala

Estación Experimental del Zaidín, CSIC (Granada) España

1 Resumen

Biotic and abiotic stress usually causes alterations in plant metabolism that can be detected even in the absence of symptoms using imaging techniques. They provide information about physiological processes in plants and can be applied in whole leaves, plants or even crops. Therefore, proximal and remote imaging sensors are particularly valuable tools in plant phenotyping and plant breeding programs.

Classifiers are algorithms trained on numerical data obtained from images and, according to the information provided, able to classify new samples as “healthy” vs “stressed/infected”. Such an approach leads to the automation of the whole process, from data acquisition to interpretation and decision making. Moreover, this strategy reduces the time of response and allows for targeted rather than whole-field actions, minimizing the environmental impact.

We have applied thermography, variable chlorophyll fluorescence (Chl-FI) and multicolour fluorescence imaging (MCFI) -providing information about transpiration, photosynthesis and secondary metabolism- to monitor infections on melon and zucchini plants caused by two pathogens, *Podosphaera xanthii* and *Dickeya dadantii*. Firstly, the impact of pathogens on plant physiology was evaluated. Then, three different algorithms were trained in order to identify infected plants before the appearance of visual symptoms: binomial linear regression analysis (LRA), support vector machines (SVM) and artificial neural networks (ANN). The performance of the classifications was evaluated in terms of specificity, sensitivity, accuracy and F1 score. The success of the method employed was related to the type of numerical information selected for feeding the classifiers, and to the degree of vicinity to the inoculation sites.
C0121 THE PGPR BURKHOLDERIA SP. (AU4I) PROTECTS ARABIDOPSIS THALIANA AGAINST FUNGAL PATHOGENS THROUGH DIRECT INHIBITION AND MODULATION OF DEFENSE AND PLANT CELL WALL METABOLISM

María Belén Colavolpe¹, Andrés Gárriz¹, Natalia M. Villerreal¹, Franco R. Rossi¹, Fernando M. Romero¹, Oscar A. Ruiz¹, Adesh Saini², Maria Marina¹

¹IIB-INTECH (CONICET-UNSAM) (Buenos Aires) Argentina
²Faculty of Applied Sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences (Solan) India

1 Resumen
Burkholderia sp. (AU4i), a strain which can acts as PGPR (Plant Growth-Promoting Rhizobacteria) in plants, was previously isolated from pea. We demonstrated that AU4i is able to grow endophytically on Arabidopsis plants and promote Arabidopsis growth increasing the number of lateral roots as well as root and shoot dry weight. To evaluate the potential of AU4i as a biocontrol agent, leaves inoculated with AU4i were challenged with the necrotrophic pathogens Botrytis cinerea and Sclerotinia sclerotiorum. The necrotic lesion areas provoked by both pathogens were reduced in leaves previously inoculated by AU4i. Additionally, AU4i was capable to inhibit the in vitro growth of B. cinerea, but not of S. sclerotiorum. In other hand, AU4i was able to induce the expression of the jasmonate responsive marker gene PDF1.2, but not the expression of salicylic acid marker gene (PR-1). The protective effect of AU4i against fungal pathogens was evaluated in Arabidopsis defense signaling pathways mutant plants, coi1 (defective in jasmonate perception) and sid2-2 (defective in salicylic acid synthesis). Similar protective effect was observed in both lines in comparison to Col-0 plants, suggesting the independence of the AU4i protective effect and plant signaling pathways. As well, the effect of AU4i inoculation on plant cell wall metabolism during that endophytic interaction was evaluated. As a result it was revealed that AU4i modulates host cell wall metabolism, through the down-regulation of genes involved in hemicellulose and pectins lateral chains degradation (AtExp8 and AtAra1, respectively) and the up-regulation of AtPME3, a gene encoding a putative pectin methylesterase involved in pectin main chain esterification. Taken together, our results suggest that Burkholderia sp. AU4i might be a good candidate as a biocontrol agent.
C0150 TWO NRAMP6 ISOFORMS FUNCTION AS IRON AND MANGANESE TRANSPORTERS AND CONTRIBUTE TO DISEASE RESISTANCE IN RICE

Ferran Sanchez Sanuy1, Cristina Peris Peris1, Albert Serra Cardona2, Joaquin Ariño Carmona2, Blanca San Segundo de los Mozos1, Sonia Campo Sánchez1

1 CRAG Bellaterra (Barcelona) España
2 IBB (Barcelona) España

1 Resumen
Metal ions are essential elements for all living organisms. However, metals can be toxic when present in excess. In plants, metal homeostasis is partly achieved through the function of metal transporters, including the diverse natural resistance-associated macrophage proteins (NRAMP). Among them, the OsNramp6 gene encodes a previously uncharacterized member of the rice NRAMP family that undergoes alternative splicing to produce different NRAMP6 proteins. In this work, we determined the metal transport activity and biological role of the full-length and the shortest NRAMP6 proteins (I-NRAMP6 and s-NRAMP6, respectively). Both I-NRAMP6 and s-NRAMP6 are plasma membrane-localized proteins that function as iron and manganese transporters. The expression of I-Nramp6 and s-Nramp6 is regulated during infection with the fungal pathogen Magnaporthe oryzae, albeit with different kinetics. Rice plants grown under high iron supply show stronger induction of rice defense genes and enhanced resistance to M. oryzae infection. Also, loss of function of OsNramp6 results in enhanced resistance to M. oryzae, supporting the idea that OsNramp6 negatively regulates rice immunity. Furthermore, nramp6 plants showed reduced biomass, pointing to a role of OsNramp6 in plant growth. A better understanding of OsNramp6-mediated mechanisms underlying disease resistance in rice will help in developing appropriate strategies for crop protection.
C0172 IDENTIFICATION OF MECHANISMS OF SPECIATION/ADAPTATION THAT ALLOW TETRANYCHID SPECIES TO USE TOMATO AS A HOST

Cristina Rioja¹, Kristie Bruinsma Bruinsma², María Estrella Santamaría Fernández³, Vladimir Zurov³, Catherine Martel², Vicente Arbona⁴, Isabel Díaz³, Miodrag Grbic², Vojislava Grbic²

¹University of Copenhagen Frederiksberg, Copenhagen (Copenhagen City) Denmark
²Western University (Ontario) Canada
³Centro de Biotecnología y Genómica de Plantas-Universidad Politécnica de Madrid (Madrid) Spain
⁴Universidad Jaume I (Castellón) Spain

1 Resumen

Plant-pest interactions result from an elaborate evolutionary interplay, where plants have developed strategies to deter attackers or reduce pest fitness and pests have evolved to overcome plant defenses. Tetranychid mites provide an excellent experimental system to study the plant-herbivore interaction as it harbors T. evansi (TE) –a specialist feeding only on Solanaceas–, and T. urticae (TU) –an extreme generalist able to feed on a wide range of plant hosts–. As both species feed on tomato (Solanum lycopersicum), we can compare plant and mite responses characterizing TU-tomato and TE-tomato interactions. In these comparisons we use TU population (reared on beans, and thus non-adapted to tomato) and TE population (reared on Solanum nigrum and adapted to tomato). We show that tomato plants effectively deter TU feeding by inducing defense response (incompatible interaction), whereas tomato plants are unable to restrict TE fitness and, as a result, they become susceptible to its herbivory (compatible interaction).

Manipulation of host responses and biochemical adaptations have been proposed as mechanisms of mite adaptation to host plants. Several studies have demonstrated that TU and TE are able to manipulate plant induced responses to adapt to tomato as a host (Alba et al., 2015; Wybouw et al., 2015). Despite this, it has not yet been demonstrated whether the suppression of plant induced responses contributes to increase mite performance and whether the detoxification of plant metabolites contributes to mite host adaptation.

We have characterized tomato early transcriptomic responses upon TU or TE feeding and identified tomato responses that are attenuated by the specialist TE. In further analysis, we demonstrate that both manipulation of host responses and detoxification of tomato allelochemicals contribute to TE’s adaptation/speciation to tomato. The full understanding of these adaptation mechanisms will help to design new strategies to improve crop’s ability to efficiently respond to herbivory attack.
C0199 AMI1, A NOVEL MOLECULAR HUB AT THE CROSSROADS OF GROWTH-DEFENCE TRADEOFFS

Beatriz Sánchez Parra, Leticia Martín Torres, Lucía Jordá Miró, Stephan Pollmann

Centro de Biotecnología y Genómica de Plantas (UPM-INIA) (Madrid) España

1 Resumen

Plants are highly prone to injury by pathogens, herbivores, and mechanical stresses jeopardizing their tissue integrity. In order to maintain fitness, plants have to adequately respond to these threats. In this regard, they largely rely on plant hormone crosstalk and a complex signal transduction network that connects damage-associated signals with appropriate adjustments of metabolic processes in the short-term and changes in plant growth and development in the long-term. These wound induced adaptive responses are nearly exclusively triggered by de novo biosynthesis of the plant hormone jasmonic acid (JA). However, recently, we were able to demonstrate that auxin biosynthesis, and therewith plant growth responses, are seemingly tightly linked to this process. Here, we present the functional characterization of AMI1, an Arabidopsis thaliana IAM amidohydrolase contributing to cellular auxin homeostasis, that apparently acts as a novel molecular hub connecting the energy status of the plant with auxin levels and defence fitness. We were able to detect that AMI1 expression levels are controlled by glucose and sucrose, but most intriguingly we found that the ami1 knockout line contains significantly increased JA contents and shows a higher resistance towards biotic predators.
C0293 A CALCIUM DEPENDENT PROTEIN KINASE MEDIATES BOTH Drought Tolerance AND BLAST DISEASE RESISTANCE IN RICE PLANTS

Mireia Bundó, María Coca

Centre for Research in Agricultural Genomics, CRAG (Barcelona) Spain

1 Resumen

Rice is the major cereal food crop for an increasing world population. Blast disease caused by the ascomycete fungus *Magnaporthe oryzae* and drought are the most important adverse factors limiting rice productivity. Improving the acclimation capacity of rice plants to adversity might stabilize yields and guarantee food security. Rice plant acclimation responses are activated upon sensing stress conditions. A complex network of signaling pathways orchestrates these acclimation responses that involve changes at molecular, cellular and physiological levels. These signaling pathways may have synergistic or antagonistic effects on one another, which are responsible for crosstolerances and tradeoffs between stresses. Calcium-dependent protein kinases (CPKs) are plant proteins that function as signaling components in multiple stress acclimation responses. CPKs are both calcium sensors and protein kinase effectors in one single molecule, and as such they transduce calcium signals into phosphorylation cascades to further downstream signaling events. We have identified the OsCPK10 isoform from the multigene family of rice CPKs as a signaling component that positively mediates drought tolerance and blast disease resistance. We show that OsCPK10 enhances the antioxidant capacity of rice plants and protects them from the reactive oxygen species (ROS) damage associated to both stresses. Drought tolerance is achieved by modulating the accumulation of catalase proteins, which reduces the extent of lipid peroxidation and prevents the integrity of cell membranes. Similarly, OsCPK10 reduces the accumulation of ROS during blast fungus infection interfering with its necrotrophic growth and leading to blast disease resistance. Furthermore, we demonstrate that OsCPK10 is a plasma membrane protein that physically interacts in vivo with catalase A. These studies show that OsCPK10 could be a good molecular target to provide tolerance to multiple distinct stresses in the economically relevant rice crop.
C0181 PGK ISOFORMS INTERACT TO MAINTAIN METABOLIC HOMEOSTASIS IN ARABIDOPSIS

Sara Rosa Téllez1, Armand D. Anoman2, Sergio Nebauer2, Saleh Alseekh3, Maria Flores Tomnero1, Jesús Muñoz Bertomeu1, Juan Segura2, Alisdair Fernie5, Roc Ros Palau1

1ERI de Biotecnologia y Biomedicina. Departamento de Biología Vegetal. Facultad de Farmacia. Burjassot (Valencia) Spain
2Departamento de Producción Vegetal. Departamento de Producción Vegetal. Universitat Politècnica de València, (Valencia) Spain
3Max Planck Institut für Molekulare Pflanzenphysiologie, 14476, (Potsdam-Golm) Germany
4Departamento de Biología Vegetal. Facultad de Farmacia. (Burjassot) Spain
5Max Planck Institut für Molekulare Pflanzenphysiologie, (Potsdam-Golm) Germany

1 Resumen
In plants, Phosphoglycerate kinases (PGK) not only catalyze the conversion of 1,3 bis-phosphoglycerate into 3-phosphoglycerate (3-PGA) in glycolysis but also the reverse reaction in the Calvin-Benson Cycle. We followed a loss-of-function approach to functionally characterize PGK isoforms at the physiological and molecular level. In the databases we found three genes encoding putative PGKs. PGK1 was localized exclusively in the chloroplasts of photosynthetic tissues while PGK2 was expressed in the plastid/chloroplast of photosynthetic and non-photosynthetic cells. PGK3 was ubiquitously expressed in the cytosol of all cell types studied. Measurements of carbohydrate content and photosynthetic activities in PGK mutants and silenced lines corroborated that PGK1 was the photosynthetic isoform while PGK2 and PGK3 were the plastidial and cytosolic glycolytic isoforms respectively. Expression studies in PGK mutants showed that the PGK1 and PGK3 were transcriptionally co-regulated in order to achieve metabolic adjustment. In this sense pgk1.1 pgk3.2 double mutants displayed an intermediate phenotype between pgk1.1 and pgk3.2 indicating a compensatory effect. In addition, double knock-out mutants of PGK3 and the triose phosphate transporter (pgk3.2 tpt3) displayed a drastic growth phenotype but were viable. Our results provide new insight about the functions of PGK isoforms and how they are regulated. The study of double mutants support the complexity and the plasticity of the primary metabolic networks. In this sense, it is emphasized that plastidial and cytosolic glycolysis interacts and co-regulates to each other. Therefore, results obtained in this work support that the plastidial and cytosolic metabolism are intimately connected and that there are regulatory mechanisms that tend to maintain the balance between catabolic and anabolic reactions in the carbon metabolism of plants.

Acknowledgements: This work has been funded by the Spanish Government (BFU2015 and FPI fellowship BES-2010-040265), the European Union (64204R-FEDER) and the Generalitat Valenciana (PROMETEO II/2014/052).
C0070 GRAPEVINE PHENOLOGY, VIGOR AND GRAPE YIELD UNDER CLIMATE CHANGE SCENARIOS (ELEVATED CO2 AND TEMPERATURE): RESPONSE OF FIVE ACCESSIONS OF TEMPRANILLO

Marta Arrizabalaga Arriazu1, Fermín Morales Iribas2, Eric Gomès3, Juan José Irigoyen Iparrea4, Ghislaine Hilbert5, Inmaculada Pascual Elizalde6

1Universidad de Navarra. Plant Stress Physiology Group, Associated Unit to CSIC (EEAD, Zaragoza, and ICVV, Logroño)/Institut des Sciences de la Vigne et du Vin. Bordeaux. UMR 1287 Ecophysiologie et génomique fonctionnelle de la vigne (Navarra/Gironde) Spain/France
2Estación Experimental de Aula Dei (EEAD). CSIC. Department of Plant Nutrition, Zaragoza (Zaragoza) España
3Université de Bordeaux, Institut des Sciences de la Vigne et du Vin. Unité Mixte de Recherche, 1287 Ecophysiologie et Génomique Fonctionnelle de la Vigne. Villenave d’Ormon (Gironde) France
4Universidad de Navarra. Faculty of Sciences. Plant Stress Physiology Group, Associated Unit to CSIC (EEAD, Zaragoza, and ICVV, Logroño), Pamplona (Navarra) Spain
5Institut National de la Recherche Agronomique, Institut des Sciences de la Vigne et du Vin. Unité Mixte de Recherche, 1287 Ecophysiologie et génomique fonctionnelle de la vigne, Villenave d’Ormon (Gironde) France

1 Resumen
Atmospheric CO2 levels and global temperatures are expected to rise in the next decades. Grapevine (Vitis vinifera L.) has been shown to respond to single climate change factors, but the interactive effects of environmental factors needs further research. In this context, exploiting the intra-varietal diversity of grapevine can be a useful tool to adapt viticulture to climate change. The objective was to study the effect of high levels of CO2 and elevated temperature, both individually and combined, on the growth, yield and phenology of five Tempranillo somatic variants. Fruit-bearing cuttings of Tempranillo (accessions 306, T3, 43, 1084 and VN31) were grown from fruit set to maturity, under two temperature regimes (ambient vs ambient + 4°C), combined with two CO2 levels (400 ppm vs 700 ppm), in temperature-gradient greenhouses. The accessions studied showed differences in phenology and vigor, with the 1084 accession showing a longer ripening period and increased vegetative growth at maturity. In terms of grape yield, the accessions VN31, 306 and 43 were the most productive. Considering all the accessions, elevated temperature hastened the ripening period and increased leaf and root dry weight, irrespective of CO2. In contrast, plants grown under high temperature had lower bunch weight and number of berries per bunch, most likely associated with the high temperatures recorded in this treatment (16 days above 40°C). Considering all the accessions, elevated CO2 hastened plant phenology, with a greater effect before veraison. Also, elevated CO2 increased photosynthesis and, consequently, vegetative growth, irrespective of temperature. The results suggest that temperature and CO2 levels representative of the conditions expected for the end of the 21st century may advance berry maturity and increase plant vigor, without marked interactive effects of CO2, temperature and accession.

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C0071 PLANT GROWTH PROMOTION BY ARBUSCULAR MYCORRHIZAL SYMBIOSIS IS CONDITIONED BY N FORM AND FERTILIZATION LEVEL

Gabriela Quiroga García, Gorka Erice Soreasu, Ricardo Aroca Álvarez, Juan Manuel Ruiz Lozano
Estación Experimental del Zaidín Granada (Granada) España

1 Resumen

Most commonly used N fertilization forms are nitrate, ammonium (NH$_4^+$) and urea, being urea the most widespread N fertilizer. In soil urea is degraded to ammonium rapidly. Ammonium, if not taken by plants, is processed to nitrate by soil microorganisms. The aim of this study was to evaluate the regulation of maize growth and metabolism by arbuscular mycorrhizal (AM) fungi in presence of two nitrogen sources, (ammonium and urea) at two contrasting levels and under drought stress. Maize plants were inoculated or not with the AM fungus *Rhizophagus irregularis* and grown for 8 weeks. Half of plants were drought stressed for 2 weeks by irrigating them with half the water volume of well-watered plants. Plants were fertilized using N-free Hoagland solution with three levels of NH$_4^+$ (0, 6 µM and 20 mM) or urea (0, 3µM and 10mM). MA inoculation generally enhanced shoot (SFW) and root growth (RFW) in N-deprived plants and in those cultivated with low NH$_4^+$ or urea concentrations. Nevertheless, AM led to SFW and RFW reductions in well-watered plants fertilized with high NH$_4^+$ and RFW dropped in droughted plants grown at high urea level. Initial (pre-drought) net photosynthesis (A) was enhanced by AM at high NH$_4^+$ and urea. Similar pattern was found 7 days after starting the water treatment in well-watered plants. However, in droughted plants fertilized with high urea, AM made drop A and gs. This trend was more marked 14 days after starting the water treatment, regardless of water regime. Results highlight the importance of deeping on the study of the interactive effects of AM symbiosis with plant N nutrition and to elucidate if the AM symbiosis alters N mobilization through regulation of plant aquaporins and fungal or AM-inducible plant N transporters, conditioning, thus, plant metabolism and growth potential.
C0078 EVALUATION OF THREE CAX1 BRASSICA RAPA MUTANTS GROWN UNDER DIFFERENT CA DOSES

Eloy Navarro León, Luis Romero Monreal, Juan Manuel Ruiz Sáez, Begoña Blasco León
Facultad de Ciencias Granada (Granada) España

1 Resumen

Plant cation/ H+ exchangers (CAXs) are important in the removal of Ca from the cytosol after a peak in response of perturbations in cells. CAXs transporters play a key role in the modulation of cytosolic Ca and in the generation of different Ca profiles to respond environmental cues or signalling process. Furthermore, they store Ca in vacuoles so they could be one possible target gene to raise Ca concentration in crops. Future research should find mutations which maximize Ca accumulation but minimize deleterious phenotypes. Three missense mutations has been generated and identified through TILLING (Targeting Induced Local Lesions In Genomes) in Brassica rapa Ca transporter CAX1: BraA.cax1a-4, BraA.cax1a-7, and BraA.cax1a-12. The aim of this work is to assess the biomass as well as physiological state of these independent alleles grown with different Ca doses, in order to make an initial evaluation for a possible improvement of B. rapa and related crops. To achieve this objective, mutants and the original R-o-18 parent line were grown with different Ca doses: 4 mM CaCl₂ as control, 0.4 mM CaCl₂ as low Ca dose, and 40 mM CaCl₂ as high Ca dose. Ca and chlorophyll concentrations, and PSII fluorescence parameters were determined in mature leaves. The results showed that mutants accumulated more Ca under control and high Ca conditions, and BraA.cax1a-7 and BraA.cax1a-12 presented more foliar biomass under low Ca conditions. However, BraA.cax1a-4 and BraA.cax1a-7 plants registered lower chlorophyll concentrations and PSII fluorescence indicated an altered photosynthetic performance with higher Fo, Fm and K-peak levels and lower Fv/Fm, PI abs, and RC/ABS values. BraA.cax1a-12 mutant was the one with higher foliar biomass under high Ca conditions and only slight effects were observed in their chlorophyll concentrations and its photosynthetic performance. Therefore we might identify BraA.cax1a-12 as a potential mutant for crop breeding.
C0323 ZMBHLH80, ZMBHLH90 AND ZMORPHAN94 ARE NOVEL REGULATORS OF ZMPEPC1 GENE EXPRESSION IN MAIZE

Alicja Gorska1, Paulo Gouveia1, Ana Rita Borba1, Anna Zimmermani1, Tania Serra1, Margarida Oliveira1, Christoph Peterhaenseli2, Nelson Saiboi1

1 ITQB NOVA Oeiras (Lisboa) Portugal
2 Leibniz Universität (Hannover) Germany

1 Resumen

C4 photosynthesis evolved from the C3 metabolism and C4 plants show an improved photosynthetic efficiency. The advantage of these plants results from a specific leaf anatomy and compartmentation of the photosynthetic reactions between two leaf cell types: mesophyll (M) and bundle sheath (BS). The compartmentation of the photosynthetic reactions is achieved by the cell specific expression of major C4 enzymes, such as the maize Phosphoenolpyruvate carboxylase 1 (ZmPEPC1). The ZmPEPC1 transcripts accumulate exclusively in maize mesophyll cells. Previous studies have shown that ZmPEPC1 promoter also directs mesophyll specific and light regulated gene expression in rice, a C3 plant, indicating that rice has the transcription factors (TFs) necessary to regulate ZmPEPC1 cell – specific gene expression. Here, we report the identification and characterization of novel TFs regulating ZmPEPC1 gene expression. Using a Yeast One Hybrid approach, we have identified three maize (ZmOrphan94, ZmCPP8, ZmHB87) and one rice (OsbHLH112) TFs binding to the maize ZmPEPC1 promoter. In addition, we have shown that the maize orthologs (ZmbHLH80 and ZmbHLH90) of the identified rice OsbHLH112 also interact with ZmPEPC1 promoter, highlighting the importance of this regulation in ZmPEPC1 expression. Using a Bimolecular Fluorescence Complementation assay, we have shown that the identified TFs form homodimers and heterodimers. A trans-activation assays in maize protoplasts revealed that ZmbHLH90 acts as activator, whereas ZmbHLH80 and ZmOrphan94 act as repressors of ZmPEPC1 gene expression. Moreover, co-expression of ZmbHLH90 with ZmbHLH80 or ZmOrphan94 suppress the ZmPEPC1 activation induced by ZmbHLH90. Gene expression analysis revealed that ZmbHLH90 is equally expressed between mesophyll and bundle sheath cells, whereas ZmbHLH80 and ZmOrphan94 show higher transcripts accumulations in bundle sheath cells. Furthermore, all the three TFs showed similar gene expression patterns along the day. Altogether, our results suggest that the interplay between the identified maize TFs may contribute to mesophyll cell specific ZmPEPC1 expression.
Photosynthesis is tightly regulated by energy capture and processing. Changes in illumination alter thylakoid protein phosphorylation and reorganization of the photosynthetic machinery. The activity of the STN7 kinase, which is mainly involved in the phosphorylation of the light-harvesting complex II (LHCII) proteins, has been reported to be cooperatively regulated by the redox state of the plastoquinone (PQ)-pool and the ferredoxin-thioredoxin system. The present study aims to investigate the putative role of plastid thioredoxins (Trx) on the regulation of the STN7 kinase and its impact in photosynthesis. This analysis was performed in tobacco plants overexpressing Trx f or m from the plastid genome, or expressing mutant variants of these Trxs carrying double cysteine-serine exchanges in the active site. Our findings demonstrate that overexpression of Trx m, but not Trx f, was associated with a complete loss of LHCII phosphorylation, which was not correlated with a decrease in STN7 levels. The redox state of the PQ-pool in these lines was monitored by gas exchange and OJIP fluorescence transient measurements. The absence of phosphorylation in Trx m-overexpressing plants impeded migration of LHCII from photosystem (PS) II to PSI, with the concomitant loss of PSI-LHCI-LHCII supercomplex formation. Consequently, the thylakoid ultrastructure was altered in this plant, exhibiting a decrease in the number of grana and stacked layers. Moreover, the overexpression of Trx m negatively affected photosynthetic rate and electron transport. We also demonstrated a putative protein-protein interaction between Trx m and STN7 proteins, either directly or through associated partners such as cytochrome b6f complex. Tobacco plants overexpressing the redox mutant of Trx m reverted this phenotype. Our results indicate that the overexpressed Trx m inhibits the STN7 kinase activity in a redox-dependent way and impairs the photosynthetic performance of tobacco plants.
C0259 RAV GENES CONTROL HEADING DATE AND CARPEL DEVELOPMENT IN THE CROP SPECIES RICE

Michela Osnato

CRAG Cerdanyola del Vallès (Barcelona) España

1 Resumen

Among the plant-specific RAV family of transcription factors, TEMPRANILLO 1 (TEM1) and TEM2 were previously shown to negatively regulate the floral transition in Arabidopsis thaliana through direct repression of FLOWERING LOCUS T and AtGA3oxidase1/2 encoding major components of the florigen. Here we identify and study RAV genes from the crop species rice (Oryza sativa) and unravel their regulatory roles in key steps of reproductive development. Our data suggest that like TEMs, OsRAV9/OsTEM1 plays a conserved function as repressor of flowering upstream of the floral activators OsMADS14 and Hd3a through a mechanism reminiscent to that underlying floral transition in temperate cereals. Furthermore, OsRAV11 and OsRAV12 acquired a novel function in the differentiation of the carpel, likely downstream of floral homeotic factors. Our findings reveal conservation of RAV gene function in the regulation of flowering time in monocot and dicot plants, but also uncover new roles in the development of the gynoecium in rice.
Resumen

Thiol-based redox regulation is essential for the rapid adaptation of chloroplast metabolism to dark-light transitions. A classic example of redox-regulated process in chloroplasts is the CO₂ fixation by the Calvin-Benson cycle in which key regulatory enzymes are reduced and fully active during the day and oxidized, hence inactive, during the night. The mechanism of reduction is well-known: photo-reduced ferredoxin (FdB) donates reducing equivalents, via a Fd-dependent Trx reductase (FTR), to the pool of chloroplast thioredoxins (Trxs), which in turn catalyze the light-dependent reduction of biosynthetic enzymes. However, very little is known about the mechanism of oxidative inactivation. Chloroplasts are also equipped with an NADPH-dependent Trx reductase (NTRC), which is the most efficient reductant of the thiol-dependent peroxidase 2-Cys peroxiredoxin (2-Cys Prx), a hydrogen peroxide scavenging enzyme and our group has proposed that NTRC and 2-Cys Prx play a key role of maintaining the reducing capacity of the pool of Trxs thus being essential for chloroplast redox regulation. Since 2-Cys Prxs transfer reducing equivalents from thiolic groups to hydrogen peroxide, we established the hypothesis that these enzymes participate in the oxidative deactivation of biosynthetic enzymes upon darkness. To address this issue, we generated Arabidopsis double mutants knockout for the two 2-Cys Prxs, A and B, present in this plant. The 2cpa-2cpc double mutant showed delayed oxidation of Calvin-Benson cycle enzymes during light-to-dark transitions in contrast to the rapid and complete oxidation observed in wild type plants. Indeed, these enzymes remained partially reduced during the whole period of night. In vitro studies with the purified components suggest that reduced enzymes are oxidized by donating reducing equivalents to Trxs and then to hydrogen peroxide via 2-Cys Prxs. The severe phenotype of 2cpa-2cpc seedlings indicates that this redox system plays an essential role at early stages of plant development.
ROOM: GRAN SALÓN CATALUNYA
17:15-18:00

SESSION 7. METABOLISM AND BIOCHEMISTRY

C0116 XYLOGLUCAN EXOGLYCOSIDASES IN THE GRASS MODEL BRACHYPODIUM DISTACHYON

Diego Rubianes, Elene R Valdivia, Gloria Revilla, Ignacio Zarra Zarra Cameselle, Javier Sampedro

Universidad de santiago de Compostela (A Coruña) España

1 Resumen

Plant growth is a tug-of-war between turgor pressure and the mechanical resistance of the primary wall, which depends on the interaction between cellulose microfibrils and a surrounding matrix of flexible and metabolically-active polysaccharides. In Arabidopsis xyloglucan forms a large part of the matrix and appears to play a role in regulating growth. In grasses and closely related families the amount of xyloglucan is reduced and its role is much less clear.

As a first step in the study of xyloglucan metabolism in grasses, we set out to identify xyloglucan exoglycosidases in the model species Brachypodium distachyon. This species has single orthologs of Arabidopsis α-xylosidase and β-galactosidase genes, which we have named BdXYL1 and BdBGAL1. When BdXYL1 was expressed in an Arabidopsis mutant deficient in xylosidase, xyloglucan composition was restored to almost wild type values. Similarly BdBGAL1 was able to complement a galactosidase-deficient mutant.

Arabidopsis has both soluble and GPI-anchored xyloglucan β-glucosidases. We identified several putative orthologs in Brachypodium. BdBGLC1 appears to be a soluble protein and it can complement an Arabidopsis mutant deficient in glucosidases. BdBGLC2, on the other hand, has a putative GPI-anchored signal. Full length BdBGLC2 had no effect on the mutant, but a version without the anchor sequence partially complemented it. In addition expression of full-length BdBGLC2 resulted in glucosidase activity concentrated in microsomes, suggesting that Brachypodium also has soluble and GPI-anchored β-glucosidases involved in xyloglucan metabolism.

Finally Brachypodium has two orthologs of Arabidopsis xyloglucan fucosidase and both appear to lack a signal peptide, as do orthologs in most other species. BdXFUC1 expression resulted in weak complementation of a fucosidase-deficient mutant, while BdXFUC2 had no effect. Localization studies in tobacco suggest that xyloglucan fucosidases lacking signal peptides can still be exported to the apoplast, possibly through an unconventional pathway that may not work efficiently in Arabidopsis.
C0156 REGULATION OF PEROXULE FORMATION INDUCED BY CADMIUM BY PEROXISOMAL REACTIVE OXYGEN SPECIES

Cristina López1, Antonio Garrido1, Rosa Luque1, Maria Rodríguez-Serrano1, Adela Olmedilla1, Maria C. Romero-Puertas1, Luisa M Sandalio Gonzalez2

1Estación Experimental del Zaidín (Granada) España
2Departamento de Bioquímica, Biología Celular y Molecular de Plantas. Estacion Experimental del Zaidin, CSIC Granada (Granada) España

1 Resumen
Peroxisomes are highly dynamic and metabolically active organelles which play an important role in cellular functions, including the reactive oxygen species (ROS) metabolism. Peroxisomal dynamics, such as proliferation and movement, have been associated with reactive oxygen species (ROS) in plant cells [1]. Recently, we demonstrated that peroxules, (highly dynamic peroxisomal extensions) can be induced by treatment with Cd and As [1]. These structures are regulated by ROS produced by NADPH oxidases, and its function is related to ROS detoxification and signalling transduction [1]. To determine whether peroxules are regulated by other sources of ROS, we analysed the formation of these structures in different Arabidopsis mutants showing altered ROS production associated with peroxisomal photorespiration (glycolate oxidase, GOX) and fatty acid β-oxidation (Acyl CoA oxidase, ACX). Firstly, we characterized the phenotype of Arabidopsis mutants deficient in GOX2 (gox2), the GOX overexpressor, (35S:GOX2) and deficient in ACX1, (acx1). These lines were crossed with Arabidopsis lines expressing the CFP reporter in peroxisomes (px-ck). Confocal analysis of gox2px-ck, 35S:GOX2px-ck and acx1px-ck showed a significant reduction in peroxisomal size in all lines under control conditions. The percentage of peroxule-producing peroxisomes was considerably smaller in 35S:GOX2, but insignificant in gox2. Interestingly, the acx1 lines did not show peroxules under stress conditions. Analysis of PEX11a expression, which regulates peroxule formation, in the different mutants suggests that, in addition to transcriptional regulation of PEX11a, posttranscriptional modifications of this protein may be involved in the regulation of peroxule formation. We therefore concluded that disturbances in peroxisomal metabolism promote changes in the size, number and dynamics of peroxisomes. These organelles discriminate between different ROS sources, which could play an important role in the perception of specific stimuli.


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C0200 METHODS FOR DETECTION OF AUTOPHagy IN SORGHUM bicolor l.

Guillermo Baena Vaca¹, Brett Williams², Liu Ning³, Sagadevan Mundree², José Antonio Monreal¹, Sofía García-Mauriño¹

¹Department of Plant Biology and Ecology, Faculty of Biology, University of Seville (Seville) Spain
²Centre for Tropical Crops and Biocommodities, Queensland University of Technology (Brisbane, Queensland) Australia
³Central Analytical Research Facility (CARF), Queensland University of Technology (Brisbane, Queensland) Australia

1 Resumen
Autophagy is a highly conserved process that is used for the bulk degradation of cellular components following encapsulation in double-membraned vesicles, termed autophagosomes, and degradation in the lysosome (animals) or vacuole (plants). The relevance of autophagy in plant development and responses to stress is widely recognized. In addition, recent experimental evidences show that up-regulation of autophagy by overexpression of autophagy-related genes (ATG) impacts positively on crop production. The functioning of autophagy has been thoroughly studied in the model plant arabidopsis and several crop plants. However, no information regarding the mechanics of autophagy is available for sorghum. Sorghum, a C₄ plant is the fifth cereal crop in the world and has uses as human and animal food as well as biomass for biofuel production. The sorghum genome has been fully sequenced and is available in public databases.

The aim of the work was to detect the activation of autophagy in sorghum using different methods to develop a suite of tools that allow the role of autophagy in development and productivity to be investigated. Sorghum plants were subjected to stressful conditions including nutritional stresses, oxidative stress and salinity that have been reported to activate autophagy in other species.

Autophagy was measured by confocal and TEM, immunodetection of ATG8-PE and real-time qPCR of SbATG6a, SbATG6b and SbATG18a in shoots and roots. Confocal and TEM studies showed an increment in autophagy under most of the stressful conditions, and similar results were obtained by immunodetection of ATG8-PE. Expression of ATG genes correlated well with microscopy and immunoblotting results in some stresses as carbon starvation and salinity, but not upon nitrogen deprivation.

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THE ROLE OF THIOREDOXIN M IN THE REGULATION OF CARBON AND NITROGEN METABOLISM IN TOBACCO PLANTS

**Maria Ancín**, Luis Larraya, Ives Gibon, Inmaculada Farran Blanch

1 Instituto de Agrobiotecnología (Universidad Pública de Navarra-CSIC) (Navarra) Spain
2 Institut National de la Recherche Agronomique and Université de Bordeaux (Aquitaine) France

1 Resumen
Plant carbon (C) and nitrogen (N) metabolisms must be tightly coordinated to sustain optimal growth and development. Primary carbon metabolism requires nitrogen assimilation to build the proteins and chlorophyll of the photosynthetic apparatus and, conversely, nitrogen assimilation requires a continuous supply of energy and carbon skeletons. This means photosynthetic products must be partitioned between carbohydrate synthesis and the synthesis of amino acids. The control of this partitioning constitutes an extremely complex network, involving signals emanating from different metabolites and hormones. In addition, modulation of the redox status of the cell is another important factor that might impact coordination of C and N assimilation. This includes the role of thioredoxins (Trx), small ubiquitous disulfide reductases originally discovered in plants in the context of photosynthesis. Proteomic studies are uncovering a wide range of potential targets for thioredoxins, which could be involved in the regulation of C/N metabolism. The present study aims to investigate the role of plastid Trx m in the C/N metabolism. For that purpose the Trx m gene was overexpressed from the plastid genome of tobacco plants and subsequently analyzed. Compared to wild type, Trx m-overexpressing plants showed a significant decrease in leaf starch and sugar accumulation both in light and dark conditions. However, protein and amino acid levels were significantly higher in these transgenic plants. To further analyze this fact we conducted a metabolomic analysis as well as an enzymatic activity study of the main proteins involved in C and N metabolism. Taking together, our results suggest that Trx m plays a role in the intricate regulatory machinery that coordinates C and N partitioning in plants.
Biologically fixed nitrogen is vital for ecosystems and sustainable agriculture. Capacity for nitrogen fixation relies solely upon the nitrogenase enzyme, which is produced by several bacteria and archaea known as diazotrophs, and they accordingly play a key role in the global ecosystem. This enzyme allows these organisms to fix the atmospheric dinitrogen by catalyzing its reduction into ammonia. This reaction consumes high-energetic compounds, such as ATP, and requires strong biological reductants, making it one of the most metabolically expensive processes in biology. The nitrogenase complex consists of two distinct proteins, dinitrogenase, that binds and reduces N\textsubscript{2} or other substrates, and dinitrogenase reductase, which has the specific role of passing electrons one at a time to dinitrogenase. Most Biological nitrogen fixation is produced in symbiotic associations, since plants provide the high energetic requirements for the dinitrogen fixation. The principal symbioses are those between rhizobia and legumes, the actinomycete Frankia and actinorhizal plants, and Cyanobacterial-plant interactions (Azolla, Gunnera, Cycads). The low redox potential and the high reactivity of nitrogenases demand a reducing environment with an absence of O\textsubscript{2}, as this molecule irreversibly denatures nitrogenases by reacting with the metal clusters of the enzyme and disrupting the proteins that supply them with reductant. Diazotrophs have devised an important variety of strategies to protect the enzyme from molecular oxygen. These strategies can be summarized as: avoidance of an aerobic environment; physical barriers around the nitrogenase enzyme; prevention of oxygen diffusing to the enzyme; metabolic removal of oxygen by increasing the respiratory rate by nitrogenases itself or via hydrogenases reducing hydrogen ions to molecular hydrogen; and the use of antioxidant systems such as superoxide dismutases (SODs) to reduce active oxygen species; by conformational change of nitrogenase under high oxygen levels, rendering the enzyme oxygen-tolerant, but catalytically inactive; and temporal separation of nitrogen fixation and respiration.
C0274 A SPECIFIC ROLE OF TOMATO PIF1A IN SENESCENCE?

Miguel Simón-Moya¹, Daniele Rosado², Lucio D Andrea¹, Giovanna Gramegna², Magdalena Rossi², Manuel Rodríguez-Concepción¹

¹Centre for Research in Agricultural Genomics Bellaterra (Cerdanyola del Vallés) (Barcelona) España
²Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo (São Paulo) Brazil

1. Resumen

Phytochrome-Interacting Factors (PIFs) are a family of basic helix–loop–helix (bHLH) transcription factors that play different roles during plant development. In Arabidopsis thaliana, PIFs are involved in seedling deetiolation, shade avoidance, flowering, chloroplast development, elongation growth, or leaf senescence, among other light-regulated processes. However, little is known about the function of PIFs in other plants. Previous work in our lab showed that one member of the PIF family in tomato, PIF1a, is involved in the regulation of carotenoid accumulation during tomato fruit ripening. A careful examination of transgenic lines with a constitutively silenced PIF1a gene further suggested that this transcription factor might be involved in senescence. First, tomato fruit with reduced PIF1a activity did not show senescence symptoms when incubated in the dark. Second, transient expression experiments in Nicotiana benthamiana leaves confirmed that the PIF1a protein (but not other tomato PIF homologues) is able to promote senescence. In contrast with these results, the Arabidopsis pif1 mutant shows no senescence-associated phenotypes, whereas mutants for PIF3, PIF4, and PIF5 showed a delayed senescence of dark-incubated leaves. Experiments to address the molecular basis of such differences between Arabidopsis and tomato PIF homologues are in progress.
C0276 METABOLIC CHANNELING OF PHE FOR LIGNIN BIOSYNTHESIS IN MARITIME PINE

Fernando de la Torre Fazio¹, Jorge El-Azaz Ciudad¹, Belén Pascual Moreno¹, Jean-François Trontin², Sandrine Debille², Francis Canlet², Concepción Ávila Sáez¹, Francisco M. Cánovas Ramos¹

¹Universidad de Málaga (Málaga) España
²FCBA Technological Institute (Pierroton) France

1 Resumen
Phenylalanine (Phe) is the main precursor of phenylpropanoids biosynthesis in plants. This vast family of Phe-derived compounds can represent more than 30% of captured photosynthetic carbon, playing essential roles in plants such as cell wall components, defense molecules, pigments and flavors. In addition to its physiological importance, phenylpropanoids and particularly lignin, a component of wood, are targets in plant biotechnology.

The arogenate pathway has been proposed as the main pathway for Phe biosynthesis in plants (Maeda et al., 2010). The final step in Phe biosynthesis, catalyzed by the enzyme arogenate dehydratase (ADT), has been considered as a key regulatory point in Phe biosynthesis, due to its key branch position in the pathway, the multiple isoenzymes identified in plants and the existence of a feedback inhibition mechanism by Phe. So far, the regulatory mechanisms underlying ADT genes expression have been poorly characterized, although a strong regulation of the Phe metabolic flux should be expected depending on its alternative use for protein biosynthesis versus phenylpropanoid biosynthesis. This second fate involves a massive carbon flux compared to the first one.

Here we report our current research activities in the transcriptional regulation of ADT genes by MYB transcription factors in Pinus pinaster. The conifers channels massive amounts of photosynthetic carbon for phenylpropanoid biosynthesis during wood formation. We have identified the complete ADT gene family in maritime pine (El-Azaz et al., 2016) and a set of ADT isoforms specifically related with the lignification process. The potential control of transcription factors previously reported as key regulators in pine wood formation (Craven-Bartle et al., 2013) will be presented.
C0318 CHARACTERIZATION OF TOMATO UDP-GLUCOSE STEROL GLYCOSYLTRANSFERASES

Karla Ramírez Estrada1, Nidia Castillo1, Juan Alejandro Lara1, Daniel Torres1, Montserrat Arró2, Albert Boronat3, Albert Ferrer2, Teresa Altabella4

1 C.R.A.G. Consorcio CSIC-IRTA-UAB Bellaterra (Barcelona) España
2 Departamento de Bioquímica y Fisiología. Facultad de Farmacia. Universidad de Barcelona CRAG. Consorcio CSIC-IRTA-UAB (Barcelona) España
3 Departamento de Bioquímica y Biomedicina Molecular, Facultad de Biología. Universidad de Barcelona. CRAG. Consorcio CSIC-IRTA-UAB (Barcelona) España
4 Departamento de Biología, Sanidad y Medio Ambiente. Facultad de Farmacia. Universidad de Barcelona. Consorcio CSIC-IRTA-UAB (Barcelona) España

1 Resumen
Sterol glycosyltransferases (SGTs) catalyze the glycosylation of the free hydroxyl group at C-3 position of sterols to produce sterol glycosides. Glycosylated sterols and free sterols are primarily located in cell membranes where in combination with other membrane-bound lipids play a key role in modulating their properties and functioning. In contrast to most plant species, plants of the genus Solanum contain very high levels of glycosylated sterols, which in the case of tomato may account for more than 85% of the total sterol content. In this work we report the identification and functional characterization of the four members of the tomato (Solanum lycopersicum cv MicroTom) SGT gene family. Expression of recombinant SlSGT proteins in E. coli followed by in vitro enzyme activity assays demonstrated the ability of the four enzymes to glycosylate brassicasterol, campesterol, stigmasterol and b-sitosterol from UDP-glucose as a sugar moiety donor. Subcellular localization studies based on fluorescence recovery after photobleaching and cell fractionation analysis, revealed a loose association of SlSGT1 and SlSGT3 with the plasma membrane whereas SlSGT2 and SlSGT4 behave as more soluble cytosolic proteins. The SlSGT genes have specialized but still largely overlapping expression patterns in different organs of tomato plants and throughout the different stages of fruit development and ripening. Moreover, they are differentially regulated in response to biotic and abiotic stress conditions. SlSGT4 and SlSGT2expression increases markedly in response to osmotic, salt and cold stress, as well as upon treatment with abscisic acid. On the contrary, SlSGT1 and SlSGT3 expression remains unaltered under most of the stress conditions tested. Overall, this study contributes to broaden the current knowledge on plant SGTs and provides support to the view that tomato SGTs play overlapping but not completely redundant biological functions involved in mediating developmental and stress responses.
C0064 THE COMBINATION OF IRRIGATION STRATEGY AND MYCORRHIZAL INOCULATION CAN IMPROVE BERRY QUALITY OF TEMPRANILLO GRAPEVINES IN A CLIMATE CHANGE SCENARIO

Nazareth Torres Molina, Nieves Goicoechea Preboste, M. Carmen Antolín Bellver

Universidad de Navarra Pamplona (Navarra) España

1 Resumen

Over South Europe Mediterranean region, the projected warming combined with severe droughts in the growing season is expected to have detrimental impacts on grape berry quality. Within this new scenario, deficit irrigation has emerged as a potential strategy to withstand water stress. Besides, symbiotic association with arbuscular mycorrhizal fungi (AMF) produces numerous benefits to host plants and can help them to cope with abiotic stresses. Therefore, the aims of this research were: 1) to characterize the response of three clones of Vitis vinifera L. cv. Tempranillo to different irrigation programs under elevated temperatures, and 2) to determine whether AMF inoculation can improve berry antioxidant properties under these conditions. The study was carried out on three fruit-bearing cuttings clones of cv. Tempranillo (CL-260, CL-1089 and CL-843) inoculated (+M) or not (-M) with AMF and subjected to two temperature regimes (24/14°C and 28/18°C (day/night)) combined with three irrigation regimes during berry ripening. Irrigation treatments were: (i) water deficit from fruit set to veraison (early deficit, ED); (ii) water deficit from veraison to maturity (late deficit, LD); and (iii) full irrigation (FI). Although each clone of Tempranillo seemed to have different abilities to respond to elevated temperatures and water supply, in general, at 24/14°C LD treatment performed better than ED. Differences among clones were attenuated at 28/18°C. In addition, potential benefits of LD treatment were improved by AMF inoculation. Thus, in all clones the loss of anthocyanins at 28/18°C detected in –M plants after applying LD did not occur in the +M treatment. Moreover, AMF inoculation increased (CL-1089) or maintained (CL-843) must antioxidant capacity at 28/18°C regardless of irrigation system applied. Our results suggest that the implementation of measures to promote the association of grapevines with AMF could contribute to optimize effects of irrigation strategy on berry properties under future warming conditions.
C0104 WATER AVAILABILITY IN LEAVES OF CITRUS PLANTS DETERMINES THE ABA ACCUMULATION IN ROOTS UNDER WATER STRESS

Matias Manzi, Marta Pitarach-Bielsa, Carlos de Ollas, Damián Balfagón, Vicent Arbona
Ecofisiología y Biotecnología. Dept. Ciencias Agrarias y del Medio Natural. Universitat Jaume I Castellón (Castellón) Spain

1 Resumen
The reduction in soil water potential appears to be the signal to induce the abscisic acid (ABA) accumulation at the entire plant level. Under this context the root system become essential in sensing the decrease in water availability. However, it is established that leaves are able to detect the changes in the evaporative demand and trigger the ABA accumulation without any involvement of roots. In this work, the influence of leaf water status on the ABA accumulation in roots was evaluated by keeping the canopy of citrus plants under a high humidity atmosphere and subjecting the roots to dehydration. Whereas in leaves and roots of totally dehydrated plants ABA increased as expected, plants with turgid leaves and dehydrated roots were unable to show a similar response and ABA remained almost unaltered in both organs. The relative gene expression of the 9-cis-epoxycarotenoid dioxygenase1 (NCED1), the rate-limiting carotenoid cleavage enzyme in the ABA pathway, was strongly influenced by the water availability in the leaves and showed a marginal influence of the water status of roots. In this sense, the NCED1 expression in roots was induced in completely dehydrated plants whereas in turgid leaves/dehydrated roots treatment the expression was unchanged. Results suggest that, in contrast to previous reports, leaves trigger the ABA accumulation at whole plant level in a concerted manner after the reduction in water availability in this organ. Overall, data agree with previous finding indicating that leaves are a relevant source of ABA to the roots during water deprivation.

Keywords: ABA transport, carotenoids, drought, 9-cis-epoxycarotenoid dioxygenase (NCED), shoot-to-root transport, water stress.

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C0125 MODULATION OF THE ANTIOXIDANT SYSTEM IS ASSOCIATED TO CITRUS TOLERANCE TO COMBINED CONDITIONS OF ABIOTIC STRESS

Damián Balfagón Sanmartín, Sara Izquierdo Zandalinas, Vicent Arbona Mengual, Carlos de Ollas, Marta Pitarch-Bielsa

UJI (Castellón) España

1 Resumen
Drought and high temperatures are two major abiotic stress factors that often occur simultaneously in nature, affecting negatively crop performance and yield. Moreover, these environmental challenges induce oxidative stress in plants through the production of reactive oxygen species (ROS). Carrizo citrange and Cleopatra mandarin are two citrus genotypes with contrasting ability to cope with the combination of drought and heat stress. In this work, a direct relationship between an increased antioxidant activity and stress tolerance is reported. According to our results, the ability of Carrizo plants to efficiently coordinate SOD, CAT and APX activities involved in ROS detoxification along with the maintenance of a favorable GSH/GSSG ratio could be related to their relative tolerance to this stress combination. On the other hand, the increment of SOD activity along with the lack of CAT and APX activation in Cleopatra plants in response to situations of combined stress, could contribute to the increased oxidative stress and the higher sensibility of this citrus genotype to the combination of drought and high temperatures.

This work has been supported by MINECO (AGL2016-76574-R) and UJI (B2016-23/B2016-24).
C0225 EXOGENOUS SALICYLIC ACID AS AN OLIVE TREE PHYSIOLOGICAL REGULATOR DURING DROUGHT AND POST-DROUGHT RECOVERY

Cátia Brito1, Lia-Tania Dinis1, Glória Ferreira1, Mónica Mejón3, Luís Valledor3, José Moutinho-Pereira1, Carlos Correia1

1Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (Vila Real) Portugal
2Department of Biology & CESAM – Centre for Environmental and Marine Studies, University of Aveiro, Campus Universitário de Santiago (Aveiro) Portugal
3Plant Physiology, Department B.O.S., Faculty of Biology, University of Oviedo (Oviedo, Asturias) Spain

1 Resumen
In Mediterranean-type ecosystems is recurrent the occurrence of drought and rewatering periods, a growing problem regarding the increased risk of extreme events, compromising olive tree development processes. Therefore, must be implemented agronomic strategies displaying the ability to offset the negative effects of drought and to improve the respective recovery capacity. We propose the use of salicylic acid (SA), a signaling phytohormone that plays a key role in stress responses. During the summer season, in Vila Real, Portugal, three-years-old potted olive trees (cv. Cobrançosa) sprayed with 0 (D) and 100 µM SA (DSA) were subjected to three cycles of drought, by withholding water, and rewatering, while others, sprayed with 0 µM SA, were continuously irrigated (CI). Physiological responses were monitored throughout the experiment and in the end biomass accumulation was accessed. The results revealed that SA ameliorates olive tree physiological activity during drought, including net photosynthesis and water status, concomitant with higher concentration of chlorophylls, soluble sugars and proteins and starch depletion in leaves. SA also induced a faster recovery of net photosynthesis and water status after stress relief. After the recovery phase, previously droughted plants still exhibited an intense abscisic acid immunohistochemical signal in leaves, revealing D plants a special accumulation close to the upper epidermis. Concerning the indole-3-acetic acid, was observed a crescent signal intensity with the order D, DSA and CI, suggesting a parallel active growth. Finally, SA alleviates the drought-induced decline in biomass accumulation. In conclusion, the results demonstrate the role of SA in regulating drought and recovery responses of olive trees, and claim that SA application is a very promising short-term solution to implement in rainfed olive growing areas.
C0189 DURATION OF DEVELOPMENTAL PHASES, AND DYNAMICS OF LEAF APPEARANCE AND TILLERING, AS AFFECTED BY SOURCE AND DOSES OF PHOTOPERIOD INSENSITIVITY ALLELES IN WHEAT UNDER FIELD CONDITIONS

Helga Ochagavia Orbeagoa1, Paula Prieto1, Roxana Savin1, Simon Griffiths2, Gustavo Slafer1

1Universidad De Lleida (Lleida) España
2John Innes Centre (Norwich) United Kingdom

1 Resumen

Variation in photoperiod sensitivity in wheat plays a major role in the crop adaptation to wide agronomic environments. Photoperiod insensitivity is provided by Ppd-Aa, Ppd-B1a and Ppd-D1a alleles. Effects of the genome, doses and source of the particular Ppd-1a alleles on time to anthesis has not been analysed simultaneously in the same experiments, even less under field conditions; and the effects on particular phases rather than on considering only the total time to anthesis and on phyllochron have not been considered for this range of allele combinations. We carried out field experiments during two consecutive growing seasons to assess differences in time to anthesis, in its component phases, in final leaf number, phyllochron and tillering across wheat isogenic lines differing in specific Ppd-1a alleles (genome), doses, and source of one of the alleles. Beyond confirming that the introgression of Ppd-1a alleles advanced anthesis time, we found that the effects of particular alleles largely depended on the source and Ppd-B1a could tend to be stronger or be clearly weaker than Ppd-D1a depending on the donor considered. All components of time to flowering (the particular sub-phases as well final leaf number and phyllochron of late-appearing leaves) were sensitive to Ppd-1a alleles, but the strength of particular alleles on particular components was noticeably different, so that similar adjustments in time to anthesis could be achieved with different partitioning of developmental time between the considered phases. Also, beyond confirming the effect of these alleles on final leaf number, we found that although they did not affect phyllochron of the first 7 leaves, that of the leaves appearing later was consistently reduced when Ppd-1a alleles were introgressed. Tillering was sensitive too, but not final number of spikes due to compensations between tillering and tiller mortality.
C0262 ELEVATED AIR HUMIDITY INCREASES UV-MEDIATED DAMAGE IN PISUM SATIVUM DUE TO CHANGES IN EPICUTICULAR WAX AND REDUCED FLAVONOID CONTENT

Sheona Noemi Innes¹, Louise E. Arve², Line Nybakken¹, Tone Melby¹, Boris Zimmerman¹, Knut Asbjørn Solhaug¹, Jorunn E. Olsen¹, Sissel Torre¹

¹Norwegian University of Life Sciences (Akershus) Norway
²Norwegian Food Safety Authority (Oslo) Norway

1 Resumen

Growth in high air humidity (RH, > 85%) affects plant stomatal function and causes diminished response to closing signals. We hypothesised that exposure to UV radiation may induce stomatal closure in a background of high RH and thereby re-establish stomatal function. We tested the effects of UV exposure on pea plants (Pisum sativum) grown in moderate (60%) or high (90%) RH. Stomatal conductance analyses supported our hypothesis through indication of reduced stomatal conductance in plants exposed to UV radiation. However, UV exposed plants also showed signs of injury, including leaf curling and chlorosis, which was significantly greater in plants grown in high RH. CPD-DNA quantification indicated that UV exposed plants grown in high RH had significantly more DNA damage than any other treatments. HPLC analysis of flavonoid content in leaves indicated that plants grown in high RH had lower flavonoid content than those grown in moderate RH, and plants exposed to UV radiation had less than non-UV exposed controls. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to determine how UV radiation affects the epicuticular wax layer, and whether or not this was affected by background RH. The results indicate an effect of both RH and UV radiation on epicuticular wax composition.
C0305 EFFECT OF ELEVATED INORGANIC CARBON ON THE CYTOSOLIC HOMEOSTASIS OF NITRATE IN THE MARINE ANGIOSPERM POSIDONIA OCEANICA (L.) DELILE.

Lourdes Rubio1, Delia García Pérez, José Antonio Fernández García

Facultad de Ciencias. Universidad de Málaga (Málaga) España

1 Resumen

The marine angiosperm Posidonia oceanica is a mediterranean endemism of great ecological significance. As other marine plants, P. oceanica has adapted secondarily to the marine environment and develop anew different mechanisms to colonize it. Among others, this plant has developed a plasma membrane system for the direct uptake of bicarbonate. In this work we have developed both NO3- and Cl- selective microelectrodes for the continuous monitoring of the intracellular (cytosolic) NO3- and Cl-. In the light, leaf mesophyll cells show a cytosolic NO3- concentration of 5.7±0.2 mM (n=10), while in the dark cytosolic NO3- raises up to 8.7±1.1 mM; these values are in the range of concentrations quoted for Arabidopsis thaliana (Cookson et al., 2005). The enrichment of natural seawater (NSW) with 3 mM NaHCO3 caused a decrease of the cytosolic NO3- concentration of 1 mM and a decrease of the cytosolic concentration of Cl- of 3.5 mM. The saturation of NSW with 1000 µL CO2 L-1 produced a lower diminution of the cytosolic NO3- (0.3 mM).

In the presence of 0.1 mM of the plasma membrane permeable inhibitor of the carbonic anhydrase (EZ) the diminution of cytosolic NO3- caused by the same concentration of CO2 was much lower, 0.1 mM. The addition of inorganic carbon, either HCO3- or CO2, has an effect on the cytosolic mechanisms for anionic homeostasis, one of which is the opening of the slow anion channels. These channels are permeable to NO3- and Cl- and could elicit the efflux of these ions. In P. oceanica, the response in the presence of EZ points out that the inorganic carbon species that cause the NO3-/Cl- efflux is HCO3-. This effect could contribute to plant biomass N dilution observed in elevated CO2.

References:

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C0315 GAS EXCHANGE AND CHLOROPHYLL FLUORESCENCE OF “GOLDEN DELICIOUS” AND “FUJI” APPLE TREES PROTECTED BY A GREY ANTI-HAIL COVER

Carlos Correia, Ermelinda Silva, Cátia Brito, Luis Pinto, Helena Ferreira, Ana Luzio, Lia Dinis, José Moutinho Pereira, Manuel A. Rodrigues, Alexandre Gonçalves

1Universidade de Trás-os-Montes e Alto Douro Vila Real (Trás-os-Montes e Alto Douro) Portugal
2Instituto Politécnico de Bragança (Trás-os-Montes e Alto Douro) Portugal

1 Resumen

The frequency and severity of hail events in some apple growing areas of the Iberian Peninsula may increase in the future in the context of climate change. One technological option to minimise potential damages is the use of over-tree netting. Moreover, anti-hail nets can also provide additional benefits by reducing plant stress, sunburn and wind speed. In this work, carried out during 2016, was tested the application of a grey anti-hail net in a commercial orchard with two cultivars (Golden Delicious and Fuji) of apple tree (Malus domestica Borkh.). Although it reduced the photosynthetically active radiation, the grey net had no negative effects on the photosynthetic performance of both apple tree cultivars during the summer season. On contrary, the anti-hail net contributed to the improvement of net photosynthetic rate of “Fuji” plants close to harvest, namely during the afternoon period, reducing the degree of photosynthesis depression from the morning to the afternoon. A strictly association was observed between photosynthesis and stomatal conductance, although non-stomatal limitations to photosynthesis, that include changes in effective photochemical quantum yield of PSII and in electron transport rate, also occur in open sky plants. Therefore, the use of a grey anti-hail net on apple orchards appears as an interesting tool for the protection of apple trees against hail ensuring an adequate photosynthetic activity.

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SESSION 9. ABIOTIC STRESS

C0044 PRIMING EFFECT OF MENADIONE SODIUM BISULPHITE AGAINST SALINITY STRESS IN ARABIDOPSIS INVOLVES EPIGENETIC CHANGES IN GENES CONTROLLING PROLINE METABOLISM

David Jiménez-Arias¹, Juan C. Luis Jorge², Francisco Valdés², Francisco J. García-Machado¹, Luisa M. Sandalio³, José A. Pérez³, Andrés A. Borges Rodríguez¹

¹Instituto de Productos Naturales y Agrobiología - CSIC, Campus de Anchieta, La Laguna (Santa Cruz de Tenerife) España
²Grupo de Biología Vegetal Aplicada, Departamento de Biología Vegetal—Facultad de Farmacia, Universidad de La Laguna, Tenerife, Canary Islands, Spain (Santa Cruz de Tenerife) España
³Departamento de Bioquímica, Biología celular y Molecular de Plantas, Estación Experimental del Zaidín-CSIC, Granada, Spain (Granada) España

1 Resumen

Plants are able to develop numerous defence strategies to face stress. Amongst these, higher plants are capable of demonstrating stress imprint, a mechanism related with the phenomenon of priming. This is usually defined as genetic or biochemical modifications induced by a first stress exposure that leads to enhanced resistance to a later stress. Menadione sodium bisulphite (MSB), a water-soluble addition compound of vitamin K3, was first studied as a plant growth regulator and has been later widely shown to function as plant defence activator against several pathogens in a number of plant species. We recently reported that treating Arabidopsis seeds with MSB primes salt tolerance by inducing an early acclimation to salt stress. Here we describe the analysis of the effect of MSB on cytosine methylation in a salt stress background demonstrating that one of the mechanisms underlying this early acclimation to salt stress is an epigenetic mark. Specifically, MSB leads to a hypomethylation state at the promoter region of genes involved in the biosynthesis (P5CS1) and degradation (ERD5) of proline, affecting mainly CHG and CHH sites (where H is any nucleotide except G). The epigenetic changes detected are correlated with the observed expression patterns of P5CS1 (upregulation) and ERD5 (downregulation) genes and the increase in proline accumulation.

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C0106 ARGININE, POLYAMINES AND GABA ACCUMULATION REMARK THE ROLE OF UREA CYCLE ON M. TRUNCATULA SEEDLINGS GROWN UNDER DIFFERENT N SOURCES

Daniel Cerdan1, Javier Buezo1, Raquel Esteban2, Alfonso Cornejo3, Victor Martinez-Merino3, Maria Jose Gil1, Jose Fernando Moran Juez4, Beatriz Royo1

1Institute of Agrobiotechnology, IdAB-CSIC-UPNA-Government of Navarre, Pamplona, Spain
2Universidad del País Vasco, Bilbao, Spain
3Public University of Navarra, Department of Chemistry (Pamplona) Spain
4Instituto de Agrobiotecnologia Multiva (Navarra) España

1 Resumen

Plants may be affected by environmental stresses disturbing their grown and development. As a consequence, they have evolved various regulatory strategies, such as the production of molecules with protective functions. Polyamines (PA) are a group of nitrogenous compounds that play roles in cell growth and development, as well as, in relation to stress-associated processes, which functions are not well understood. The most commonly PAs found in higher plants are putrescine (Put), spermidine (Spd) and spermine (Spm). Interestingly, PA catabolism may produce hydrogen peroxide. Therefore, we aimed to identify the differential effect of NH4+ and urea as the sole N source in comparison to NO3-based nutrition on the urea cycle and polyamines synthetic pathways in 15-days old M. truncatula plants grown under axenic cultures to avoid interference from microbial signaling. Our results indicate that NH4+-grown plants significantly accumulated higher amounts of PA. In shoots, Spd and Put were mainly found, whereas Put clearly was the prevalent PA in roots. The relative content of Arg was significantly higher in both tissues of plants cultured at high doses of NH4+. Besides, these plants exhibited the lowest relative content of GABA. No remarkable differences were found in ROS content. Our data evidences that the oxidative metabolism of PAs is restricted in NH4+-fed plants, especially at high doses of N. Thus, under NH4+ nutrition the PAs pathway may be blocked by the effect of NH4+ as a product from the catalytic steps of PA-oxidases.

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C0127 DIFFERENTIAL RESPONSES OF ROOT MEMBRANE POTENTIAL TO SALINITY, PH, AND CARBONATE IN TWO CONTRASTING ARABIDOPSIS ACCESSIONS

Benet Gunsé Forcadell, Laura Pérez Martín, Joana Terés Gelabert, Charlotte Poschenrieder Wiens

Fisiología Vegetal. Biociències (Barcelona) España

1 Resumen
Natural populations of Arabidopsis thaliana can locally adapt to salinity or moderately carbonated soils. Combination of both factors that limit crop productivity in many coastal areas is poorly explored in this species. The aim here was to assess early root membrane responses of two A. thaliana accessions differing in salt and carbonate tolerance to pH, salinity and carbonate, alone and in combination. Plants from two A. thaliana accessions, T6 (coastal, carbonate-sensitive) and A1 (inland, moderately carbonate-tolerant) were grown in nutrient solution at pH 5.9 and exposed to Na⁺ and NaHCO₃ both at pH 5.9 and 8.9 in buffered solutions. Root membrane potential (MP) of both accessions was measured to visualize differences in the early responses to the three factors. Under control conditions, T6 had lower MP than A1. Both high pH and Na⁺ affected membrane potential of T6, while bicarbonate had a main effect when plants were exposed to high pH. MP of LLM2 was unaffected by Na⁺ at pH 5.9, but HCO₃⁻ caused a significant increase. Rising the pH increased MP in A1, while bicarbonate had no further effect.

The observation that the MP of the adapted accessions react to the corresponding stress factor in a more sensitive way than the sensitive accession suggests that lowering of MP can act as a fast stress signaling mechanism (alarm phase) allowing activation of tolerance mechanisms.

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C0148 ABSCISIC ACID AND SALINE STRESS RESPONSES IN SYMBIOTIC GREEN MICROALGA TREBOUXIA SP. TR9 ISOLATED FROM THE LICHEN RAMALINA FARINACEA

Ernesto Hinojosa Vidal1, Francisco Marco Picó2, Fernando Martínez Alberola1, Pedro Carrasco Sorlí1, Eva Barreno Rodríguez1

1Universitat de València, Inst. “Cavanilles” de Biodiversidad y Biología Evolutiva, Botánica, Fac. CC. Biológicas (Valencia) España
2Universitat de València, Dept. Biología Vegetal (Valencia) España
3Universitat de València, Dept. Bioquímica y Biología Vegetal, Fac. CC. Biológicas (Valencia) España

1 Resumen

Tolerance to saline conditions in plants involves a set of multiple stress-related genes that cross-talk with other components of stress-signaling transduction pathways. Phytohormone Abscisic acid (ABA) controls a series of downstream stress responses and integrates signaling from saline, thermal and drought stress conditions. The role of ABA in abiotic stress management has been thoroughly studied in land plants, and to an extent in halophyte, free-living microalgae, but evidence of its effects on lichen microalgae is scarce to nonexistent. Since lichen phycobionts are capable of enduring harsh, restrictive and rapidly changing environments, such as saline environments, it is necessary to study the metabolic machinery that operates in these extreme conditions. Sequencing and annotating the genome of the symbiotic microalga Trebouxia sp. TR9 allowed us to identify the genes involved in ABA biosynthesis and ABA-mediated responses to saline stress. We quantified the expression levels of this set of genes in cultures of this phycobiont subjected to a wide range of salinity concentrations, and determined the endogenous concentrations of the phytohormone. Our results suggest that when Trebouxia sp. TR9 is confronted with extreme saline stress environmental conditions the organism displays a rather different molecular response than land plants and free-living halophile microalgae, with no noticeable increase in ABA-related gene activity and no significant increase in endogenous ABA levels. Despite this, the involved genes are fully functional and responsive to exogenous ABA, suggesting that symbiotic green alga may have developed a different molecular pathway to cope with highly saline environments. (GVA, PROMETEOII/2013/021; MINECO, CGL2016-79158-P; FEDER).
C0152 ABSCISIC ACID PARTICIPATES IN ROOT DEVELOPMENTAL AND HYDRAULIC RESPONSES TO WATER DEFICIT IN ARABIDOPSIS

Miguel Angel Rosales Villegas, Julie Gasc, Christophe Maurel, Philippe Nacry

Biochemistry and Plant Molecular Biology (B&PMP), UMR5004, INRA/CNRS/Montpellier SupAgro/Université Montpellier (Montpellier) France

1 Resumen

Plants have to constantly adjust their water status during development and in response to variations in environmental conditions, recently amplified by climate change. By exploring the soil and taking up water, plant roots play a crucial role in these processes. Water uptake by roots is determined by their architecture, which results from root growth and branching, and the hydraulics of root cells and tissues. Abscisic acid (ABA) is a phytohormone that plays a major role in the adaptation of plants to drought, participating in both growth and hydraulic properties of roots. This work addresses the integration at whole root level of mechanisms that determine the root hydraulic architecture of Arabidopsis, and the role of ABA in its regulation in response to water deficit. For this purpose, root system architecture and root hydraulic conductivity (Lpr) were analyzed in Arabidopsis plants grown in hydroponics after 5 days of polyethylene glycol (PEG8000) treatments to induce different water deficit levels. Results showed a dual-response to stress: an increase in both number and length of lateral roots and in the hydraulic conductivity (Lpr) of the whole root system under mild water deficit, whereas these parameters were reduced with higher osmotic stress levels. Analysis of root architecture and hydraulics in different ABA-signaling mutants and exogenous-ABA treatments was performed to uncover the involvement of ABA in this dual-response to water deficit.
C0228 DESIGN OF AN AFFORDABLE PHENOTYPING PLATFORM TO CHARACTERIZE HORMONAL CONTROL OVER TRANSPIRATION RELATED RESPONSES IN ARABIDOPSIS THALIANA

Carlos de Ollas Valverde, Jorge Collado Dominguez, Marta Pitarch Bielsa, Vicente Vives Peris, Aurelio Gómez Cadenas

Universidad Jaume I Castellon (Castellon) España

1 Resumen

In the last decade, high-throughput screening of plant populations has become more affordable and popular. However, automated plant phenotyping platforms require a huge economic investment and trained technical expertise. Alternatives to highly automated platforms with little investment are possible. We present the design and development of a high-throughput phenotypic platform to obtain accurate and reproducible data of plants responses to variable soil moisture. Assessing the transpiration phenotype of a line is not an easy task, plants gas exchange depends of both genetic constraints and environmental conditions like soil moisture, VPD, light intensity and quality and CO₂ concentration. A standard environmental chamber controls atmospheric variables but soil moisture is one of the most critical variables affecting transpiration hence a reproducible yet easy way to establish and record soil moisture is gravimetric relative soil water content. This is achievable using peat jiffy pellets as weight among different units (both dry and under water saturation conditions) is very homogeneous. Coupling this characteristic with a simple scale allows a quick calculation of the relative amount of soil water content and plant evapotranspiration (daily or in any scale of time). Avoidance of noise from soil transpiration can be accomplished using tape sealed handmade plastic cups, allowing only the rosette out of the system. Rosette size and relative growth can be measured with a digital camera and free software as “easyleafarea” can be used to normalize transpiration per unit of area. Gas exchange can be recorded in parallel with an IR equipment (instantaneous gas exchange versus cumulative). Destructive harvesting includes shoot fresh/dry weight and material for hormonal/metabolomic and transcriptomic. A random design allows 100 individual (12 plants per line) a week with good inter-batch reproducibility. An example of the use of this platform with different mutants in the JA biosynthesis is shown.
C0301 IDENTIFICATION OF UBIQUITIN LIGASE E3 INVOLVED IN PROTEASOMAL DEGRADATION OF ARABIDOPSIS MAPKKK18

Małgorzata Tajdel-Zielinska, Małgorzata Marczak, Agnieszka Ludwikow

Adam Mickiewicz University in Poznan (Wielkopolska) Poland

1 Resumen

The ubiquitin proteasome system (UPS) function as one of the principle mechanisms for control of protein function. UPS plays major roles in regulation of many physiological processes including hormone signaling, e.g. abscisic acid (ABA). Degradation of ABA regulators is crucial for resetting ABA signaling. ABA-activated kinases and protein phosphatases, as well as other proteins are degraded by the UPS. Ubiquitination is an enzymatic process that involves covalent conjugation of ubiquitin (Ub) to a specific lysine residue(s) in a target protein. This tagging reaction, is well known however the protein components involved in this process are usually target specific. The specificity is achieved by the Ub E3 ligases that are the most important for regulation of the ubiquitination process.

Available studies demonstrate that the stability of ABA-induced MAPKKK18 is regulated by proteasomal degradation via the ABA core pathway. However, the exact mechanism of MAPKKK18 protein turnover remains to be determined. Thus our efforts are focused on identification of mechanism driving MAPKKK18 turnover. Using mass spectrometry, BiFC and pull down assays we identified specific Ub ligase E3 involved in this process. We have also mapped MAPKKK18 ubiquitination sites. Here, we present new components and regulators involved in MAPKKK18 protein turnover.
C0302 IDENTIFICATION INTERACTION SITES BETWEEN ABI1-LIKE PROTEIN PHOSPHATASES AND ACC SYNTHASE 7

Małgorzata Marczak, Małgorzata Tajdel Zielinska, Maciej Janicki, Agata Ciesła, Agnieszka Ludwikow

Adam Mickiewicz University in Poznan (Wielkopolska) Poland

1 Resumen

ABI1 and ABI2 encode PP2C-type of protein serine/threonine phosphatases that are known as negatively regulators of abscisic acid (ABA) signaling. The reversible protein phosphorylation is a crucial post-transcriptional protein modification that regulates numerous cellular processes. In the present study, we demonstrated the interaction between 1-amino-cyclopropane-1-carboxylate synthase 7 (ACS7) and ABI1/ABI2 protein phosphatase type 2C (PP2C) using mass spectrometry, in vitro pull-down assay and bimolecular fluorescence complementation (BiFC) analysis. ACS7 is an essential enzyme in plants, it catalyzes the first step in ethylene biosynthesis in which S-Adenosyl methionine (AdoMet) is converting to 1-aAminocyclopropane-1-carboxylic acid (ACC). ACS7 belongs to type III isoforms of ACS and has a truncated C-terminal MAPK and CPK phosphorylation sites. The stability of ACS isoforms is regulated by reversible phosphorylation modifications. Using cell-free degradation assay we demonstrate that ABI1 affects the stability of ACS. Computational model of ACS7-ABI1 complex was generated to identify residues dephosphorylated by ABI1. On the basis of the bioinformatics analysis four deletion constructs were generated: ?1ACS7 (1-100aa), ?2ACS7 (1-50aa), ?4ACS7 (101-447aa), ?6ACS7 (201-447aa). ACS7 deletion constructs including forms carrying single point mutations were used for analysis of interaction with ABI1/2. Data shows multiple residues on full length sequence of ACS7 that can interact with ABI1 and ABI2. Presented data indicate a few serine/threonine residues can be potentially dephosphorylated by ABI1 and ABI2. Knowledge of specific sites will allow understanding of mechanisms underlying the process of ABI1 protein dephosphorylation and ACS7 complex formation as well as the role of ABI1 PP2C in regulation of ACS7 degradation via 26S-proteasome pathway.

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**Resumen**

The rooting of stem cuttings is a common vegetative propagation practice in many ornamental plants. In cultivated carnation, adventitious root (AR) formation in the base of the cuttings is triggered by an endogenous auxin peak built-up by an active auxin transport from the leaves. To provide fundamental insights into the genetic basis of this complex trait, we studied AR formation in a collection of 194 lines derived from a cross between two hybrid cultivars, 2102-01 MFR and 2003 R 8, showing contrasting rooting performance. Time-series for several root architectural traits were quantified in sixteen of these lines displaying extreme rooting phenotypes, which were significantly correlated with the metabolomic profiles of the stem cuttings at harvesting time. Selective transcriptome profiling will allow us to identify a collection of candidate SNPs linked to rooting performance traits that might be used to establish a novel marker-assisted selection approach in this species for improved adventitious rooting.

References:

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C0123 OVEREXPRESSON OF HEMOGLOBIN GENE IN BARLEY INCREASE DROUGHT TOLERANCE THROUGH ETHYLENE PATHWAY INHIBITION

Gracia Montilla Bascón, Diego Rubiales Olmedo, Kim H Hebelstrup, Julien Mandon, Frans J.M. Harren, Simona Cristescu, Luis AJ Mur, Elena Prats Pérez

1 Instituto for Sustainable Agriculture (IAS-CSIC) Córdoba (Córdoba) España
2 Aarhus University (Slagelse) Denmark
3 Radboud University Nijmegen (Nijmegen) Netherlands
4 University of Aberystwyth (Aberystwyth) United Kingdom

1 Resumen
Drought is one of the major constraint of crops worldwide. Breeding for drought resistance is a complex task for which a sound knowledge of plant responses to drought at all level, from crop physiology to molecular changes is needed. Nitric oxide (NO) is a key messenger in plant stress responses but its exact role during drought remains unclear. Thus, we investigated the role of NO during drought by employing transgenic barley plants (UHb) overexpressing the barley non-symbiotic hemoglobin gene HvHb1 (Hb) that oxidizes NO to NO$_3^-$. Overexpression of the hemoglobin gene in UHb plants was confirmed both under well-watered and drought conditions and this was related to lower level of NO released, measured in vivo, in intact plants. Interestingly, both UHb genotypes assessed were significantly more tolerant to drought stress than wild types (WT) when monitored over 18 days’ time courses that reduced relative water content progressively up to 20%. Overexpression of Hb gene was associated with reduction in the expression of several genes of ethylene pathway, i.e. 1-aminocyclopropane-1-carboxylate [ACC] synthase (ACS), which catalyzes the first committed step in ethylene biosynthesis and 1-aminocyclopropane-1-carboxylate oxidase (ACO) that oxidizes ACC to ethylene. Downregulation of ethylene related genes observed in UHb genotypes was associated to reduction of ethylene, measured in vivo in intact plants, and with the improvement of drought related parameters in UHb plants. In addition, the higher level of ethylene observed in WT plants was associated with early senescence and reduction of chlorophyll content of the leaves under drought. Thus, we conclude that ethylene pathway inhibition mediated by NO regulation contribute to drought tolerance in barley.
C0153 CHROMATIN-DEPENDENT REGULATION OF SORBITOL SYNTHESIS IN FLOWER BUDS

Alba Lloret Compañí, Amparo Martínez Fuentes, Manuel Agustí, María Luisa Badenes, Gabino Ríos

1 Instituto Valenciano de Investigaciones Agrarias Moncada (Valencia) España
2 Instituto Agroforestal Mediterráneo (Valencia) España

1 Resumen
Buds of perennial plants containing reproductive and vegetative meristems have to cope with low temperature and water deficit stresses during the cold season. The tolerance of winter buds to environmental stresses is improved by seasonal endodormancy through mechanisms that are insufficiently known at the molecular level. We have studied the PpeS6PDH gene coding for an enzyme with sorbitol-6-phosphate dehydrogenase activity, which is differentially regulated during bud development. PpeS6PDH is highly expressed in dormant buds consistently with sorbitol accumulation. Concomitantly with PpeS6PDH down-regulation in dormancy-released flower buds, chromatin around the translation start site of the gene shows changes in the methylation state of specific residues of histone H3 (H3K4 and H3K27). These data suggest the transcriptional regulation of PpeS6PDH expression by chromatin modification mechanisms. Moreover, abiotic stresses affect PpeS6PDH expression. Low temperature treatments induce gene expression in buds and leaves, whereas desiccation up-regulates PpeS6PDH in buds and represses the gene in leaves. These data suggest the participation of PpeS6PDH in tolerance against cold and water deficit stresses in buds.
C0161 MULTIGENIC CONTROL OF STRINGLESS POD AND DEHISCENCE IN COMMON BEAN AND ITS IMPLICATIONS IN BREEDING

Ana María González Fernández¹, A Paula Rodiño Miguez¹, Fernando Yuste-Lisbona², Rafael Lozano Ruiz¹, Marta Santalla Ferradas¹

¹Misión Biológica de Galicia-CSIC Salcedo. Pontevedra (Pontevedra) España
²Centro de Investigación en Biotecnología Agroalimentaria (BITAL). Universidad De Almería (Almería) España

1 Resumen

Loss or reduction of natural seed dispersal was selected from a dehiscent wild common bean (Phaseolus vulgaris L.) ancestor during domestication. Pods of wild beans have also fibers in their sutures (string) and the pod walls. Complete loss of these fibers leads to indehiscence and lack of string of the pods in many modern bean varieties. Although the identification of the genes controlling dehiscence and absence of pod string will be useful for plant breeding, little is known about the molecular genetic basis of both mechanisms in common bean. In this study, we employed multiple experimental approaches to explore the genetic mechanism underlying the evolution of indehiscent and stringless phenotypes in domesticated common beans. Comparative genomics and genome database of soybean and its legume model plants Medicago truncatula, Lotus japonicus, Glycine max and Pisum sativum were used to identify candidate genes in common bean related to known endocarp determinant genes. A recombinant inbred (RI) mapping population was evaluated for measurements of whole pod morphology, especially those affecting hardness of cell walls, string (on a 0–9 scale) and dehiscence vs. indehiscence. The RI population was genotyped using polymorphisms (Derived Cleaved Amplified Polymorphic Sequences (dCAPS) and Simple Sequences Repeat (SSR)) found in the homologous genes of fruit dehiscence-related genes identified (AGAMOUS, FRUITFULL, SHATTERPROOF, INDEHISCENT, ALCATRAZ, SECONDARY WALL THICKENING PROMOTING FACTOR, POLYGALACTURONASE), and that were added to the linkage map to identify quantitative trait loci (QTL) for pod traits. Significant markers identified in this study provide a cost-effective and an efficient method for introgression and pyramiding of favorable alleles for pod dehiscence and lack of string via marker-assisted selection in common bean improvement programs.
C0179 GIBBERELLINS REGULATE OVULE INITIATION AND DEVELOPMENT

Maria Dolores Gómez, Maite Saura-Sánchez, Ernesto Escoms, Daniela Barro-Trastoy, Asier Briones, Inés Sánchez, Francisco Vera-Sirera, Juan José Ripoll, Esther Carrera, Isabel López-Díaz, Miguel Perez-Amador

1 IBMCP (Valencia) España
2 UCSD (California) EEUU
3 IBMCP-CSIC (Valencia) España

1 Resumen

The formation of ovules and viable seeds is an essential process in the life cycle of plants, as it ensures their correct reproduction, as well as being of great economic importance by having a direct impact on crop yield. Ovule initiation is controlled by a complex genetic (CUCs and ANT) and hormonal (auxins, cytokinins and brassinosteroids) network. Our recent data suggest that gibberellins (GAs) also play a very important role in controlling the development of ovules in both Arabidopsis and tomato. DELLA proteins (key repressors of GAs signaling) favor increase of ovule number and ensure their correct organogenesis, whereas GAs would repress ovule formation by promoting the degradation of DELLA. The DELLA loss-of-function mutants show a decrease and increase, respectively, in ovule number per pistil. On the other hand, exogenous GA treatments reduce ovule number in the same manner as DELLA loss-of-function mutants. DELLA RGA, GAI and RGL2 play a major role in this process and accordingly are present in placenta tissue. Moreover, the ectopic expression of rgaD17, a dominant allele of DELLA, in the placenta is sufficient to promote the formation of more ovules per pistil. We are currently investigating the interaction of GAs with other hormones involved in ovule formation. GAs does not regulate ovule formation by interaction with brassinosteroids, but through independent pathways. A plausible scenario is that the role of DELLA in ovule development is mediated by its direct protein-protein interaction with CUC2.
C0304 DEVELOPING TOOLS FOR THE MOLECULAR CHARACTERIZATION AND BREEDING OF GARLIC

Ricardo Parreño Montoro, Ainoa Gallego, Eva Rodríguez Alcocer, Álvaro Ferriz, Daniel Blasco Espada, Felipe Gómez del Castillo, Purificación Castillo Martínez, Héctor Candela

1 Instituto de Bioingeniería, Universidad Miguel Hernández Eliche (Alicante) Spain
2 Coopaman, S.C.L. (Cuenca) España

1 Resumen

The genus Allium consists of numerous species that are highly appreciated for their bulbs and have an important commercial value, such as garlic and onion. In order to develop resources for the molecular characterization of garlic, we have sequenced, assembled and annotated the chloroplast genome of a garlic cultivar. This genome is a circular, double-stranded DNA molecule with a length of 151,131 base pairs, which contains 90 protein-coding genes, 38 transfer RNA genes, 8 ribosomal RNA genes and 6 pseudogenes. In addition to this, we are following a similar approach to assemble the mitochondrial genome and have already assembled the transcriptome of several tissues, including shoot apical meristems, vegetative leaves, bulbs and bulbils.

For our transcriptomic studies, we are using a line of garlic with variegated bulbs that contain white and purple sectors. These sectors have sharply defined boundaries and extend along the proximal-distal axis of their tunicas (modified leaves that cover the bulbs), an observation that suggests that the distinct pigmentation is transmitted throughout cell divisions. With these transcriptomic studies, we expect to gain insight on bulb development and the pigment synthesis pathways in garlic.
C0309 OXIDATIVE STRESS SIGNATURE INDUCED BY TOXIC ELEMENTS IS ASSOCIATED WITH AUTOPHAGY INITIATION IN CHLAMYDOMONAS REINHARDTII

Angel Baron-Sola¹, Fior Martinez², Claudia Holgueras¹, Maria Arana¹, Raul Pastor¹, David Bautista¹, Cristina Ortega-Villasante¹, Luis Eduardo Hernandez²

¹Fisiologia Vegetal, Dpto. Biologia, Edif. Biologicas BS13, Campus de Cantoblanco (Madrid) España
²Universidad Autonoma de Madrid (Madrid) España

1 Resumen

Accumulation of reactive oxygen species (ROS) and changes in antioxidants are characteristic responses of plants to metal(loid) stress toxic elements like cadmium (Cd) and arsenic (As). We are studying the early responses of the unicellular photosynthetic model Chlamydomonas reinhardtii treated with up to 50 µM Cd²⁺ and 20 µM H₂AsO₄⁻ in the activity of glutathione reductase, ascorbate peroxidase and catalase. In addition, both toxic elements caused the synthesis of phytochelatins; biothiols known by their ability to decrease metal(loid) toxicity. Clear symptoms of autophagy were observed in cells treated with Cd and As, as numerous cytoplasmic vesicles were observed by Transmission Electron Microscopy (TEM); process that may be important for recycling of damaged organelles under metal(loid) stress. To test this possibility, we study by immunodetection the abundance and mobility shifts of ATG4 (Cys-protease) and/or ATG8 (chaperone-like) proteins, as markers of autophagy. Under not-stressed conditions (appropriate redox cellular balance) little autophagy occurs, since active ATG4 cleaves off ATG8 from its phosphatidylethanolamine (PE) anchor of otherwise potential autophagic vesicles. However, under oxidative stress conditions ATG4 is inhibited and therefore ATG8-PE is not cleaved, prompting vesicles for autophagy. This is the case of Chlamydomonas shortly exposed to Cd, which showed clear generation of ROS and changes in the redox cellular balance. Nevertheless, a weaker response was observed in microalgae treated with H₂AsO₄⁻ despite some symptoms of oxidative stress were also observed, implying a differential mechanism of response to each metal(loid).

²Pérez-Pérez ME et al. (2016) Control of autophagy in Chlamydomonas is mediated through redox dependent inactivation of the ATG4 protease. Plant Physiol 172:2219-2234

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**POSTERS**

**Abiotic Stress**

C0043 PROLINE BIOSYNTHESIS IN THE ROOT TIPS OF WHEAT SEEDLINGS UNDER OSMOTIC STRESS IS SUPPRESSED BY THE INHIBITION OF NITRIC OXIDE SYNTHESIS

Helga Königshofer, Lüppert Hansgeorg

University of Natural Resources and Life Sciences (Vienna) Austria

1 Resumen

The accumulation of free proline occurs in plants under a broad range of stress conditions. In addition to the role as a compatible osmolyte under water-deficit, proline may stabilize membranes and proteins, act as radical scavenger or provide carbon, nitrogen and energy upon relief from stress. The synthesis of proline under stress is catalyzed by the enzymes pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) converting glutamate to proline in two successive reactions via the intermediate pyrroline-5-carboxylate. Although these reactions have been well characterized, only limited information is available on the signalling pathway involved in the regulation of proline biosynthesis under stress. Abscisic acid, hydrogen peroxide, calcium and phospholipase C have been proposed to participate in the activation of proline biosynthesis during stress, but as yet a regulatory function of nitric oxide (NO) in proline metabolism has not been confirmed.

In this study, we monitored changes in the activities of P5CS and P5CR and the proline content in the root tips, the root maturation zone and the leaves of wheat (*Triticum aestivum* L. cv. Josef) seedlings during the first hours after the application of osmotic stress imposed by 22.5% polyethylene glycol 6000. Only in the root tips, the activities of P5CS and P5CR were markedly enhanced during stress resulting in considerable accumulation of proline already within 8 hours. The application of N^G^-nitro-L-arginine methylester (L-NAME), a competitive inhibitor of NO synthase, reduced the increase in the activities of both biosynthetic enzymes and the proline content in the stressed root tips by half.

Our results indicate that under water deficit proline synthesis is first enhanced in the root tips to protect this region active in growth. Moreover, we could show that NO seems to be involved in the transduction of the osmotic stress signal leading to the up-regulation of the proline biosynthetic enzymes.
C0049 ANTIOXIDANT RESPONSE MECHANISMS INDUCED BY ACCUMULATION OF POTENTIALLY TOXIC ELEMENTS IN RED-LEAF LETTUCE

Ines Moreira Neto, Luisa Martins Louro, Miguel Mourato

LEAF, Instituto Superior de Agronomia, Universidade de Lisboa, (Lisboa) Portugal

1 Resumen

Lettuce is a world wild used vegetable that is primarily consumed fresh or in salad mixes. It is a healthy leaf vegetable containing vitamins and phenolic compounds, like anthocyanins. On the other hand it can easily accumulate Potentially Toxic Elements (PTEs) from contaminated media and this constitutes a route of contamination of the food chain. Therefore, the purpose of this study was to identify the mechanisms of tolerance and toxicity caused by PTEs.

The effect of five PTE at two concentrations was studied: Cadmium (5 and 15 µM), Nickel (50 and 125 µM), Zinc (50 and 150 µM), Chromium (5 and 15 µM) and Lead (125 and 150 µM). After 37 days of growth red-leaf lettuce plants (Lactuca sativa L., var. capitata) were contaminated in hydroponic system and samples were collected after a further 13 days.

The total antioxidant capacity demonstrated a tendency to increase for all the studied PTE in relation to the control. Tolerance of red lettuce was mainly due to the activation of antioxidant enzyme system: cadmium and nickel mostly activated guaiacol-peroxidase activity; zinc caused a general increase in the enzymatic activity of superoxide-dismutase, guaiacol-peroxidase, ascorbate-peroxidase and glutathione-peroxidase; chromium only increased the activity of glutathione-reductase; lead increased catalase and glutathione-peroxidase activity. Total ascorbate increased for cadmium, nickel and zinc exposure and no significant differences were observed for chromium and lead. This suggests that the different PTEs triggered different antioxidant defense mechanisms. Polyphenolic compounds, anthocyanins and phenylalanine ammonia lyase activity showed an increasing trend, except for the lead contamination. H₂O₂ levels also increased indicating ROS production mainly at the higher applied concentrations. This confirms that anthocyanins are important in the ability of red-leaf lettuce to tolerate higher PTE concentrations because they can act as the scavengers of free radicals.

Keywords: red lettuce, potentially toxic elements, antioxidant response mechanisms, anthocyanins
C0051 DOES THE REGULATION OF THE SHIKIMATE PATHWAY AFTER GLYPHOSATE OR QUINATE TREATMENTS CHANGE IN AMARANTHUS PALMERI OVEREXPRESSING EPSPS?

Ainhoa Zulet González, Manuel Fernández Escalada, Miriam Gil Monreal, Ana Zabalza Aznárez, Mercedes Royuela Hernando

Universidad Pública de Navarra. Pamplona (Navarra) España

1 Resumen

Glyphosate is a herbicide that functions by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in the biosynthetic pathway of aromatic amino acids (AAA) (the shikimate pathway). Glyphosate provokes quinate accumulation and exogenous quinate application to plants shows a potential role of quinate in the toxicity of the herbicide glyphosate. Amaranthus palmeri is one of the most important weeds where populations with resistance to glyphosate have been described by amplification of the EPSPS gene. The general aim of this study was to ascertain if a common regulatory pattern of carbon flux through the shikimate pathway is detected in plants after glyphosate, quinate and both compounds applied together, and if it is dependent of the number of EPSPS gene copy number of the treated plants. In this study sensitive and glyphosate-resistant A. palmeri plants have been grown in aerated hydroponic cultured under controlled conditions. Plants were sprayed with quinate, low doses of glyphosate and both compounds simultaneously. The effect of the different treatments on the regulation of the carbon flux through the shikimate pathway was evaluated after 3 days by measuring the amount of the first enzyme (3-Deoxy-d-arabino-heptulosonate-7-phosphate synthase (DAHPS) and EPSPS by immunoblotting, chorismate mutase and anthranilate synthase by in vitro activity assay and free AAA content by capillary electrophoresis. Glyphosate application induced an increase of the DAHPS and EPSPS proteins and AAA content. The exogenous quinate induced a decrease in the amount of DAHPS protein. As expected, in both populations the effects detected after glyphosate or quinate treatments were different. Results of the combined treatment are discussed in relation to the regulation of shikimate pathway.
C0053 ANTIOXIDATIVE ENZYMATIC MECHANISMS INVOLVED IN THE RESPONSE OF 
BRASSICA NAPUS PLANTS TO TOXICITY INDUCED BY AS, CU CD AND NACL. 

Inês Leitão Barata, Miguel Mourato, Luisa Martins Louro 

Instituto Superior de Agronomia (Lisboa) Portugal 

1 Resumen 

The main objective of this work was to study the enzymatic response of Brassica napus plants to oxidative stress induced by different potentially toxic elements (PTE) in nutrient solution: arsenic (50 µM), cadmium (50 µM), copper (100 µM) and NaCl (25 mM). At the end of the experiment (46 days after treatment with each contamination) these plants were able to accumulate high concentrations of these elements, respectively, up to 2.81 mg As/kg, 290.2 mg Cd/kg, 61.1 mg Cu/kg and 2.29 g Na/kg in the leaves on a dry matter basis. 

The activities of CAT, SOD, APX, GPOD, GR and GPX in leaves (after 18, 30, 39 and 46 days after contamination) were evaluated in order to identify the main enzymes involved in the antioxidative response for each PTE studied. The total antioxidant activity and total phenol content was also determined. 

The activity of CAT only increased in NaCl contaminated plants, while that of GPOD decreased, showing that CAT is the main mechanism for H_2O_2 degradation, in this situation. In plants growing under As stress there was an increase only in GPOD activity. The activity of SOD only increased in NaCl contaminated plants. 

The total antioxidant activity increased with time, mainly for the treatments with As, Cd and Cu. There was also a significant increase in the total phenols level, mainly at the longer times, for As and NaCl. 

This work showed that different antioxidative mechanisms are involved when oxidative stress is induced by different PTE, in the same plant, under the same conditions. 

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C0066 MOLECULAR RESPONSE TO CADMIUM IN SESUVIUM PORTULACASTRUM

Mariem Wali¹, Soledad Martos², Laura Pérez Martin³, Chedly Abdelly Abdelly¹, Tahar Ghnaya¹, Charlotte Poschenrieder², Benet Gunse²

¹Centre de Biotechnologies de la Technopole de Borj-Cedria (Hammam) Tunisia.
²UAB (Barcelona) Espanya
³Fisiologia Vegetal Terrassa (Barcelona) Espanya

1 Resumen

Sesuvium portulacastrum (Aizoaceae) is a halophyte plant with important Cd tolerance, especially in high salinity soils, but the molecular mechanisms explaining this salt-induced alleviation of metal toxicity remain unknown. Seedlings of S. portulacastrum were submitted hydroponically to different Cd concentrations (0 and 25 µM Cd) in combination with low (0.09 mM), or high (200 mM) NaCl. The relative expression of SOS1 and AHA1, an anti-porter involved in Na extrusion to the apoplast, and BADH, a gene related to the synthesis of the osmoprotector glycine betaine, were analyzed in a short-term period (24h, 48h, 1w and 2w). In a long-term period (21 days) the fluxes of Na⁺ and Cd²⁺ ions in protoplasts of treated plants were also analyzed. The combination of salt and Cd induced an over-expression of SOS1, AHA1 and BADH genes. The highest expression of BADH was observed in plants treated with Cd but low salt. The composition of Cd²⁺, Cl⁻, K⁺ and Na⁺ revealed differences in short-term monitoring involving different strategies among treatments. A main strategy of S. portucalastrum seems to be extrusion of Na⁺ to the cuticle reducing the osmotic and ionic stress of Na and Cd.

The kinetics of Na⁺ and Cd²⁺ after 21 days (long-term period) confirmed that the ion fluxes depend on the previous treatment plants had received, indicating a pre-activation of specific channels.

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C0076 HOW COULD NITRIFICATION INHIBITORS AFFECT PLANT METABOLISM?

Janaina Maria Rodrigues dos Santos¹, Berta Lasa¹, Marco Bettì², Carmen Gonzalez Murua³, Pedro Aparicio Tejo¹

¹Universidad Publica de Navarra Pamplona (Navarra) España
²Facultad de Química, Universidad de Sevilla (Sevilla) España
³Universidad del País Vasco (País Vasco) España

1 Resumen

The nitrification inhibitors (NIS), chemical compounds are used as additives to nitrogenous fertilizers that block or delay the oxidation of NH$_4^+$ to NO$_3^-$ in agricultural soils [1]. The application of NIs together with ammonium-based fertilizers has been used to maintain NH$_4^+$ in the soil for longer periods of time while efficiently reducing N losses; therefore, increasing the efficiency of the nitrogen fertilization. However, the undesirable effects that NIs might have on non-target organisms, including their potential phytotoxicity have not been extensively studied and, although their toxicity is low, there is an increasing concern about the impact that continuous application of NIs in one given site may have on plant growth [2][3].

The aim of this study is to determine the metabolic pathways affected by the absorption of 3,4-dimethylpyrazole phosphate (DMPP) in plants by transcriptomic analysis. This analysis has been carried out in Lotus japonicus, considered a model plant and species chosen representative of grassland crops. L. japonicus were cultured for 4 weeks in hydroponics culture and controlled conditions. In the last week, 10 ppm of DMPP was applied. The dose was calculated to be 10 times greater than that used in agricultural fertilizers. One week after treatment the plants were immediately frozen in liquid nitrogen and stored at -80°C until use for transcriptomic analysis.

The transcriptomic analysis was carried out in leaves of control and DMPP-treated plants. A L. japonicus specific microarray was designed by Agilent (Agilent Technologies; http://www.agilent.com), and hybridized with total leaf RNA according to the manufacturer’s instructions.

The results obtained from transcriptomic analysis suggest that the genes encoding for several transcription factors related to abiotic stress were modulated by the application of DMPP.

C0131 HVPAP1 AND HVPAP19 TWO PLANT CYSTEINE PROTEASES INVOLVED IN BARLEY LEAF SENESENCENCE MEDIATED BY DROUGHT

Andrea Gómez Sánchez, Blanca Velasco Arroyo, Mercedes Díaz Mendoza, Pablo González Melendi, Manuel Martínez, Isabel Díaz

1 Centro de Biotecnología y Genómica de Plantas (UPM-INIA) Pozuelo de Alarcón (Madrid) España
2 Centro de Biotecnología y Genómica de Plantas (UPM-INIA) Parque Científico y Tecnológico de la UPM Campus de Montegancedo (Madrid) España

1 Resumen

Almost four-tenths of the world's agricultural land lies in arid or semi-arid regions. These areas are stringy expanding due to climate change. Besides, extreme temperatures, drought, perform the most harmful stress for plant productivity. Plants respond to different abiotic stress developing some molecular mechanisms associated with physiological processes like leaf senescence. This strategy includes, among other important events, protein breakdown and mobilization from stressed tissues to sink and young organs like seeds. The end of this process is to ensure an efficient offspring. It is well known that Cysteine Proteases (Cys-Prot) C1A of the papain-class are the most abundant enzymes associated with leaf senescence. Preliminary data from our lab has demonstrated the specific participation of two C1A proteases, HvPap-1 and HvPap-19, cathepsin F-like and B-like, respectively, in the proteolytic event. Transcriptomic data using barley leaves after drought treatment have revealed the induction of the HvPap-1 and HvPap-19 genes. This result has been supported by alterations in proteolytic activities and modifications in the leaf protein content. Currently, amiRNA silenced lines in both cathepsin-encoding genes are being used for deeper analyses. The main goal of this work is to decipher their protein targets, the protein location and traffic, their regulation by specific protease inhibitors named cystatins and importantly, the final effects on the whole plant through the senescence process.
Recent work has revealed that ABA receptor proteins, e.g. PYR1, PYL4 and PYL8, can be degraded via an ubiquitination-dependent mechanism through single subunit and CUL4-based E3 ligases (Bueso et al., 2014; Irigoyen et al., 2014; Belda-Palazón et al., 2016). In particular, we have identified a 10-member family of single-subunit E3 ubiquitin ligases, named RFA for RING finger ABA-related, acting as E3 ligases of the PYR/PYL/RCAR ABA receptors and therefore controlling their ubiquitination and half-life. The RFA family is different from the CULLIN4-RING E3 ubiquitin ligase (CRL4) complex that interacts with ABA receptors through the substrate adapter DDB1-ASSOCIATED1 (Irigoyen et al., 2014). RFAs are structurally characterized by the presence of three putative RING domains in tandem (Bueso et al., 2014), i.e. a canonical RING domain containing a C6HC zinc finger flanked by two RING finger-like domains and according to this structure they belong to RBR (RING between RING fingers) E3 ligases (Marin et al., 2010). We provide evidence that RFA4 interacts with ABA receptors in the nucleus and promotes their ubiquitination in vitro and their degradation in vivo. Additionally, we have identified UBC26 as the cognate nuclear E2 interacting with the RFA4 E3 ligase. Therefore, the concerted action of UBC26/RFA4 regulates half-life of ABA receptors in the nucleus. Altogether, our results reveal a sophisticated targeting of ABA receptors at different subcellular locations, which involves at least the single-subunit RBR E3 ligases and the multiple-subunit CRL4 complex.
C0140 THE MATH-BTB BPM3 AND BPM5 SUBUNITS OF CULLIN3-RING E3 UBIQUITIN LIGASES TARGET CLADE A PROTEIN PHOSPHATASES TYPE-2C AND ENHANCE DROUGHT TOLERANCE

Jose Julián Valenzuela1, Alberto Coego González1, Qian Wu2, Xu Zhang2, Jorge Lozano Juste1, Esther Lechner3, Pascal Genschik3, Pedro Luis Rodríguez Egea1

1Instituto de Biología Molecular y Celular de Plantas (Valencia) España
2The State Key Laboratory of Protein and Plant Gene Research (Beijing) China
3Institut de Biologie Moléculaire des Plantes (Strasbourg) Francia

1 Resumen

Recently, studies that address the turnover of core ABA signaling components have opened new avenues of research in ABA signaling (Bueso et al., 2014; Irigoyen et al., 2014; Kong et al., 2015; Belda-Palazón et al., 2016; Wu et al., 2016; Yu et al., 2016); however, a comprehensive understanding of the mechanisms and components that regulate receptor and clade A protein phosphatases type-2C (PP2Cs) levels is still lacking. These mechanisms can contribute to the modulation of ABA signaling in different tissues and developmental stages as well as in response to environmental cues. During the course of our proteomic studies aimed to identify putative E3 ligases that mediate the turnover of clade A PP2Cs, in addition to RGLG1 and RGLG5 E3 ligases (Wu et al., 2016), we identified MATH-BTB (BPM) proteins that interact with PP2CA. BPMs belong to a six-member BPM1-6 protein family in Arabidopsis. The BTB domain binds CUL3, a highly conserved CULLIN family member that is present in CULLIN3-RING E3 ubiquitin ligases class 3 (CRL3s). The MATH domain serves as the recognition site for substrates of the CRL3 MATH subfamily of CRLs. We will present evidence that BPM3 and BPM5 gain-of-function promotes PP2C degradation in vivo, leads to enhanced sensitivity to ABA and enhances drought tolerance. Conversely, reducing CRL3BPM function impairs stomatal closure and enhances water transpiration. Therefore degradation of clade A PP2Cs through different E3 ligases, i.e. PUB, RING and CRL3 E3 families, is a complementary mechanism to PYR/PYL/RCAR-mediated inhibition of PP2C activity to relieve repression of ABA signaling.
DIFFERENTIAL EFFECT OF CR(VI) AND PH ON PHYSIOLOGICAL STRESS PARAMETERS OF TWO SALVINIA SPECIES

Silvana Chocobar Ponce, Mariana Daniela Rosa, Carolina Prado, Fernando Prado

Laboratorio de Fisiología Vegetal . Facultad de Ciencias Naturales e Instituto Miguel Lillo San Miguel de Tucumán (Tucumán) Argentina

1 Resumen
In the nature, the Cr exists in two stable oxidation states: trivalent, Cr(III) and hexavalent, Cr(VI). Both forms are present in aquatic systems differing in their chemical properties, mobility and toxicity. Chromium toxicity upon depends of its concentration and bioavailability, being this last dependent of both the solution pH and redox potential. Because its extreme toxicity, chromium must be remove from polluted aquatic systems to avoid deleterious environmental effects. Chromium removal by aquatic macrophytes has become in inexpensive and easily applicable method for water decontamination. In this work we analyzed the effect of Cr(VI) and pH on three physiological stress parameters: content of soluble (SP) and insoluble (IP) phenolics, accumulation of malondialdehyde (MDA) and plasma membrane damage (electrolyte leakage, EL) in S. minima and S. rotundifolia, exposed to 5 and 20 mg l⁻¹ of K₂Cr₂O₇ at pH 4.0, 6.0 and 7.6. Results show that studied parameters were differentially affected by both Cr(VI) and pH in both salvinia species. Plant responses also depend on analyzed organs (fronds or lacinias). SP accumulation profiles were different and pH-dependent in both species. IP decreased with increasing pH, but increased in presence of Cr(VI). EL and MDA, also showed differential results according to metal concentration and pH value, being lacinias the most sensitive organ for both species. However, visual damage was only observed in S. minima fronds. Additive or synergic effects between metal and pH value seem to be occur in both Salvinia species. Evaluated parameters enhance the knowledge on the performance of Salvinia species to adverse environmental conditions which can constrain the sustainability of phytoremediation processes under a changing pH scenario.
C0204 SEASONALITY AND CR(VI) AFFECT STARCH-SUCROSE PARTITIONING AND RELATED ENZYMES IN FLOATING LEAVES OF SALVINIA MINIMA

Mariana Rosa¹, Carolina Prado², Silvana Chocobar Ponce², Eduardo Pagano³, Fernando Prado³

¹Facultad de Ciencias Naturales e IML- UNT San Miguel de Tucumán (Tucumán) Argentina
²Cátedra de Fisiología Vegetal. FCN e IML - INBIOFIV (CONICET-UNT) (Tucumán) Argentina
³UBA (Buenos Aires) Argentina

1 Resumen

The effect of both seasonality and increasing concentrations of Cr(VI) on starch-sucrose partitioning and enzyme activities involved in starch and sucrose metabolism were analyzed in fronds of Salvinia minima. Carbohydrate contents and enzyme activities of Cr-exposed fronds show different patterns in winter and summer seasons. Total soluble sugars, starch, glucose and fructose were higher in winter fronds, whereas sucrose concentration showed a seasonal inverse pattern with the higher content in summer fronds. Carbohydrates, except glucose, increased under increasing Cr(VI) concentrations in winter fronds, while in summer ones only sucrose increased under Cr(VI) exposure. In summer fronds the contents of starch, total soluble sugars, fructose and glucose practically stayed without changes in all assayed Cr(VI) concentrations. Most of enzyme activities (e.g. ADPGase, SPS, SS and AI) related to starch and sucrose metabolisms were higher in winter than in summer, whereas only amylase and cFBPase activities were higher in this last. Except ADPGase activity, Cr(VI) treatment increased enzyme activities in both winter and summer fronds, but a no clear pattern was observed. Data of this study show clearly that carbohydrate metabolism is differently perturbed by both seasonality and Cr(VI) stress, which affects the carbon partitioning of fronds in terms of SLA.
C0206 EFFECTS OF ANTIMONY ON OXIDANT AND ANTIOXIDANT DEFENCE SYSTEMS IN DITRICHIA VISCOSA PLANTS GROWING IN MINING SOILS IN BADAJOZ (SPAIN)

Alfonso Ortega Garrido, Inmaculada Garrido Carballo, María Carmen Álvarez Tinaut, Francisco Espinosa Borreguero

Grupo FBCMP (Badajoz) España

1 Resumen

The alterations induced by the toxicity of antimony (Sb) in the leaves of Ditrichia viscosa plants were determined. The plants growing in mining soils in the “San Antonio” mine (La Codosera, BA) under different concentrations of Sb. Leaves of D. viscosa from 3 selected localizations from the mine area were used: wastes, muds and reference area, in all three cases soil samples were taken to determine their content in Sb. The amount of Sb was determined by ICP-MS. The O2.- generation, superoxidisedismutase (SOD), peroxidase (POX), glutathione reductase (GR) and dehydroascorbate reductase (DHAR) activities, the total ascorbate and glutathione, the photosynthetic pigments, the total phenolic, flavonoids and phenylpropanoids glicosides compounds and polyphenoloxidase (PPO) activity were determined. The mobile Sb content in soil samples were 28.5 mg/kg and 39.6 mg/kg for wastes and muds, respectively. The Sb content in leaves were 2.39 mg/kg and 9.63 mg/kg for plants located in wastes and muds, respectively. In the soil and leaves reference area the Sb content is not detected. Chlorophyll content did not altered, but carotenoids increased under Sb toxicity. The total content of phenolics, flavonoids, and phenylpropanoid glycosides rose, and PPO activity decrease, evidence of their participation in the defence response. Sb induced increases in the amount of superoxide anion generation, and also an strong increase in the antioxidant SOD, POX and DHAR activities, but GR activity remained unaltered. These increases prevent the lipid peroxidation in leaves. The total amount of ascorbate and glutathione increased in all cases. This oxidative stress induced by Sb affects both the phenolic and the antioxidant systems, which seems to implicate their involvement in the plant's defence and response to the stress.

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C0207 CHARACTERIZATION OF CADMIUM ACCUMULATION BY SPINACH PLANTS (SPINACEA OLERACEA L.)
Filipa Pinto, Joana Sales, Miguel Mourato, Luisa Louro Martins
LEAF-ISA (Lisboa) Portugal

1 Resumen
Cadmium is a heavy metal that has no known biological function and can be toxic to both plants and animals. Spinach plants are highly tolerant to Cd and can accumulate high amounts of this metal without visible signs of toxicity. The consumption of contaminated spinach plants can lead to contamination of the food chain.
We studied the accumulation of Cd by spinach plants exposed to different concentrations (0, 5, 10, 25, 50 and 100 µM) and the effect on the growth and development of young plants (30 days old) and mature plants (70 days old). The Cd concentration in leaves and roots, chlorophyll content, shoot and root length and biomass were measured.
Spinach leaves showed a considerable Cd accumulation capacity, higher for younger plants (612.7 ± 31.1 mg Cd kg\(^{-1}\) DW) than for mature ones (179.5 ± 12.4 mg Cd kg\(^{-1}\) DW), when exposed to 100 µM Cd for 21 days. The variation of Cd concentration with time in leaves and roots follows a hyperbolic curve, with the exception of adult plant leaves.
Cadmium also affected other vegetative parameters mainly for the longest time of exposure and higher Cd concentration. The shoot and root length increased over time with the exception of plants exposed to 50 µM of Cd after 21 days and 100 µM after 13 days. The biomass decreased in contaminated plants after 13 days of contamination. Chlorophyll was most affected in young plants.
Spinach plants were able to grow even at the highest concentration of 100 µM Cd and after 13 days of exposure and, although it accumulated high Cd levels in the leaves, it still looked healthy enough to be potentially consumed and this could cause a food contamination hazard.
Financial support from FCT (SFRH/BD/81080/2011) and LEAF (UID/AGR/04129/2013).
C0209 EFFECT OF COPPER ON THE GERMINATION AND SEEDLING DEVELOPMENT OF TWO TOMATO VARIETIES

Joana Sales, Filipa Pinto, Inês Leitão, Luisa Louro Martins, Miguel Mourato
LEAF-ISA (Lisboa) Portugal

1 Resumen

The main objective of this work was to study the effects of different concentrations of copper (Cu) on seed germination and seedling growth of two varieties of tomato (Solanum lycopersicum), Lusitano and Nemabrix (high lycopene content), in the initial stages of the plant development.

The effect of each Cu concentration was evaluated on 30 tomato seeds, in quadruplicate, for 15 days, for each one of the varieties. The Cu concentrations used were: 0, 5, 10, 25, 50, 100, 150, 250, 350, 500, 750, 1000, 1250, 1500 and 2000 µM.

To evaluate the effect of the metal on germination and growth of tomato seedlings the following determinations were performed: germination rate (%), viable seedling, root length (cm), shoot and root biomass (g), Cu content (mg.kg⁻¹) and tolerance index of the seedlings (%).

For both varieties, with increasing Cu concentration, there were no significant differences in germination rate or viable seedling, due to the barrier effect of the seed coating, preventing the metal from contacting the developing embryo. However root length was significantly affected by concentrations greater than 250 µM for both varieties.

The evaluation of seedlings development showed that the Lusitano variety had a greater tolerance to copper toxicity, with a reduction of 25 % in shoot biomass and 79 % in the root biomass while the Nemabrix variety showed a biomass reduction of 61 % and 82 % respectively.

Regarding metal accumulation, the Nemabrix variety showed greater accumulation in the shoots, while the Lusitano variety presented greater accumulation of the metal in the roots.

With this work, we can conclude that copper does not affect the germination rate, but negatively affects variables like root length and shoot and root biomass. The Lusitano variety is apparently more tolerant to the presence of Copper than the Nemabrix variety.

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C0278 FOLIAR APPLICATION OF MELATONIN TO MITIGATE OLIVE TREE SUMMER STRESS

Alexandre Gonçalves1, Ermelinda Silva1, Sandra Martins1, Cátia Brito1, Luís Pinto1, Luis Rocha1, Ivo Pavia1, Ana Luzio1, Lia Dinis1, M. Ângelo Rodrigues2, José Moutinho-Pereira1, Carlos M. Correia1

1 Centre for the Research and Technology of Agro-Environmental and Biological Sciences, University of Trás-os-Montes and Alto Douro, (UTAD-CITAB), Quinta de Prados, (Vila Real) Portugal
2 Mountain Research Centre (CIMO) – ESA, Polytechnic Institute of Bragança, (Bragança) Portugal

1 Resumen

Olive crop is widely distributed in the Mediterranean region and has a distinct socioeconomic importance in Portugal, namely in the Northeast region, where mostly rainfed olive orchards cover over 75000 ha and comprise approximately 36000 producers. The projected climate change characterized by severe summer conditions, with low rainfall, excessive heat load and high irradiance levels might affect this crop, notwithstanding the defense strategies that olive trees dispose against summer stress. In this work we aimed to investigate a new cropping practice with the introduction of a foliar application of melatonin (N-acetyl-5-methoxytryptamine; 100 µM) that have shown a central role in stress tolerance in different plant species. The study was conducted in an organic orchard of seven-years-old at Quinta do Prado, Lodões, Vila Flor (41°20’13.3”N, 7°05’54.2”W), Portugal, and shows the impact on leaf gas exchange, chlorophyll a fluorescence, relative water content, concentration of photosynthetic pigments and fruits total phenolic content at 6 different times between July and October 2016. Melatonin sprayed plants consistently showed higher stomatal conductance (18-73% during morning measurements and 11-136% at midday), net photosynthesis (25-81% morning and 32-130% at midday), electron transport rate (12-23% morning and 0.1-13% at midday) and photochemical yield of photosystem II (12-24% morning and 0-13% at midday). The better physiological status of the melatonin sprayed olive trees was reflected in a decrease in fruits total phenolic content (21%). The results suggest that the foliar application of melatonin boost the physiological activity of olive trees, giving new insights about the effect of this new cropping practice in olive orchards. Future studies to optimize the concentration and the number of applications of this metabolite must be pursued in order to get a better performance of olive tree under the harsh climatic conditions projected for the Mediterranean region.
Resumen

Pinus halepensis es una especie de planta que ha sido ampliamente utilizada en la restauración de ecosistemas degradados semi-áridos en la región mediterránea. Las especies de pino también han sido propuestas como bioindicadores de la disponibilidad de metales en sitios contaminados [1]. Los severos condiciones que prevalecen en las minas de tailings en el sur de España se esperan provocar alteraciones en el status redox de las plantas que pueden reflejarse en la expresión de sistemas antioxidantes. En el presente trabajo, reportamos los niveles de algunos componentes de ambos sistemas antioxidantes enzimáticos y no enzimáticos determinados en hojas de cinco poblaciones de P. halepensis (cuatro de ellas creciendo en minas de tailings y el resto en una zona no minera de la proximidad).

Los muestras fueron recolectadas en primavera y verano en dos años consecutivos. Más de 30 parámetros diferentes fueron determinados en las hojas, incluyendo especies de oxígeno reactivo, productos de oxidación macromolecular, compuestos C-, N- y S- con actividad antioxidante, oligómeros y polímeros fenólicos, antioxidantes enzimáticos, pigmentos y ácido salicílico, y un análisis multivariado fue realizado.

Los resultados obtenidos muestran que los patrones de expresión de los parámetros analizados eran connotativamente diferentes en mayo y septiembre, y más aún, estos patrones de expresión difirieron entre poblaciones, independientemente del tiempo de muestreo considerado, con plantas de minas de tailings exhibiendo niveles similares de ciertos parámetros que fueron significativamente más altos que los encontrados en plantas no mineras. Dado que las condiciones meteorológicas pueden ser descartadas para explicar las diferencias observadas, las condiciones edáficas deben ser los principales determinantes de la fitness de la planta. En este contexto, se muestran algunas correlaciones claras entre los parámetros del suelo y los de la planta y se discuten en este trabajo. [1] Párraga-Aguado et al. (2013). Ecological Engineering, 58: 84-90. Este trabajo fue apoyado por el MINECO y FEDER (CGL2014-54029-R). AL-O obtiene un aporte del MECD (AP2012-2559). Parte de este trabajo fue llevado a cabo en el Instituto de Biotecnología Vegetal (UPCT).
C0300 ARSENIC STRESS IN MEDICAGO SATIVA: LINK AMONG OXIDATIVE RESPONSE, HORMONS AND BIOTHIOL METABOLISM

Alejandro Navazas, Carmen Cascón, Laura Gómez, Luis Eduardo Hernández, Isabel Feito, Aida González Díaz

1Universidad de Oviedo (Asturias) España
2Universidad Autónoma de Madrid (Madrid) España
3SERIDA (Asturias) España
4Fisiología Vegetal. Dpto. B.O.S..Fac. Biología Oviedo (Asturias) Spain

1 Resumen
Environmental and human health are seriously threatened by the accumulation of toxic metal(loid)s due to various natural and, mainly, anthropogenic origins. Plant ability to extract mineral nutrients from the medium can be exploited to develop phytoremediation technologies able to alleviate the negative impact of toxic elements in natural ecosystems. In this study, the essential biothiol metabolism for detoxification of As and its link to oxidative stress together with hormonal endogenous factors was investigated. To achieve this, alfalfa (Medicago sativa) seedlings were used and grown in hydroponic culture under As exposure (0, 5, 30, 70 µM) for 24 h. Growth, As accumulation, oxidative stress, hormones concentration and phytochelatins synthesis were measured. There were increases in anthocyanins concentration, cellular reactive oxygen species (ROS) production and extracellular H₂O₂ formation at increasing As concentrations. Hormone levels in leaves were increased under As exposure, with larger abscisic acid and isopentenyladenosine accumulation. Exposure to higher As levels resulted in a higher homophytochelatins or thiols concentration in both, roots and leaves. Apart from control treatment, under As exposure the total non-protein thiols (NPT) concentration was higher in roots than in leaves. De novo synthesis of NPT in roots exceeded that in leaves, with appearance of PC₂, hPC₂, PC₃ and hPC₃ in roots and only PC₂ in leaves. Thus, this increase in NPT can be considered as a main cellular response to As detoxification in order to alleviate the phytotoxicity of As in the plant, while hormones do also seem to play a role in the oxidative stress generated by As in M. sativa. This experiment adds nuance to our understanding of metal(loid)s-plant interactions under As exposure.

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C0312 Influence of AM fungi on the growth and physiological status of Cleopatra mandarin plants under deficit irrigation

Joséfa María Navarro, Juan Gabriel Pérez-Pérez, Eva María Arques, Juan Miguel Robles, Ian Dodd and Asunción Morte

1 Departamento de Riego y Fisiología del Estrés, IMIDA, Murcia, Spain
2 The Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom.
3 Departamento de Biología Vegetal y Botánica, Facultad de Biología, Universidad de Murcia, Spain

1 Resumen
The behavior of Cleopatra mandarin seedlings was studied to determine whether mycorrhizal symbiosis modifies their physiological response under deficit irrigation (DI). The seedlings were inoculated (+AM) with arbuscular mycorrhizal (AM) fungi (Rhizophagus irregularis + Funneliformis mosseae) or were non-inoculated (-AM). The irrigation treatments were started 75 days later and were maintained for 45 days. They consisted of irrigation with 100% ETc (Control) or with 60% ETc (DI). In spite of the negative effect of DI on plant growth, the +AM treatment partially recovered most of the growth parameters. The increased [ABA]root and [ABA]leaf in plants under DI produced stomatal closure and therefore diminished the photosynthesis values, that were not recovered by AM symbiosis. So, the growth stimulation of +AM plants under DI was not due to better gas exchange. The DI treatment also reduced root and leaf water potential, without affecting the plant turgor. No positive effect of AM symbiosis on plant water status was observed in plants under DI. The strong reduction of non-structural carbohydrates in roots of +AM plants caused by DI was due in part to a loss of starch, but mainly to a decline in soluble sugars. This was probably due to the sink effect of the AM fungus, demanding carbohydrates from the plants - a demand that the plants were unable to satisfy under these stressful conditions. The better growth response of +AM plants under DI could be due to superior leaf mineral nutrition regarding some essential elements, such as N, K, Cu, and Zn, and to higher concentrations of Ca, Fe, Cu, and Zn in roots. In conclusion, AM colonization changed Cleopatra mandarin growth under DI conditions, conferring a greater degree of tolerance not by improving the plant water status or the gas exchange but by improvement of plant nutrition under these stressful conditions.
C0329 EFFECTS OF SALT STRESS ON PLANT GROWTH AND FOLIAR NUTRIENT
CHANGES IN MANGO PLANTS

Jalel Mahouachi Mahouachi

Departamento de Ingeniería Agraria, Náutica, Civil y Marítima, Universidad de La Laguna (Santa Cruz de Tenerife)
España

1 Resumen
To characterize the magnitude of tolerance or susceptibility of mango (Mangifera indica L.) plants to salt stress, seedlings of two rootstocks ('13/1' and 'Gomera-3') were exposed to 50 mM NaCl in water solution during long-term period. Data showed that salt concentration differentially affected the functional leaf number and leaf biomass in the studied rootstocks. On the other hand, salinity increased Na⁺ and Cl⁻ concentration in both rootstocks, and K⁺ only in ‘13/1’; however, did not alter many of the analyzed nutrients. In addition, salt stress induced proline production in the rootstocks, although irrespective of treatments, proline content was higher in ‘Gomera-3’ than in ‘13/1’ plants. Taken together, data suggest that ‘13/1’ rootstocks appear more tolerant to the applied salt stress conditions than ‘Gomera-3’.
**Applied Plant Physiology and Molecular Breeding**

**C0082 NOVEL GENE REGULATORY NETWORKS UNVEILED DURING ADVENTITIOUS ROOT DEVELOPMENT IN TOMATO HYPOCOTYLS AFTER WOUNDING**

Sergio Ibañez Lopez¹, Aurora Alaguero¹, Antonio Cano², Joan Villanova¹, Ana Belén Sánchez-García¹, Manuel Acosta², José Manuel Pérez-Pérez²

¹Instituto de Bioingeniería, Universidad Miguel Hernández Elche (Alicante) España
²Departamento de Biología Vegetal (Fisiología Vegetal), Universidad de Murcia (Murcia) España

**1 Resumen**

Adventitious roots (ARs) are formed from non-root tissues, such as stems or leaves, in response to some stresses (i.e. flooding) or after wounding. The formation of ARs is a complex genetic process regulated by both environmental and endogenous factors, among which the plant hormone auxin plays a central role¹. We investigated the temporal course of gene expression profiling and auxin and cytokinin accumulation along the apical-basal axis of hypocotyl explants. Among the differential expressed genes at earlier time points, significant enrichment was found for those encoding auxin signaling and sugar metabolism components. Polar auxin transport inhibition impairs AR formation from hypocotyl explants whereas exogenous auxin supply enhances AR formation. Our results indicate that active polar auxin transport through the hypocotyl leads to a localized auxin gradient required for AR formation in the hypocotyl base.

The identification of the genetic networks involved in AR formation will contribute to our basic understanding of the molecular events leading to this complex developmental response.

**References:**


Work funded by MINECO/FEDER (AGL2012-33610 and BIO2015-64255-R)
C0103 MORPHOLOGICAL CHARACTERIZATION AND ASSESSMENT OF VARIABILITY IN ADVENTITIOUS ROOT FORMATION IN DIVERSE TOMATO GENOTYPES

Aurora Alaguero Cordovilla¹, Alfonso Azorín¹, Francisco Javier Gran¹, Lázaro Eustáquio Pereira Peres², José Manuel Pérez-Pérez²

¹Universidad Miguel Hernández Elche (Alicante) España
²Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (Piracicaba) Brasil

Adventitious roots (ARs) are ectopic roots that arise either naturally or in response to stress from various plant tissues, such as stems and leaves; they may also be induced by mechanical damage or following in vitro tissue culture regeneration.

Tomato is an attractive model to study the genetic basis of adventitious organ formation. To explore the phenotypic space of this trait in tomato, we characterized AR formation in excised hypocotyls in a genotypically diverse set of 10 wild tomato species, 7 commercial cultivars, and 20 tomato mutants affected in known genes involved in light or hormonal signaling. A combination of semi-automated image capture and quantitative histology methods allowed us to define the cellular dynamics during the early stages of AR initiation. By studying a collection of Solanum pennellii introgression lines³, we identified several genomic regions that might include genes involved in AR development. Mendelization of the causal genes will allow us to understand the genetic basis of AR variability within the tomato clade.

References:
¹Arikita et al. (2013) Plant Sci, 199-200: 121

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C0108 IDENTIFICATION OF WRKY TRANSCRIPTION FACTORS IN CITRUS AND THEIR INVOLVEMENT IN PLANT RESPONSES TO HORMONAL TREATMENTS AND ABIOTIC STRESSES

Vicente Vives Peris, Jorge Collado Domínguez, Damián Balfagón Sanmartín, Aurelio Gómez Cadenas, Rosa María Pérez Clemente

Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I, (Castellon De La Plana) Spain

1 Resumen

WRKY transcription factors (TF) family is involved in a huge variety of plant processes, including seed germination, plant development, phytohormone signaling and defense against biotic or abiotic stress conditions. In this work, WRKY TF family has been characterized in citrus. The relative expression of CsWRKYs was analyzed in shoots and roots of in vitro cultured plants treated with abscisic acid (ABA), salicylic acid (SA) and methyl jasmonate (MeJA). Expression of CsWRKYs was determined in roots of commercial citrus rootstocks subjected to osmotic and salt stress. A total amount of 50 CsWRKYs have been identified and classified in the different groups of WRKY family according to the sequences of the WRKY domain. The major differences in WRKY gene expression after the application of phytohormones were shown in roots, and it was found that whereas treatments with ABA and SA generally repressed CsWRKYs expression, MeJA induced their overexpression. Osmotic stress repressed the expression of most of the studied CsWRKYs, while salt stress induced their expression. Moreover, salt stress induced higher increases in CsWRKYs expression in the tolerant rootstock C. macrophylla in comparison with the salt sensitive Carrizo citrange, suggesting that these TFs may play an important role in the response to this stress. The present investigation demonstrates that a number of CsWRKY genes are involved in abiotic stress responses, and provides clues for the selection of candidate genes to be used in future breeding programs.

This work has been supported by MINECO (AGL2016-76574-R) and UJI (B2016-23/B2016-24). V.V.-P. was recipient of a predoctoral contract from the Universitat Jaume I (PREDOC/2013/31).
C0109 EXPRESSION PROFILES IN ROSA HYBRID CV KNOCKOUT UNDER COLD STRESS USING CDNA MICROARRAY

Michele Valquiria Reis¹, Laura Michelle Vaughn Rouhana², Patricia Duarte Oliveira Paiva¹, Schuyler S. Korban³

¹Federal University of Lavras (Minhas Gerais) Brazil
²Wright State University (Ohio) USA
³University of Massachusetts Boston (Boston) USA

1 Resumen
Low temperatures adversely affect crop production by restraining plant growth and productivity. Several studies have been performed to better understand the complex regulatory processes that occur during the cold acclimation. However, there are limited information about gene regulation and signaling pathways related to cold stress response in ornamental plants, specifically in the reproductive organs. Rose flowers represent a good experimental model to investigate these responses, due to the short generation time and small genome size. So, it was uses an apple microarray to investigate global gene expression profiles in rose’s floral buds under low night temperature. Expression profiles were captured in floral buds at two different times after cold temperature exposure (4°C) for 2 and 12 h, and a control, 0 h. The qRT-PCR analysis the mRNA accumulation changes were made for a selection of 12 genes, up or down regulated in microarray hybridization. The tubby, dead box and pas 2 amplicons of Rosa sp. were cloned into pDrive vector, and nucleotide sequence was determined. Additionally, it was analyzed pattern genes expression of some genes of AP2 family comparing floral buds and leaves during cold stress. In this study was revealed 318 differentially expressed genes, in which 134 genes were up-regulated and 184 down-regulated. The expression patterns of the cold responsive transcripts identified by Microarray were confirmed by qRT-PCR analysis. Sequence analysis of TUBBY, DEAD BOX and PAS 2 revealed a higher level of similarity with Fragaria vesca (strawberry). The AP2/ERFs genes were more inducible in leaves compared with floral buds tissues. A set of the differentially expressed genes identified in this study will facilitate the better understand of cold stress response in rose floral buds.

Keywords: Rosa hybrid cv Knockout. Gene expression. Floral bud. Low night temperature. Stress tolerance. AP2 gene family.

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C0124 DROUGHT TOLERANCE INVOLVE FINE MODULATION OF JASMONATES IN OAT ROOTS AND LEAVES

Francisco José Canales Castilla¹, Vicent Arbona², Aurelio Gómez-Cadenas², Elena Prats¹

¹Instituto de Agricultura Sostenible - Consejo Superior de Investigaciones Científicas (IAS - CSIC) (Córdoba) España
²Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I (Castellón) Spain

1 Resumen
Jasmonates (JA), include a diverse group of jasmonic acid derivatives, such as Ile-jasmonic or dihydrojasmonic acid and its biosynthetic intermediate, 12-OPDA. Recent data indicate an involvement of JA in drought tolerance responses. However, controversy has arisen regarding the effect of JA in drought tolerance since some studies have reported improved drought tolerance whereas others reported negative effects associated with growth and yield reductions. Observed differences rely mainly in the plant species and tissue, and in the intensity and duration of the drought stress imposed. In this work, we investigated dynamic changes in JA profile of oat plants, including roots and leaves of two well-characterized genotypes for drought resistance, over a 20 days-time course. In leaves, Jasmonic acid and Ile-jasmonic increased only in the resistant genotype as relative water content in the soil (sRWC) decreased from 40% downward, whereas both reduced their content progressively in roots in a similar proportion in both genotypes. No changes were observed regarding dihydrojasmonic acid. Interestingly, a similar increase of OPDA was detected in leaves of both genotypes while it accumulated differently in roots. Whereas a dramatic reduction was observed in the tolerant genotype an increase of OPDA was observed in the susceptible one. Since JA and particularly OPDA have been linked with root growth inhibition, their reduction in roots could explained the already observed increased root growth of the tolerant genotype. Currently experiments are being carried out to confirm this hypothesis.
C0158 A NEW ROOTSTOCK ALLEVIATES POLYETHYLENE GLYCOL-INDUCED WATER STRESS IN GRAFTED PEPPER: AN ANALYSIS OF ROOT-TO-SHOOT SIGNALLING

Lidia López-Serrano1, Guillermo Canet1, Consuelo Penella1, Gabriela Vuletin Selak2, Alberto San Bautista1, Salvador López-Galarza1, Ángeles Calatayud2

1Instituto Valenciano de Investigaciones Agrarias. Centro de Citricultura y Producción Vegetal, Departamento de Horticultura Moncada (Valencia) Spain
2Institute for Adriatic Crops and Karst Reclamation, Department of Plant Science, (Split) Croatia
3Universitat Politècnica de València. Departamento de Producción Vegetal. (Valencia) Spain

1 Resumen

In agriculture, water stress is one of the most limiting factors for crops growth and development. Suitable commercial fruit characteristics are normally difficult to be combined with resistance to abiotic stress. In addition, there is a lack of public acceptance of genetic engineering, so it is necessary to improve techniques to overcome these stresses. Actually, grafting technique is an environ-friendly-technique to avoid or reduce losses in commercial yields. Based on previous screening studies, we used pepper accession A25 as tolerant rootstock to evaluate the potential mechanisms to cope water stress resulting from grafting. Three pepper combinations were used: Adige commercial variety (A), A grafted onto itself (A/A) and A grafted onto A25 accession (A/A25). Water stress treatment was induced by the addition of 5% PEG. The objective was deep in the rootstock/scion physiological-signalling traits that contribute to improve tolerance to scion through of photosynthesis, signalling molecules level like H₂O₂, nitrate reductase (NR) and biomass. All measurements were performed at 7 days after the inoculation of stress. In general, it is possible to see maintenance of biomass in A/A25 pepper plants related to ability to limit (or protect) the loss of CO₂ assimilation; a conservative NR activity and a lower H₂O₂ level. A pronounced decrease in gas exchange measurements in A and A/A combinations closed to a reduction of their biomass and loss of NR activity in leaves join a considerable H₂O₂ increase. To sum up, the responsible mechanisms of water stress tolerance when plants are grafted onto A25 were the maintenance of the photosynthetic capacity and a sink activity of the growing organs mediated by absence of oxidative damage, probably by a higher buffer capacity of A/A25 plants under water stress.
C0240 PSEUDOMONAS STUTZERI MJL19, A BENEFICIAL RHIZOBACTERIUM FOR SOYBEAN IN SALINE CONDITIONS

Maria Jesus Lami1, Mariana Rosa2, Vicente Vives Peris3, Carolina Prado3, Fernando Prado2, Enrique Paz Garcia1, Jesus De La Torre6, Manuel Espinosa Urgel6, Paula Andrea Vincent1, Ricardo Ezequiel De Cristobal1

1Instituto Superior de Investigaciones Biológicas (INSIBIO-CONICET-UNT), Instituto de Quimica Biológica “Dr. Bernabé Bloj” San Miguel De Tucuman (Tucuman) Argentina
2Catedra de Fisiología Vegetal, Facultad de Ciencias Naturales e IML, Universidad Nacional de Tucumán; INBIOFIV (CONICET-UNT) (Tucuman) Argentina
3Departament de Ciències Agràries i del Medi Natural, Universitat Jaume I, (Castellon De La Plana) España
6Departamento de Protección Ambiental, Estación Experimental Del Zaidín. (EEZ- CSIC) (Granada) España

1 Resumen
Saline environments cause cellular dehydration, which leads to osmotic stress and removal of water from the cytoplasm, resulting in a reduction of the cytosolic and vacuolar volumes. Salt stress often creates both ionic as well as osmotic stress in plants, resulting in growth decrease and accumulation of specific metabolites. However, the use of plant growth promoting rhizobacteria (PGPR) can improve plant growth by enhancing the resistance against various abiotic stresses. This study aimed to determine the influence of Pseudomonas stutzeri MJL19, a PGPR isolated in Argentina, on growth and physiology of soybean subjected to salinity. Changes in biomass, osmolytes accumulation (sucrose and proline), lipid peroxidation (accumulation of malondialdehyde, MDA) and abscisic acid (ABA) were measured to analyze the plant response under salinity. Results showed that seedling biomass decreased with salinity, whereas seedlings inoculated with MJL19, had a slight increase in this parameter. Inoculated seedling roots showed higher sucrose and proline content than non-inoculated ones. MDA level was higher in hypocotyls than in roots and increased under salinity with or without MJL19. Stressed seedlings had higher levels of ABA than controls, but these differences were lower in inoculated seedlings. Results suggest that inoculation with P. stutzeri MJL19 could improve the defense response to saline stress in soybean seedlings.

Work supported in part through a grant from the EMHE-CSIC program (MHE-200019)
C0258 PLANT ENDOGENOUS FACTORS AS ETHYLENE AND CITOKININS MODULATE THE REDOX BALANCE UNDER METAL(LOID)S STRESS

Cristina Ortega Villasante, Angel Baron Sola, Carlos M Conesa Quintana, David Bautista Spadin, Claudia Holgueras Martin, Carlos Granda, John Hulton, Flor Martinez Diez, Luis E. Hernández Rodríguez

Fisiología Vegetal Dpto Biología Universidad Autonoma de Madrid (Madrid) España

1 Resumen

Soil pollution with toxic metal(loid)s as mercury (Hg) or arsenic (As) constitutes a serious environmental problem, and may pose a food security threat. Understanding the mechanisms of metal(loid) accumulation, metabolization and transfer to the plant edible parts is critical to attenuate risks for human and animal health.

Biothiols, Cys-containing metabolites, are fundamental for metal(loid) cellular homeostasis and speciation in plants. GSH (γ-Glu-Cys-Gly) plays a dual role in the plant cell, controlling the redox balance along with other antioxidants, and as precursor of phytochelatins (PCs). Biothiols bind toxic metal(loid)s forming complexes that are stored in the vacuole and determine their distribution in the plant body.

Biothiols metabolism is controlled both by plant hormones and cellular redox balance. Cytokinins (CKs) down-regulate the sulfur assimilatory pathway that maintains the concentration of GSH, and are depleted under nutrient deficiency and abiotic stress. Ethylene (ET) signaling is up-regulated in plants exposed to toxic metal(loid)s, and may mediate the oxidative stress responses induced by these pollutants.

Supporting these observations, our previous work in Medicago sativa seedlings exposed to 3 µM Hg also revealed alterations in the expression genes responding to ET and CKs. Therefore, we are testing the implication of these hormones in biothiols metabolism, metal(loid)s homeostasis and stress tolerance with special focus on cellular redox balance. Using pharmacological approaches using specific inhibitors of hormone signaling (1-methylcyclopropene) or Arabidopsis thaliana mutants insensitive to CKs (CKs receptors double mutant ahk2ahk3) or ethylene (ein2-5, altered in ET signaling cascade) which could affect the tolerance to toxic metal(loid)s, we have analyzed reactive oxygen species (ROS) accumulation, cellular redox balance alterations, antioxidant enzymes activity, biothiols profiles, etc. We will present our latest results in the characterization of plant tolerance to As and Hg and the implication of CKs and ET in the mechanisms of tolerance to these elements.
C0330 QTL ANALYSIS AND WHOLE GENOME SEQUENCING FOR SOLUBLE SOLIDS CONTENT, MATURITY DATE AND MEALINESS IN A SEGREGATING POPULATION OF PEACH [PRUNUS PERSICA (L.) BATSCH]

Gerardo Nunez-Lillo, Verónica Arenas, Christofer Jaque, Cristian Vergara, María Teresa Dettori, Ignazio Verde, Claudio Meneses, Reinaldo Campos-Vargas

1 Resumen

Prunus persica is one of the most important temperate fruit trees in the world considering production and cultivated area. The consumer acceptance is the principal objective of the breeding programs and is dependent on many factors such as the soluble solids content (SSC) and mealiness (M) or lack of the juice due to long cold storage. Besides, maturity date (MD) is an important trait because has been reported a pleiotropic effect with M and SSC. The aim of this work was to identify candidate genes for SSC, MD and M based on a QTL analysis and whole genome sequencing. For this, we used an F1 progeny of 194 siblings which was obtained from the interspecific cross between ‘O’Henry’ x ‘NR-053’ (OxN) evaluated for three seasons. We constructed a genetic linkage map with 498 markers spanning 717.6 cM with an average distance between markers of 1.4 cM/marker and using the phenotypic data of three evaluation seasons we obtained consistent QTL for SSC, MD and M in the LG1, LG2, LG5 and LG6 with a large subset of candidate genes related to each trait. Finally, we reduced the number of candidate genes using a whole genome sequencing of the parents and a subsequent SNP identification on the candidate genes.

This work is the first step to identify genes responsible to the control of SSC, MD and M in this progeny. This work was supported by CORFO Consorcio Biofrutales 13CTI-21520-SP03 & 13CTI-21520-SP04, FONDECYT 1160584 and FONDEF G13i10005.
Biotic Stress, Plant-Pathogen Interactions

C0048 BRASSICACEAE: UNABLE-MYCORRHIZAL PLANTS, BUT WITH OTHER FUNGAL-SYMBIOTIC RELATIONSHIPS. DIFFERENTIAL DEFENSIVE RESPONSES

Jorge Poveda Arias¹, Ana Alonso-Ramírez², Rosa Hermosa², Enrique Monte², Carlos Nicolás²

¹Departamento de Botánica y Fisiología Vegetal, Universidad de Salamanca; Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca (Salamanca) España
²Departamento de Microbiología y Genética, Universidad de Salamanca; Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Universidad de Salamanca (Salamanca) España

1 Resumen

The brasicaceae or cruciferous are a group of plants that lost the ability to form symbiotic relationships with mycorrhizal fungi throughout their evolutionary history, due to the soils on which these plants were originally developed. This kind of fungi provides to the plants with which it can do mycorrhizal-roots-relations numerous benefits, from the supply of mineral nutrients and water, to protection against biotic and abiotic stresses.

Due to the numerous crops of great economic importance that are included within this family of plants (cabbage, cauliflower, cabbage, rapeseed, broccoli, radish, etc.), it originates a great interest to imagine the possibility of returning to these plants the capacity to do mycorrhizal-roots-relations, reducing the use of fertilizers, water and pesticides in their cultivation. To this end, we intend to determine the hormone-defensive differences of the plant model Arabidopsis thaliana (cruciferous) in its symbiotic relationship with the beneficial fungus Trichoderma harzianum, and against the presence of mycorrhizal fungi of the genus Glomus, separately and simultaneously.

Understanding the defensive differences existing in a cruciferous faces to both types of fungi and comparing it with existing data on other crops with this capacity, such as tomato, can give us the keys to the way to forward to give these plants a symbiotic capacity, which would have a significant increase in the efficiency of its cultivation, doing it more sustainable and cost-effective.

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C0052 MICROBIAL VOLATILES MODULATE RAPID RESPONSES IN ARABIDOPSIS THROUGH THIOL OXIDATION OF CYSTEINES AS REVEALED BY QUANTITATIVE SITE-SPECIFIC REDOX PROTEOMICS

Kinia Ameztoy Del Amo1, Marouane Baslama2, Francisco José Muñoz1, Ángela María Sánchez-López1, Abdellatif Bahaji1, Goizeder Almagro1, Edurne Baroja-Fernández1, Toshiaki Mitsui2, Javier Pozueta-Romero1

1Instituto Agrobiotecnología (CSIC/UPNA/Gobierno de Navarra) Mutriku (Navarra) España
2Graduate School of Science and Technology and Department of Applied Biological Chemistry, Niigata University (Niigata) Japón

1 Resumen
We have recently shown that volatile compounds (VCs) emitted by phylogenetically diverse microorganisms (including plant pathogens) promote photosynthesis, growth, early flowering, starch over-accumulation as well as broad transcriptome and proteome changes in plants (Sánchez-López et al. 2016, Plant Cell Environ. 39: 2592-2608; Sánchez-López et al. 2016, Plant Physiol. 172: 1989-2001). In this work we show that VCs promoted increase of photosynthesis and accumulation of photosynthates (starch, soluble sugars and amino acids) occurs soon after the exposure of plants to microbial VCs. Rapid and reversible protein thiol oxidation in response to environmental changes is a fundamental redox regulatory mechanism of photosynthesis, cellular metabolism and gene expression in photosynthetic organisms. To investigate the possible involvement of redox-proteome changes in the rapid response of plants to VCs we conducted an OxiTRAQ-based proteome-wide quantitative and site-specific profiling analysis of in vivo thiol oxidation in Arabidopsis plants shortly exposed to VCs emitted by the fungal phytopathogen Alternaria alternata. This analysis identified 396 Cys-containing peptides from 384 proteins involved in biological processes such as photosynthesis, primary carbohydrate, cell wall and lipid metabolisms, redox regulation and hormone signalling whose thiols underwent oxidative modifications following VCs treatment. Among the 396 redox-sensitive peptides, 261 cysteines in 240 different proteins became more reduced in the VCs-treated plants than in the control, whereas 179 cysteines in 169 different proteins were in a more oxidized state in the VCs-treated plants. In silico analyses using the DIANNA software predicted that 66% of the redox-sensitive peptides potentially form intramolecular disulfide bonds. The overall results indicate that rapid redox-proteome changes can be involved in the initial response of plants to microbial VCs.
C0061 MECHANISMS OF METAL DEFENCE AGAINST ALTERNARIA BRASSICICOLA IN ZINC HYPERACCUMULATOR NOCCAEA CAERULESCENS

Berta Gállego1, Soledad Martos1, Charlotte Poschenrieder Wiens1, Catalina Cabot2, Juan Barceló1

1Universitat Autònoma de Barcelona (Barcelona) España
2Universitat de les Illes Balears (Palma de Mallorca) España

1 Resumen
Metal defence against biotic stress is one of the proposed hypotheses concerning the evolutionary advantage of metal hyperaccumulation in plants. The high tissue metal concentrations tolerated by hyperaccumulators may contribute to defence against biotic stress through different mechanisms: direct metal toxicity to the biotic stress factor, more efficient stress signalling, or metal-induced structural fortification. Here the influence of low, medium and high Zn concentrations on the responses of Noccaea caerulescens (Zn hyperaccumulator) to Alternaria brassicicola (necrotrophic fungus) were studied. Twenty-four hours after fungal attack, both the Zn transporter gene HMA4 and defence-related genes (PR1, BGL2, PDF1.2, LOX2, and CHIB) were activated in all infected plants. However, after 1 week, protection against fungal invasion was only achieved in plants receiving high Zn supply (102 µM). Plants growing under low (2 µM) or medium (12 µM) Zn supply exhibited a compatible interaction. This susceptible reaction was apparently not due to mistakes in stress signalling, but due to the failure to activate a defence mechanism effective against A. brassiccola. Zinc hyperaccumulation, possibly along with high glucosinolate concentrations, was able to substitute for this defect yielding an incompatible interaction.

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1 Resumen

ST proteínas, caracterizadas por DUF2775, son una familia de proteínas con repetición de tandem 1. Se ha elegido el Grupo de seis miembros de la familia de Proteínas ST de Medicago truncatula como un modelo para estudiar estas proteínas in vivo, aquí nos enfocamos en ST1.

Los transcritos de ST1 están considerablemente más abundantes en raíces que en órganos aéreos a lo largo del desarrollo del plant. Igualmente, plantas de M. truncatula transgénicas expresando la fusión de gen pST1::GUS muestran una actividad de pST1 en raíces más alta que en cualquier otro órgano. Está activa en el stele de los raíces principales y los raíces laterales, ADICIONAR ENLACE a la referencia cognitiva.

Previos estudios indicaron que los transcritos de ST1 están abundantes en librerías de cDNA realizadas a partir de raíces de plantas estableciendo interacciones con bacterias nitrogenadoras 1 y también datos de chips microarrays disponibles en línea mostraron un aumento de los transcritos de ST1 en tales interacciones beneficiosas.

Para confirmar estos resultados in vivo, monitoreamos la actividad de pST1 durante la interacción entre M. truncatula y Sinorhizobium meliloti y midimos el nivel de acumulación de mRNA de ST1. La actividad de pST1 es inducida en las raíces después de la infección, permaneciendo activa durante el desarrollo del nódulo y en las nódulos maduros que fijan el nitrógeno. Ante la estipulación de la activación visible de pST1 durante la interacción solo pequeños cambios en los niveles de transcripción de ST1 se han detectado en raíces colonizadas cuando se comparan con sus respectivos controles. ADICIONAR ENLACE a la referencia cognitiva.

Estos resultados sugieren un papel para ST1 en el organogénesis de raíces y nódulos, ya que hay superposición entre los caminos controlando estos procesos 2. Sin embargo, debe ser investigado adicionalmente si ST1 también tiene un rol en otros aspectos de la simbiosis.

C0075 CHARACTERIZATION OF RHIZOSPHERIC BACTERIA IN NATURAL POPULATION OF ARABIDOPSIS THALIANA PLANTS TO IMPROVE PLANT GROWTH IN PROBLEMATIC SOILS

Miquel Llimós1, Soledad Martos Arias2, Christian Huber3, Viviane Radl3, Peter Schröder3, Charlotte Poschenrieder1

1Plant Physiology Laboratory, Bioscience Faculty, Universitat Autònoma de Barcelona (Barcelona) Spain
2Universitat Autònoma de Barcelona Bellaterra (Cerdanyola del Vallès) (Barcelona) España
3Plant Endophyte Physiology, Research Unit Microbe-Plant Interactions, Helmholtz Zentrum München GmbH, German Research Center for Environmental Health (Munich) Germany

1 Resumen

Fast increasing food demand requires improved crops and crop management techniques to extend cultivation on problematic soils. The use of nutrient efficient varieties, along with microorganisms facilitating nutrient availability are key strategies for optimizing production systems in a sustainable way. Zinc is an essential micronutrient and Zn deficiency not only affects crop yields, but also nutritional quality and human health. Microbial transformation of unavailable forms of soil zinc to plant available Zn is an important approach contributing to plant Zn nutrition and growth promotion. The aim of this project is to obtain efficient Zn solubilization bacteria to improve the Zn availability to plants. Natural populations of Arabidopsis thaliana are investigated to select demes with different Zn efficiency and to characterize the associated microbiome thus to meet complex plant-microbe-soil as a single interconnected entity.

Zinc solubilizing bacteria were isolated from rhizospheres of natural demes of A. thaliana. After quantification, different bacterial strains were selected using medium with soluble Zn in the form of Zn–phosphate. Bacterial strains able to mobilize Zn were characterized for optimum pH, optimum temperature, IAA production and siderophore production. Sequencing of 16SrRNA was performed to identify the isolated strains. The rhizosphere of an A. thaliana deme with high Zn uptake ability hosted a Serratia marcescens with the highest Zn solubilization capacity. Furthermore, next generation sequencing technique is being applied to the microbiome of different rhizosphere and endophytic bacteria of A. thaliana demes, cultivated in soils with different Zn availability. Data analysis is in progress.

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C0079 CHARACTERIZATION OF THE RESISTANCE TO CUCUMBER MOSAIC VIRUS AGGRESSIVE STRAINS IN MELON

Jinqiang Yan, Marta Pujol, Ana Montserrat Martín Hernandez
IRTA, Centre for Research in Agricultural Genomics CSIC-IRTA-UAB-UB (CRAG) Cerdanyola del Vallès (Barcelona)
Spain

1 Resumen

Infections by *Cucumber mosaic virus* (CMV) can cause complete harvest loss in more than 1000 plant species, including important crop plants such as cucumber, melon, tomato and pepper. CMV is classified in two subgroups (I and II) showing 70% nucleotide homology. In the melon accession PI161375, cultivar Songwhan Charmi (SC), the resistance to CMV is a complex trait with several genes involved. One single gene, *cmv1*, confers by itself total recessive resistance to strains of subgroup II by preventing viral transport from the bundle sheath cells, that surround the vein, to the phloem. To confer resistance to the subgroup I strains, at least two other QTLs (*qwcmv3.1* and *qwcmv10.1*) must act together and cooperatively with *cmv1*.

We are characterizing the resistance given by these QTLs and have established that it is acting at the level of virus transport. Melon lines with different numbers and combinations of QTLs *cmv1*, *qwcmv3.1* and *qwcmv10.1*, showed a delay in the production of systemic symptoms. Our results indicate that the two QTLs collaborate with *cmv1* in restricting the transport of the virus from the bundle sheath cells to the phloem, rather than in restricting virus transport within the phloem.

The same set of melon lines is being interrogated to search for the determinant of virulence that allows some CMV strains (like FNY), but not others (like M6), to overcome the resistance conferred by the three QTLs. Infection of the plants with reassortants generated with combinations of RNAs from FNY and M6 will uncover the viral factor that determines the virulence.
C0081 ARABIDOPSIS Responds to volatile compounds emitted by the fungal pathogen Alternaria alternata by triggering plastidic phosphoglucone isomerase-independent mechanisms

Francisco Jose Muñoz Perez1, Ángela María Sánchez López1, Abdellatif Bahaji1, Nuria Nuria De Diego2, Marouane Baslam1, Goizeder Almagro1, Adriana Ricarte Bermejo1, Lukás Spíchal2, Karel Doležal2, Sergio Ciordia3, Edurne Baroja Fernández1, Javier Pozueta Romero1

1Instituto de Agrobiotecnología (CSIC/UPNA/Gobierno de Navarra) Mutitua (Navarra) España
2Centre of the Region Haná for Biotechnological and Agricultural Research (Olomouc) Czech Republic
3Unidad de Proteómica Centro Nacional de Biotecnología (CSIC) (Madrid) España

1 Resumen

Volatile compounds (VCs) emitted by phylogenetically diverse microorganisms (including plant pathogens and microbes that do not normally interact mutualistically with plants) promote photosynthesis, growth and the accumulation of high levels of starch in leaves through cytokinin (CK) regulated processes. In the Arabidopsis plants not exposed to VCs, plastidic phosphoglucone isomerase (pPGI) acts as an important determinant of photosynthesis and growth, likely as a consequence of its involvement in the synthesis of plastidic CKs in roots. Moreover, this enzyme plays an important role in connecting the Calvin Benson cycle with the starch biosynthetic pathway in leaves. To elucidate the mechanisms involved in the responses of plants to microbial VCs, and to investigate the extent of pPGI involvement, we characterized pPGI null pgi1-2 Arabidopsis plants cultured in the presence or absence of VCs emitted by Alternaria alternata. We found that volatile emissions from this fungal phytopathogen promote growth, photosynthesis and the accumulation of plastidic CKs in pgi1-2 leaves. Notably, the mesophyll cells of pgi1-2 leaves accumulated exceptionally high levels of starch following VC exposure. Proteomic analyses revealed that VCs promote global changes in the expression of proteins involved in photosynthesis, starch metabolism and growth that can account for the observed responses in pgi1-2 plants. The overall data show that Arabidopsis plants can respond to VCs emitted by phytopathogenic microorganisms by triggering pPGI-independent mechanisms.
C0083 GLUTATHIONE INDUCED RESISTANCE TO TOBACCO MOSAIC VIRUS IN SALICYLIC ACID-DEFICIENT TOBACCO

András Künstler¹, Zsuzsanna Csontos², Réka Albert³, Lóránt Király¹

¹Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences Budapest (Pest) Hungary
²Budapest University of Technology and Economics, Faculty of Chemical Technology and Biotechnology (Pest) Hungary

1 Resumen

The role of salicylic acid (SA) in plant disease resistance mechanisms are well defined, primarily it is well-known as a mediator of plant defense responses to biotrophic pathogens. The glutathione are also well-known as an antioxidant and as a signal molecule in plant defense responses. Recent publications showed that SA and glutathione may interplay in plant defense mechanisms. Ghanta and coworkers showed that tobacco plants with high glutathione content the SA levels and the pathogen resistance to Pseudomonas syringae pv. tabaci are also increased (Ghanta et al., 2011). Genetic inhibition of glutathione accumulation in Arabidopsis thaliana led to decreased SA levels (Han et al., 2013). In our recent project we want to study that artificial elevation of glutathione in SA deficient tobacco (Nicotiana tabacum cv. Xanthi nahG) are able to induce resistance to tobacco mosaic virus (TMV).

Artificial elevation of glutathione levels in intact tobacco leafs are executed by injection with reduced glutathione (GSH) and (R)-(-)-2-Oxothiazolidine-4-carboxylic acid (OTC) 2 and 4 mM concentration and 2 or 3 days before TMV inoculation. Detection of TMV and the expression of different defense related genes are detected by RTqPCR.

Our results showed that artificial elevation of GSH and OTC could induce resistance in SA deficient tobacco compared to the untreated control: in GSH and OTC treated plants showed reduced TMV levels. Reduced virus level are accompanied by the reduction of gene expression of pathogenesis related (NtPR1a, NtPRB1b)and glutathione-S-transferase (NtGSTPhi, NtGSTTau1) genes. It seems that GSH and one of his precursor OTC could increase the injured resistance of the SA deficient tobacco, which accompanied by the reduction of gene expression of defense/stress related genes.

Used literature
Ghanta et al., 2011, Planta 233, 895-910.
Han et al., 2013, Antioxidants & Redox Signaling 18, 2106-2121.
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C0084 COMPARATIVE CROSS-SPECIES SENSITIVITY TO GAMMA RADIATION AND EFFECTS OF COMBINED GAMMA-UV-B EXPOSURE IN TERRESTRIAL PLANTS

Dajana Blagojevic1, YeonKyeong Lee1, Dag Brede Anders2, Ole Lind Christian2, Brit Salbu2, Knut Solhaug Asbjørn3, Line Nybakken2, Jorunn Olsen Elisabeth1

1 IPV, NMBU Ås (Norway) Norway  
2 IMV, NMBU (Norway) Norway  
3 INA, NMBU (Norway) Norway

1 Resumen
Plant exposure to gamma radiation may have a strong impact on growth and development. Although earlier studies indicated different sensitivity to gamma radiation among plant species, few systematic, comparative studies have been performed, and information about the underlying mechanisms is limited. In this study, young seedlings of Scots pine (Pinus sylvestris), Norway spruce (Picea abies) and Arabidopsis thaliana were exposed to gamma radiation with dose rates ranging from 0-540 mGy h\(^{-1}\) for 6 days. In addition, A. thaliana seedlings were exposed for 15 days. To evaluate effects of gamma radiation at termination of exposure, analyses of DNA damage using the COMET assay were performed, and subsequent growth and development and expression of DNA repair genes assessed. Consistent with increasing genotoxicity and ROS production, the size of Scots pine and Norway spruce seedlings decreased with increasing gamma dose rate. In A. thaliana, no such effect on shoot growth was observed in spite of increasing genotoxicity. During subsequent growing, shoot and root growth were negatively affected in the conifers with increasingly disorganised shoot and root apical meristems with increasing dose rate above \(10 \text{ mGy h}^{-1}\), demonstrating adversely affected cell division pattern. In surviving conifer plants, genotoxicity persisted after termination of gamma-exposure. Despite persistent genotoxicity also in A. thaliana, most growth parameters were not affected, except delayed floral bud formation at dose rates \(\geq 400 \text{ mGy h}^{-1}\). Gamma-UV-B-co-exposure of Scots pine reduced seedling size further in a dose-rate-dependent manner with preliminary results showing additive genotoxic effect at higher but not lower dose rates. Taken together, both Scots pine and Norway spruce seedlings show high sensitivity to gamma as compared to the highly resistant A. thaliana. The results on persistent genotoxicity in response to gamma radiation is consistent with higher tolerance to genotoxicity in A. thaliana than in Scots pine and Norway spruce.
C0126 CONTRIBUTION OF ARBUSCULAR MYCORRHIZAL SYMBIOSIS TO ALLEVIATE STRESS CAUSED BY NACOBUS ABERRANS NEMATODE

Sebastián Andrés Garita, Juan Ignacio Ripodas, Marcela Fabiana Ruscitti, María Cecilia Arango, Valeria Fernanda Bernardo

Instituto de Fisiología Vegetal, CONICET-UNLP La Plata (Buenos Aires) Argentina

1 Resumen

Nacobbus aberrans is a parasitic root-knot nematode that causes yield losses in horticultural crops across the Americas, even leading to the death of plants in the case of severe attacks. The products currently used for its control have a high economic cost, lack specificity and have a high degree of toxicity. Symbiosis with arbuscular mycorrhizal fungi (AMF) has been shown to have a protective effect against several soil pathogens. Plants of Solanum lycopersicum Cv. Platense both with and without mycorrhizal colonization by Funneliformis mosseae were cultivated in soil artificially inoculated and not inoculated with N. aberrans. Four months after the transplant, the plants were extracted and it could be observed that the mobile forms of N. aberrans had penetrated into the roots, destroying the cortical parenchyma, causing necrotic lesions, barking of the root and the death of rootlets. As a consequence of this damage in cell membranes, malondialdehyde levels were significantly higher (1.9 nmoles.g⁻¹) in comparison to tests treatments where the pathogen was absent (1.2 nmoles.g⁻¹). In mycorrhizal plants, the number of lesions was significantly lower than in non-mycorrhizal plants. Adult females were hosted by the roots forming galls, breaking the continuity of conductive tissues and altering the normal flow of water. As a consequence, infected plants suffered water stress, leading to an increase in proline levels (↑ 50%), and a reduction of protein content (↓ 20%). In mycorrhizal plants, water stress indicators had significantly lower values than in non-mycorrhizal plants. In addition, the number of nematode eggs collected in mycorrhizal roots was 80% lower than in non-mycorrhizal plants, indicating that this practice is beneficial to both attenuating the stress caused by the pathogen and reducing its population.
C0128 ARE SERINE PROTEASES INHIBITORS INVOLVED IN PLANT DEFENCE AGAINST SPIDER MITE?

Ana Arnaiz Alonso\textsuperscript{1}, M\textsuperscript{a} Estrella Santamaria\textsuperscript{1}, Lucia Talavera-Mateo\textsuperscript{1}, Manuel Martinez\textsuperscript{1}, Isabel Diaz\textsuperscript{1}

\textsuperscript{1}Centro de Biotecnología y Genómica de Plantas (UPM-INIA) Pozuelo de Alarcón (Madrid) España

\textbf{1 Resumen}

The spider mite \textit{Tetranychus urticae} is one of the most destructive agriculture pest worldwide. It is a polyphagous acari that feeds on a broad range of hosts. Its short generation cycle and high fecundity, facilitate rapid and easy adaptation to a diversity of toxins and pesticides. Moreover, climate change predictions presume that \textit{T. urticae} will develop quicker leading a great reduction in crop yields. \textit{T. urticae} is able to rear on Arabidopsis plants and its genome is available which offer important advantages for basic research in genetics and molecular biology.

After mite infestation, plants activate a complex signaling network to generate a combination of constitutive and basal defences. Among them, protease inhibitors (PIs) are final defence molecules with anti-nutritional properties against herbivores. In the case of \textit{T. urticae}, serine- and cysteine- proteases are numerous and essential for the mite physiology. It has been reported the role of cysteine proteases in hydrolytic digestion nevertheless the role of serine proteases in spider mite physiology remains unknown.

We have focused our attention in deciphering the molecular mechanisms used by the plant to synthesize serine-protease inhibitors in Arabidopsis in response to mite feeding. Five serine PIs have been identified in Arabidopsis genome, being four of them induced by \textit{T. urticae} infestation. We are currently characterizing these four PI-encoding genes. Preliminary data from bioassays with independent Arabidopsis mutant plants have demonstrated that the absence of serine PIs increase the foliar damage produced by the spider mite. These results suggest their role for plant defence. Further research will clarify the mode of action, protease targets and regulation of the defence process.
C0205 DISTINCT ACTIVITY CHANGES OF DEFENSE (PATHOGENESIS) RELATED GENES AND ANTIOXIDANTS DURING SYMPTOMLESS (EXTREME) VS. LOCAL NECROTIC (HYPERSENSITIVE) PLANT VIRUS RESISTANCE

Lóránt Király1, Orsolya Zsemberi2, András Künstler1, Réka Albert1

1 Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences Budapest (Pest) Hungary
2 Szent István University, Faculty of Horticultural Science (Budapest) Hungary

1 Resumen
Infection of Potato virus X (PVX) may elicit a symptomless, so-called extreme resistance (ER) in its plant hosts governed by Rx resistance genes, while N gene-mediated resistance to Tobacco mosaic virus (TMV) is associated with localized cell and tissue death (hypersensitive response, HR). The antiviral mechanism operating during symptomless ER, including the possible role of defense-related genes, reactive oxygen species (ROS) and ROS-neutralizing antioxidants is not known. By using tobacco (Nicotiana tabacum cv. Samsun NN, Rx) as a model plant, our aim was to monitor and compare changes in expression/activity of certain defense-related (e.g. pathogenesis-related, PR) genes and enzymatic/non-enzymatic antioxidants during virus-elicited symptomless ER and local necrotic HR.

Extreme resistance elicited by PVX was clearly detectable already at 6 hours after virus inoculation (by real time RT-qPCR) and has fully developed by the first day of PVX infection. Overall, no significant activity of defense-related genes and antioxidants occurred in PVX-inoculated plants exhibiting ER. Although the early induction of three defense-related/stress marker genes (NtPR-1a, NtPRB-1b and NtGSTphi) and three cell death and ROS-regulator genes (NtBI-1, NtAOX1-2, NgCat1) signaled the onset of resistance, gene expression either did not change or decreased following the development of ER. On the other hand, beginning from the first day after TMV inoculation (DAI), expression of all these genes has significantly increased, concomitant with the development of visible local necrotic lesions (HR). Furthermore, in case of TMV-induced HR, amounts of the non-enzymatic antioxidant and signaling agent, glutathione and activities of glutathione-associated antioxidant enzymes (glutathione reductase and glutathione S-transferase) has markedly increased 4 DAI, while no such changes occurred during PVX-induced ER. Our results demonstrate that the extreme resistance elicited by PVX is fully developed and completed by the first day of PVX infection and accompanied by distinct activity changes of defense-related (PR) genes and antioxidants.

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C0216 THE ROLE OF A MIRNA INVOLVED IN FLOWERING ON THE ARABIDOPSIS-ROOT-KNOT NEMATODES INTERACTION
Ana Cláudia Silva, Fernando E. Díaz-Manzano, Javier Cabrera, Carmen Fenoll, Carolina Escobar

Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla-La Mancha Toledo (Toledo) España

1 Resumen
Plant sedentary endoparasitic nematodes represent nowadays a major threat to agricultural production. Among those, the root-knot nematodes, *Meloidogyne* spp., are obligate parasites that are firstly attracted by plant exudates to the roots, where they penetrate and establish in the vascular cylinder. They form a pseudo-organ called gall containing their feeding cells, the giant cells (GCs; Escobar et al., 2015). Our group studied the transcriptome of those GCs isolated by laser microdissection compared to non-infected vascular tissues, interestingly, a high number of genes were repressed in early-developing GCs (Barcala et al., 2010). Although, the mechanisms mediating this gene repression are still not fully understood, massive sequencing of small RNAs (sRNAs) has demonstrated that 24-nt-sRNAs, which are involved in epigenetic regulation, were mostly upregulated in galls at 3 days post infection (Cabrera et al., 2016). This, strongly suggest that gene silencing could be mediated via 24-nt-sRNAs epigenetic regulation.

The miRNA 172 (miR172), which is known to regulate positively flowering, tuberization and fruit development in Arabidopsis (Abelenda et al., 2011; Khan et al., 2014), is induced in GCs. MiR172 regulates AP2-like transcription factors such as TOE1 that is repressed in GCs. Hence, we analysed the promoter of 5 genes coding miR172 (MIR172A, B, C, D; E), all showing different activation patterns in plants and in galls formed by *Meloidogyne* spp. MIR172C and D show a restricted activation pattern within galls. We will also discuss functional implications of miR172 and TOE1 after studying loss of function lines. Therefore, the participation of the gene module miRNA172-TOE1-FT on galls/GCs development will be evaluated.

*Both first and second authors have contributed equally to this work.*
C0256 HOST-SPECIFIC PROTEOMIC AND GROWTH ANALYSIS OF MAIZE AND TOMATO ROOTS INOCULATED WITH AZOSPIRILLUM BRASILENSE SP 7

Carla Roman1, Ana Isabel Cueto-Ginzo2, Ricardo Rodriguez1, Paula Meler, Ester Sin1, Maria Angels Achon1, Laura Arcal1, Vicente Medina3

1Universitat de Lleida (Lleida) Espana
2Universitat de Lleida, Agrotecnio Center (Lleida) Espana
3Universitat de Lleida, Programa Udl-IMPULS (Lleida) Espana

1 Resumen

Azospirillum brasilense Sp7 is a diazotrophic, free-living plant growth promoting rhizobacteria (PGPR) that is increasingly important for its ability to reduce stress and improve nutrient up-take by plants, thus moderating the need for chemical fertilizer application. To test the hypothesis that A. brasilense Sp7 interacts differently with the primary metabolism in C3 and C4 plants, differential proteomics was employed to study protein expression in A. brasilense Sp7-inoculated tomato and maize plants grown in greenhouse conditions. The proteomes of the two inoculated species were compared with each other and alongside their non-inoculated counterparts at various (early) vegetative growth stages in an effort to determine temporal proteome changes in response to inoculation. Plant height and root growth parameters were also monitored and analyzed to accompany proteomic data. Protein levels of 42 and 44 spots were significantly modulated by A. brasilense Sp7 root interaction with tomato and maize, respectively. Ferredoxin NADP+ reductase, glyceraldehyde-3-phosphate dehydrogenase and large subunit Rubisco were regulated in both species, while host-specific protein expression patterns provide insight into species-specific interactions. This study provides an integrated perspective on how A. brasilense Sp7 inoculation interacts with C3 and C4 plants to modulate primary metabolism, photosynthesis, and stress-response.
ROOT DEVELOPMENTAL PROCESSES ALTERED BY ENDOPARASITIC NEMATODES FOR THE ESTABLISHMENT OF THEIR FEEDING SITES.

Rocio Olmo López1, Javier Cabrera1, Virginia Ruiz-Ferrer1, Alejandra Garcia1, Miguel A. Moreno-Risueno1, Maria Fe Andrés3, Carmen Fenoll1, Carolina Escobar2

1Universidad de Castilla-La Macha Toledo España
2Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid-INIA (Madrid) Spain
3Departamento Protección Vegetal, Instituto Ciencias Agrarias CSIC (Madrid) Spain

1 Resumen
Root-knot nematodes (RKNs; Meloidogyne spp.) are a group of phyto-endoparasitic nematodes that constitute a major threat for agriculture due to their impact on plant productivity and to the gradual banishment of effective but contaminant chemical nematicides. RKNs induce a group of feeding cells, called giant cells (GCs) from vascular cell precursors by means of a battery of effector molecules. GCs expands and suffer numerous other changes, e.g., a modified cell cycle with repeated mitosis and aborted cytokinesis becoming transfer-like cells. Additionally, a gall is formed by divisions and hypertrophy of several tissues within the root around the GCs (Cabrera et al., 2015). We obtained the transcriptomes of early-developing Arabidopsis GCs/galls after M. javanica infection by laser capture microdissection (Barcala et al., 2010). The specific transcriptomes of GCs and galls were compared to those characteristics of different root cell types (Brady et al., 2007). Results indicated that the transcriptomes of undifferentiated root cell types, as those from the quiescent centre (QC) and the lateral root (LR) initial cells, shared characteristics with that of GCs and gall transcriptomes. Moreover, transcripts characteristic of developing xylem were overrepresented specifically in GCs, whereas transcripts from the endodermis were enriched in galls (Cabrera et al., 2014). Hence, we studied the expression and function of key genes for these cell types. Genes with essential roles in the root QC establishment and stem cell maintenance like Schizoriza (SCZ), Scarecrow (SCR), Short root (SHR) and WUSCHEL-RELATED HOMEBOX 5 (WOX5) were induced in Arabidopsis galls. We also analysed HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6), a xylem pole pericycle and protoxylem marker in the root apical meristem and different stages of LR development. Our main conclusion is that genes characteristics of root developmental processes are also important during gall and GC development.
C0283 LOOKING FOR NOVEL COMPOUNDS TO INCREASE YIELD CROP UNDER STRESS CONDITIONS

Francisco Javier García Machado¹, Cristina Garrido Orduña¹, Sarai Morales Sierra¹, Cristo Luis Jorge², Francisco Valdés González², Antonio Herrera González², Andrés Borges Rodríguez², Alicia Boto Castro¹, David Jiménez Arias¹

¹Instituto de Productos Naturales y Agrobiología-CSIC (Santa Cruz de Tenerife) Spain
²Universidad de La Laguna (Santa Cruz de Tenerife) Spain

1 Resumen

Biotic and abiotic stresses cause the majority of yield losses in agriculture. In order to prevent the downfall in the productivity traditionally labours uses different chemicals solutions (pesticide) to prevent biotic insults. By the other hand, abiotic stress is the difficult to manage because need cooperation between producers and public administration. Some of the traditional methods to fight against stress may cause problems in human health or environmental complications. Nowadays, the EU, in order to prevent these unlike secondary effect, has launched a specific normative about the formulations used in field. As a consequence is necessary to design new compositions with high activity but with low toxicity. On this sense, our research group study different compounds capable to induce a sensitisation or primed state which helps plants to face biotic and abiotic insults. Our background on the priming research line leads us to develop different strategies to test novel compounds using in-vitro and in-planta screenings. Here, we present some of these screenings and several of our new compounds to protect crops against biotic and abiotic stresses.
C0288 REGULATION OF ROOT COLONIZATION BY DIFFERENT ARBUSCULAR
MYCORRHIZAL FUNGI UNDER DIFFERENT STRESS CONDITIONS.

Javier Lidoy Logroño, Clara Amate, Jorge Prieto, Juan Manuel García Lidoy, Concepción Azcón-Aguilar, María J. Pozo

Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín – Consejo Superior de
Investigaciones Científicas (CSIC) Granada (Granada) España

1 Resumen

The arbuscular mycorrhizal symbiosis between plant roots and arbuscular mycorrhizal fungi (AMF) has an
enormous potential in modern and sustainable agriculture, improving plant nutrition and enhancing plant
fitness through boosted plant resistance to pathogens and pests, as well as tolerance to diverse abiotic
stresses. A functional symbiosis requires a very precise and fine-tuned regulation of plant responses,
mainly orchestrated by phytohormones. Thus, a holistic approach is required to understand the key
regulatory signals involved in the interaction under different conditions, and how they affect different AMF
genotypes. The aim of this work is to explore how the regulation of plant defense signaling impacts the
plant interaction with different AMF strains. Using Solanum esculentum as a model, we evaluate two
different AMF, Funneliformis mosseae and Rhizoglomus irregularis, amply distributed in most ecosystems
and commonly used as bioinoculants in commercial products. Both fungi were compared in their ability to
colonize tomato roots when they were applied independently or in combination in a 6-week assay under
greenhouse conditions, and the host plant was subjected to high salinity conditions (NaCl treatment) or to
defense activation by the pulverization of aboveground tissues with the main defense related hormones,
Methyl Jasmonate (MeJA), Abscisic Acid (ABA) and Salicylic Acid (SA). These stress treatments were
applied during the establishment of the symbiosis, two weeks after inoculation, and were maintained along
the assay. The hormonal treatments where applied once a week over a 4-week period. We found
significant differences in the pattern of colonization between the F. mosseae and R. irregularis depending
on the stress or hormonal treatments, ABA and NaCl treatments enhancing the colonization by F.
mosseae and MeJA inhibiting R. irregularis colonization. The combined AMF treatment showed also a
different pattern of mycorrhizal colonization compared to the individual fungi; MeJA, ABA and SA inhibited
the colonization.
Ecophysiology and Climate Change

C0107 NACL PROTECTS AGAINST CD AND CU- INDUCED TOXICITY IN THE HALOPHYTE ATRIPLEX HALIMUS

Insaf Bankaji¹, Jorge Collado Dominguez², Noomene Sleimi¹, Vicente Vives², Rosa Mª Perez², Aurelio Gómez²

¹Faculté des Sciences de Bizerte (Bizerte) Tunes
²Departamento de Ciencias Agrarias y del Medio Natural.Universidad Jaume I Castellón de la Plana (Castellón) España

1 Resumen
Toxic trace elements such as Cadmium (Cd²⁺) and Copper (Cu²⁺) are bioavailable to plants in the soil. When plants absorb trace elements can suffer a phytotoxic process such as biomass reduction, inefficient photosynthesis process, oxidative stress and reduced shoot growth. The objective of the present work was to evaluate the extent of Cd- and Cu-induced oxidative stress and the antioxidant response triggered in the halophyte species Atriplex halimus after metallic trace elements exposure. Plants were treated for one month with Cd²⁺ or Cu²⁺ (400 µM) in the absence or presence of 200 mM NaCl in the irrigation solution. The interaction between salinity and heavy metal stress was analyzed in relation to plant growth, tissue ion contents (Na+, K+ and Mg²⁺), oxidative damage (Malondialdehyde (MDA)) and antioxidative metabolism (Ascorbate peroxidase (APX), Catalase (CAT), Guaiacol peroxidase (GPX) and Reduced glutathione (GSH)). Data indicate that shoot and root weight significantly decreased as a consequence of Cd²⁺-or Cu²⁺-induced stress. Metallic stress leads to unbalanced nutrient uptake by reducing the translocation of K⁺ and Mg²⁺ from the root to the shoot. The levels of malondialdehyde increased in root tissue when Cd, and especially Cu, were added to the irrigation solution, indicating that oxidative damage occurred. Results showed that NaCl gave a partial protection against Cd and Cu induced toxicity, although these contaminants had distinct influence on plant physiology.

In conclusion, salinity drastically modified heavy metal absorption and improved plant growth. Salinity also decreased oxidative damage, but differently in plants exposed to Cd or Cu stress.

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C0113 LACTUCA SATIVA, A MODEL PLANT TO EVALUATE THE EFFECT OF IRON NANOPARTICLES (NPS) ON CONTAMINATED SOILS

Carmen Martín Fernández¹, Carmen Fajardo², Gerardo Mengs³, Mar Nande³, Margarita Martín⁴

¹ETSI Agronómica, Alimentaria y de Biosistemas. Universidad Politécnica de Madrid (Madrid) España
²Facultad de Veterinaria, Universidad Complutense (Madrid) Spain

1 Resumen

NPs can enter the soil through various pathways such as agricultural amendments, atmospheric deposition, landfills, and accidental spills during industrial production, or due to intentional applications in the context of soil remediation. Nanoscale zero-valent iron (nZVI) is introduced into soil by the latter pathway, since it has the potential to remediate diverse environmental contaminants, such as chlorinated organic compounds inorganic anions, metals, and metalloids. This remediation strategy has been mainly used for the decontamination of groundwater, although its application in soil remediation is being considered more frequently (Fajardo et al., 2012). However, it is necessary to check the effect of these remedial measures on living organisms. In the case of the effect of the NPs on plants, Lactuca sativa has been chosen as a model to evaluate the impact that its presence at different concentrations can have. The effect on their germination capacity has been studied as well as the expression of genes involved in the stress response (phenylalanine ammonia-lyase (PAL) and 4-hydroxyphenylpyruvate dioxygenase (HPPD)).

Germination of L. sativa was carried out on soil contaminated with two concentrations of heavy metals (Pb, Zn and Cd) without NPs and with 2 concentrations of NPs (1% and 5%). The inhibition of germination in the samples corresponding to the highest concentration of heavy metals was evident regardless of the presence of NPs. Differences were also detected in the stress levels of the different samples.
C0159 EFFECT OF HIGH SOLAR RADIATION ON POLYAMINES LEVELS ON APPLE EXOCARP

Laura Vita, Santiago Maiale, Valentín Tassile, Graciela Colavita

1Facultad de Ciencias Agrarias Cinco Saltos (Río Negro) Argentina
2Unidad de Biotecnología- Instituto Tecnológico de Chascomús-UNSAM-CONICET (Buenos Aires) Argentina
3Facultad de Ciencias y Tecnología de los Alimentos-UNCo (Río Negro) Argentina
4Centro de Investigaciones en Toxicología Ambiental y Agrobiotecnología del Comahue-UNCo-CONICET (Río Negro) Argentina

1 Resumen
High solar radiation stress causes an excessive production of reactive oxygen species that can not be counteracted by the antioxidant mechanisms in apple exocarp. This imbalance alters exocarp oxidative metabolism, maturity indices and pigments. Within the antioxidant plants defense under adverse environmental conditions, polyamines (PAs), such as Putrescine (Put), Spermidine (Spd) and Spermine (Spm) have been reported. However, there is limited information about PAs content in apple exocarp exposed to high solar radiation. At fruit maturity state, lipid peroxidation (thiobarbituric acid-reactive substances, TBARs), antioxidant activity (DPPH radical-scavenging) and free PAs content were analyzed in high solar radiation exposed exocarp (E) and non-exposed exocarp (NE) of Granny Smith apples. High solar radiation stress produced an increase in lipid peroxidation in E (59.8 nmol.g⁻¹FW) compared to NE (43.8 nmol.g⁻¹FW). Antioxidant capacity also increased 31.6% in E. PAs content increased significantly 39.7% in E in regard to NE. Put was the polyamine found in highest proportion followed by Spd and Spm. Put concentration was 159.0 nmol.g⁻¹FW in NE, while in E was 201.4 nmol.g⁻¹FW. Spd and Spm increased 63.6% and 40.8% respectively in E compared to NE. Chlorophyll content decreased from 62.5 µg.g⁻¹FW in NE to 28.5 µg.g⁻¹FW in E. Since Spd and Spm have been reported as direct free radical scavenger, it is possible that the improvement of the antioxidant capacity in apple exposed exocarp is related to Spd and Spm increase. However, this higher antioxidant capacity and particularly PAs level were not adequate to avoid higher lipid peroxidation, chlorophyll degradation and high solar radiation symptoms expression.
C0168 FUNCTIONAL STATUS OF LONG-LIVED MONUMENTAL PINUS CANARIENSIS TREES

Patricia Brito Sánchez¹, Eva María Pérez-Martín¹, Alicia V. Perera-Castro², J Roberto Lorenzo Martín¹, Águeda María González-Rodríguez¹

¹Department of Botany, Ecology and Plant Physiology, Universidad de La Laguna San Cristóbal de La Laguna (Santa Cruz de Tenerife) España
²Laboratori de Fisiologia Vegetal, Universitat de les Illes Balears (UIB) (Baleares) España

1 Resumen

Pinus canariensis Sweet ex Spreng is an endemic species to the outer Canary Islands, where forms one of the most representative natural forests. Beyond its singular fire resistance, this pine is one of the most paradigmatic species and its relevance has been reflected in numerous historical and cultural traditions. On many occasions, these popular beliefs are brought a focus in specific individuals, commonly known as “big pines” (“pinos gordos”), extremely long-lived monumental trees that stand out due to their DBH and height. These large trees are scarce in the forest due to extensive past exploitation.

In recent years, symptoms of decline have been observed in some of these individuals standing out the attention of scientific community and managers. Therefore, to provide information on the functional status of these unique individuals is an imminent requirement. The aims of this study were: to determine the functional status of monumental trees and to recognize trees die-off identifying useful physiological markers of this decline. Morphological and physiological characteristics (specific leaf area, relative water content, chlorophyll fluorescence parameters, chlorophyll and carotenoids pigments and tocopherols contents) were analyzed in the four canopy orientations and different heights of current and one-year needles of 6 monumental trees.

In general, all sampled trees showed lower chlorophyll contents than general range describe to this species. Nevertheless, most of the pines showed healthy physiological status (relative high content in chlorophyll and carotenoids, and fluorescence parameters in optimum range). Nevertheless, “Pino Bonito” growing in Gran Canaria Island showed a significant different functional response with evidences of canopy damage (high α-tocopherol and low photosynthetic pigments, Fv/Fm, Rfd and PI fluorescence parameters). This should be taken into account in management decisions.

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Cellular reactive oxygen species (ROS) accumulation is regulated by enzymatic and non-enzymatic antioxidants. However, as some are not synthesized by humans and animals, these antioxidants need to be supplied through vegetable ingestion. We analyzed the antioxidant capacity of different wild plants growing in the Sierra Nevada Mountains (Granada). We observed higher phenol content in fresh plant material in *Lupinus angustifolius* > *Vicia disperma* > *Lens nigricans* > *Trifolium cherleri*; however, in material dried to 60°C, phenol content was considerably higher, with the highest values observed in *Lathyrus* spp. and *Vicia* spp. In fresh and dry fruits, the highest phenol content was found in *T. glomeratum*. With regard to flavonoids, in both fresh and dry whole plants, the highest values were recorded in the genus *Lathyrus*, while *Medicago* showed the lowest levels. Flavonoid content in fruits showed similar values in fresh and dry materials, with the highest level found in *Trifolium* spp., while *Vicia* spp. recorded the lowest levels. This suggests that these species would be suitable as forage hay given their high antioxidant content in dry tissue.

Total antioxidant activity (TAA) was also analyzed in whole-plant fresh material. The highest activity was observed in *T. cherleri* > *M. rigidula* > *L. angustifolius*, being found lowest values in *Lens* spp. and *Lathyrus* spp. Catalase and guaiacol peroxidases, involved in the removal of H$_2$O$_2$, were especially abundant in *Vicia* spp. Comparison of the enzymatic activities as well as phenol and flavonoid content with TAA values showed no correlation between genus *Vicia* spp. and *Medicago* spp.; this could be explained by the complex nature of the antioxidants present in vegetables and their differing contributions to TAA.

This study was supported by the Spanish Ministry of Agriculture, Food and the Environment (National Park Network) (Ref: 748), ERDF co-financed grant BIO2015-67657-P from MICINN and the Junta de Andalucía (BIO-337).
C0250 EFFECT OF COMPOUNDS RELEASED FROM AGRICULTURAL BIODEGRADABLE PLASTIC FILMS IN THE PHYSIOLOGY OF IN VITRO GROWN LETTUCE, LACTUCA SATIVA L.

Hadaly Serrano Ruiz, Adrián Gaite, Lluís Martín-Closas, Ana María Pelacho

Universidad de Lleida (Lleida) España

1 Resumen
The substitution of polyethylene agricultural mulches by biodegradable ones that can be directly degraded in the soil after harvest is intended to provide an eco-friendly alternative to alleviate the problem of plastic waste disposal. However, during their degradation, they leach compounds whose safety for the environment has to be guaranteed. Therefore, there is an urgent need to assess whether the use of these materials entails eco-toxicological effects, especially on cultivated species, which has so far not been extensively studied.

This research work has focused on evaluating the physiological stress induced by compounds released from eight biodegradable plastics (BP1 to BP8) on lettuce plants. The evaluation was carried out by in vitro cultivation of lettuce seeds on a Murashige & Skoog culture medium to which an extract with the compounds released from the plastics had been added. The extract is the result of incubating the plastic in the mineral fraction of the culture medium. After one month from germination, the levels of three physiological stress indicators were analysed in the lettuce leaves: proline concentration, catalase activity and peroxidase activity. Germination was not affected. However, the extracts from two of the biodegradable plastics tested, BP4 and BP6, modified the plant physiology as compared to that of the control cultures without plastic extracts. BP4, a biodegradable plastic film made of PBAT, poly-lactic acid and thermoplastic starch, lead to a significant increase of proline concentration, catalase and peroxidase activities, suggesting an oxidative stress situation. Furthermore, the biomass of roots and leaves was significantly lower. On the other hand, the root morphology of the plantlets growing with BP6, made of a mixture of PBAT and cereal flour, was extensively altered. Overall, these results provide evidence of the potential harmful effects of compounds released from some biodegradable plastics on plant growth.
Phenotypic Plasticity of Garlic (Allium sativum L.) in Response to Water Availability

Álvaro Sánchez Virostoa, Raúl Sánchez Vioque, David Sánchez Gómeza

Centro de Investigación Agroforestal Albaladejito (Cuenca) España

1 Resumen
Garlic is an economically important crop in Spain and especially in Castilla-La Mancha. Water availability is expected to be more limiting in the region and affect this crop as a consequence of Climate Change. Thereby, an experimental study was carried out to unravel the physiological response and phenotypic plasticity of different garlic varieties to water availability in a locality near Cuenca during 2015-16 growing season. Throughout the experiment, the variability of key functional traits including specific leaf area (SLA), stomatal conductance, photochemical efficiency and secondary metabolites (e.g. proline and polyphenols) were evaluated. At the end of the growing season, bulb yield of each of the cultivars was also evaluated in relation to the different levels of irrigation. The results showed that bulb yield was affected under moderate drought. Differences among varieties on physiological traits were also found. This variability was also reflected in phenotypic plasticity differences among varieties. These results allowed identify key traits potentially involved in drought tolerance or improved yields under drought in this crop and discuss the relevance of phenotypic plasticity for the adaptation of this vegetative propagated crop under future scenarios of increased aridity.
C0273 WILL CLIMATE CHANGE BE AN OPPORTUNITY OR A RISK FOR BARLEY GROWTH?

Ander Yoldi-Achalandabaso, Jon Miranda-Apodaca, Amaia Mena-Petite, Maite Lacuesta, Alberto Muñoz-Rueda, Usue Pérez-López

UPV/EHU (Bizkaia) España

1 Resumen

Climate is one of the main factors affecting agricultural production, and any change on it can reduce crop productivity. According to current predictions on climate change made by the IPCC, atmospheric CO₂ concentration, temperature and the intensity and duration of droughts are expected to increase, which will imply changes in the growth of crops such as barley. Barley is the fourth most important cereal in the world in terms of production. It is a moderately drought tolerant species, which makes it a good candidate to grow under future environmental conditions.

Nowadays triple interaction studies (drought, elevated CO₂ and high temperature) are very scarce and it is unclear how species, and in particular barley, will adapt to such combined conditions. Therefore, the objective of this study was to analyze the vegetative response of a malting barley (Hordeum vulgare cv. Henley) under future environmental conditions.

Plants were grown in a Conviron PGR15 controlled environment growth chamber from sowing under current (400 ppm CO₂ levels and 23/17 ºC day/night temperature) or future (700 ppm CO₂ levels and 26/20 ºC day/night temperature) environmental conditions. The drought treatment (withholding water) went on for 9 days on both growth conditions.

The results showed that future environmental conditions will enhance biomass production, mainly due to an increase in net photosynthesis, provoked by higher internal CO₂ concentration, and in leaf area both in control and drought conditions. Besides, under future environmental conditions, the negative impact of drought on plant water status will be ameliorated due to an improvement in plant water potential.

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EXOGENOUS FOLIAR APPLICATION OF ABScisic ACID INDUCED CHANGES IN
OXIDATIVE STRESS INDICATORS, PHOTOSYNTHESIS AND ANTIOXIDANT ACTIVITY OF
OLIVE TREE

Sandra Martins, Cátia Brito, Lia Dinis, Alexandre Gonçalves, Helena Ferreira, José Moutinho Pereira, Carlos M. Correia
Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes e Alto Douro (Vila Real) Portugal

1 Resumen
Abssic acid (ABA), an isoprenoid phytohormone, is a signaling mediator which regulates various physiological processes and provides adaptation to different environmental stress conditions. Therefore, these features of ABA may be important for improving plant performance under projected drier future climate in the Mediterranean region. The experiment was conducted in order to investigate the concentration of phenols, flavonoids, ascorbic acid, soluble proteins, thiols, photosynthetic pigments, carbohydrates and reactive oxygen species (ROS), the antioxidant activity and the leaf gas exchange of three-year-old olive trees (Olea europaea L. cv. Cobrançosa) sprayed with 0 and 80 µM ABA that were subjected to three cycles of drought, by withholding water. Foliar ABA ameliorates the physiological and biochemical performance of olive tree during the drought events, judging by the higher concentration of chlorophylls, soluble proteins, total thiols and soluble sugars, as well by the rise of net photosynthesis, whereas the opposite was observed for the concentration of phenols, flavonoids, ascorbic acid, starch and ROS and for antioxidant activity. These results clearly suggest that the foliar application of ABA appear to be a very promising strategy to implement in olive orchards under water scarcity.

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C0280 CHANGES ON PHYSIOLOGICAL PARAMETERS DUE TO HIGH TEMPERATURE AFFECTS TOMATO- PSEUDOMONAS SYRINGAE PATHOSYSTEM

Eugenio Llorens Vilarrocha, Marcel Pitarch Marin, Emma Fernandez Crespo, Loredana Scalschi, Ana Isabel Hernandez Gonzalez, Leonor Lapeña Barrachina, Gemma Camañes Querol, Begonya Vicedo, Pilar Garcia Agustin

Universitat Jaume I castellon (Castellón) España

1 Resumen
The increase in average global temperatures and a reduction and redistribution of the rainfall linked to climate change are forcing plants to induce adaptations against these stresses. In order to study and understand how plants manage these adaptations, changes in physiologic parameters have been analyzed in this work to perform a comparative study between tomato plants grown under normal conditions and plants subjected to high temperatures, which mimic the abiotic stress that could be produced by the climate change. To evaluate the changes observed in a climatic change scenario, tomato plants were maintained at 31ºC during the day and 27ºC during the night. Pseudomonas syringae pv. tomato strain DC 3000 was used to evaluate the plant-pathogen system under these conditions. The bacteria was grown in KB at 31ºC and subjected to three consecutive plating with the aim to adapt it to high temperatures. Tomato plants grown at high temperatures have not shown an apparent damage in the photosynthetic system. However, they have undergone changes in photosynthetic, transpiration rates and chlorophyll content. In the profile of some amino acids was demonstrated the impact of this stress on primary metabolism. In addition, it has been observed that, under these conditions, the severity of infection with Pseudomonas syringae has been reduced. The levels of the hormones involved in plant defense have undergone major changes at high temperatures in infected plants, especially ABA, which could contribute to stomatal closure. These results show a change on the tomato defense mechanisms against Pseudomonas syringae upon high temperature. However, more studies are needed to elucidate how the climate change impacts on the immune system of plants.
C0285 SUSTAINED DIACYLGLYCEROL TRANSPORT FROM THE ENDOPLASMIC RETICULUM TO THE PLASMA MEMBRANE BY SYNAPTOTAGMINS 1 AND 3 IS REQUIRED FOR FULL COLD-ACCLIMATED FREEZING TOLERANCE IN ARABIDOPSIS

Noemi Ruiz López¹, Jessica Pérez Sancho¹, Ana Alvarez Mena¹, Abel Rosado¹, Arnaldo L. Schapire¹, Sonia Osorio¹, Steffen Vanneste², Daniel Van Damme³, Lothar Willmitzer⁴, Carlos Pereá⁵, Julio Salinas⁵, Miguel Ángel Botella¹

¹Departamento de Biología Molecular y Bioquímica, IHSM (Universidad de Málaga-CSIC), (Málaga) Spain
²Department of Botany, Faculty of Sciences, University of British Columbia, (Vancouver,) Canada
³Department of Plant Systems Biology, VIB-Ghent University, (Ghent) Belgium
⁴Central Metabolism Group, Max Planck Institute of Molecular Plant Physiology, (Potsdam-Golm,) Germany
⁵Departamento de Biología Medioambiental, Centro de Investigaciones Biológicas (CSIC), (Madrid,) Spain.

1 Resumen

Cold acclimation is the capacity of certain plants to increase their freezing tolerance in response to a period of low non-freezing temperatures. Cold acclimation involves a series of biochemical and physiological adaptations, including a deep transcriptional reprogramming and drastic changes in the lipid composition of cellular membranes in order to prevent the freeze-induced damage. While a profound knowledge has been acquired on the regulation of gene expression triggered by cold-acclimation, very little is known about the mechanisms governing the cold-induced changes in membranes’ lipid composition. We report that in Arabidopsis, the constitutively expressed Synaptotagmin 1 (SYT1) and the cold-induced homolog Synaptotagmin 3 (SYT3) are essential for cold-acclimated freezing tolerance SYT1 and SYT3 are phospholipid-binding proteins located in Endoplasmic Reticulum-Plasma Membrane contact sites (ER-PM CS), conserved structures defined as regions of the cortical ER in close apposition to the PM. ER-PM CS facilitate the non-vesicular lipid transport between ER and PM in yeast and mammals, and are essential for lipid homeostasis. Confocal microscopy analyses show that during cold acclimation there is an increase of SYT1::SYT1::GFP signal as spots at the PM. High-resolution lipidome analyses show that SYT1 mainly transports diacylglycerol, indicating that PM homeostasis of this lipid is essential for cold-acclimation and freezing tolerance.
C0308 THERMIC LIMITS AND ACCLIMATION ASSESSMENT OF CANARY SUMMIT WALLFLOWER

Águeda María González Rodríguez1, Eva Mª Pérez Martín2, Patricia Brito Sánchez2

1Universidad de La Laguna San Cristobal de La Laguna (Tenerife) España
2Dpto Botánica, Ecología y Fisiología Vegetal (Tenerife) España

1 Resumen

In high mountain ecosystems, temperature is one of the most important limiting factors that determine the plant survival and fitness. Under climate change context it is expected that these ecosystems would be strongly affected causing biodiversity loss, habitat degradation and landscape modifications. Special attention is being focused in Mediterranean high mountain ecosystems, as Teide National Park (Canary Islands), where a high number of endemic species are present. The aim of this work was to determine the thermal limits and the temperature acclimation of Canary summit wallflower (Erysimum scoparium (Brouss. ex Willd.) Wettst.), an endemic species presents in the National Park. Low and high temperature resistances were assessed in a seasonal study by chlorophyll fluorescence analysis. Irreversible damage (LT_{50}) was evaluated by maximum quantum yield of PSII photochemistry (Fv/Fm) and chlorophyll fluorescence decrease ratio (Rfd) with an imaging fluorimeter.

Rfd parameter was a more sensitive index than Fv/Fm parameter to evaluate damage in low and high temperatures. E. scoparium acclimated to low temperatures with the lowest LT_{50} values achieved in February and November (~9ºC). Even under acclimation, this species was quite sensitive to freezing temperatures compared to other high-mountain species. High temperature acclimation was not observed with LT_{50} around 40ºC. Spatial distribution of damage in the leaves was different at low and high temperatures. According to Rfd evaluation, this species could be in risk of damage for low temperatures due to this threshold is within to the absolute minimum temperatures registered in Teide National Park. So freezing events could be an important risk factor for this species in this alpine ecosystem.
C0327 DIFFERENCES IN FUNCTIONAL AND XYLEM ANATOMICAL FEATURES ALLOW CISTUS SPECIES TO CO-OCCUR AND COPE DIFFERENTLY WITH DROUGHT IN THE MEDITERRANEAN REGION

José M. Torres Ruiz1, Hervé Cochard2, Elsa Fonseca3, Margarida Vaz3

1University of Bordeaux, France
2INRA, UCA, PIAF (Clermont-Ferrand) France
3Departamento de Biologia, Escola de Ciências e Tecnologia, ICAAM—Instituto de Ciências Agrarias e Ambientais Mediterrânicas, Universidade de Evora, (Evora) Portugal

1 Resumen
A significant increase in drought events frequency is predicted for the next decades induced by climate change, potentially affecting plant species mortality rates and distributions worldwide. The main trigger of plant mortality is xylem hydraulic failure due to embolism and induced by the low pressures at which water is transported through xylem. As the Mediterranean basin will be severely affected by climate change, the aim of this study was to provide novel information about drought resistance and tolerance of one of its most widely distributed and common genera as a case study: the genus Cistus. Different functional and anatomical traits were evaluated in four co-occurring Cistus species in the Mediterranean Montado ecosystem. Soil water availability for each species was also assessed to evaluate if they show different ecological niches within the area. Results showed physiological and xylem anatomical differences between the four co-occurring species, as well as in the soil water availability of the sites they occupy. Despite the significant differences in embolism resistance across species, no trade-off between hydraulic safety and efficiency was observed. Interestingly, species with narrower vessels showed lower resistance to embolism than those with higher proportions of large conduits. No correlation, however, was observed between resistance to embolism and wood density. The four species showed different water-use and drought-tolerance strategies, occupying different ecological niches that would make them cope differently with drought. These results will allow us to improve the predictions about the expected changes in vegetation dynamics in this area due to ongoing climate change.

Keywords: climate change, embolism, hydraulic safety margins, water use, xylem anatomy.
Growth and Development

C0054 LOCALIZATION OF SPHINGOLIPID ENRICHED PLASMA MEMBRANE REGIONS AND LONG-CHAIN BASE COMPOSITION DURING MATURE-FRUIT ABSCISSION IN OLIVE

Maria C. Parra Lobato1, Miguel A. Paredes1, Juana Labrador1, Mercedes Gallardo2, Maria C. Gomez-Jimenez3

1Universidad de Extremadura (Badajoz) Spain
2Universidad de Vigo (Pontevedra) Spain
3Badajoz (Badajoz) Spain

1 Resumen

Sphingolipids, found in membranes of eukaryotic cells, have been demonstrated to carry out functions in various processes in plant cells. However, the roles of these lipids in abscission processes remains to be determined in plants. Biochemical and fluorescence microscopic imaging approach has been adopted to investigate the accumulation and distribution of sphingolipids during mature-fruit abscission in olive (Olea europaea L. cv. Picual). Here, a lipid-content analysis in live protoplasts of olive abscission zone (AZ) is made with fluorescent dyes and lipid analogues, particularly plasma membrane sphingolipid-enriched domains, and their dynamics are investigated in relation to the timing of mature-fruit abscission. In olive AZ cells, the proportion measured of both polar lipids and sphingolipids increases as well as endocytosis is stimulated during mature-fruit abscission. Likewise, mature-fruit abscission resulted in quantitative and qualitative changes in sphingolipid long-chain bases (LCB) in olive AZ. The total LCB increase was due essentially to extension of t18:1(8E) LCBs, suggesting that C-4 hydroxylation and Δ8 desaturation with a preference for (E)-isomer formation are quantitatively the most important in olive AZ during abscission. However, our results also showed a specific association between the dihydroxylated LCB sphinganine (d18:0) and the mature-fruit abscission. These results indicate a clear correlation between the sphingolipid distribution and mature-fruit abscission. Moreover, measurements of endogenous sterol levels in the olive AZ revealed that it accumulates sitosterol and campesterol with a concomitant decrease in cycloartenol during abscission. In addition, underlying the distinct sterol composition of AZ during abscission, genes for key biosynthetic enzymes for sterol synthesis for obtusifoliol 14α-demethylase (CYP51) and C-24 sterol methyltransferase2 (SMT2) were up-regulated during mature-fruit abscission parallel to the increase in sitosterol content. The differences found in AZ lipid composition, and the relationships established between LCB and sterol composition, offer new insights about sphingolipids and sterols in abscission signalling.
KNO3- AND NITRIC OXIDE-INDUCED PLANT GROWTH IN PEA SEEDLINGS IS RELATED WITH ANTIOXIDANTIVE METABOLISM AND ABA/GA BALANCE

Antonia Vidal, Daniel Cantabella, Agustina Bernal-Vicente, Pedro Díaz-Vivancos, José A. Hernández Cortés

CEBAS-CSIC (Murcia) Spain

1 Resumen

Different nitrogen-containing compounds, including nitrite, nitrate (NO$_3^-$) or sodium nitroprusside (SNP, a NO-generating compound) have been associated with breaking seed dormancy and the germination process. In this work, we study the effect of some nitrogen-containing compounds, such as KNO$_3$ and SNP, in the presence and the absence of c-PTIO (a NO-scavenger), on the germination process and the early growth of pea seedling. The effect of these treatments on the antioxidant defences as well as the hormone profile in pea seedlings was studied in order to study the possible interrelation antioxidant metabolism-plant hormones during the germination process and early seedling growth.

Results showed that 10 mM KNO$_3$ and 50 µM SNP significantly increased seedling growth. The effect of SNP in seedling growth was overcome in the presence of cPTIO. However, the same effect was not observed in the combination KNO$_3$ + cPTIO.

KNO$_3$ had no important effect in the ASC-GSH cycle enzymes but increased POX and AOX activities, whereas SNP reduced MDHAR and increased SOD, POX and AOX activities. In general, the addition of cPTIO increased APX and MDHAR and reduced POX.

Both treatments increased reduced ascorbate levels, but this response was reverted in the presence of CPTIO. In SNP-treated seeds, a higher GSH level was reported, but in all cases, cPTIO reduced GSH, leading to a decrease in the redox state of glutathione.

KNO$_3$ and SNP increased the concentration of GA$_4$ in pea seedlings, but the NO-scavenger reduced its levels. Conversely, both treatments reduced ABA concentration resulting in a decrease in the ABA/GA ratio. The presence of the NO-scavenger increased ABA levels as well as the ABA/GA ratio.

Results showed a relation between KNO$_3$ and SNP treatments, antioxidant metabolism and ABA/GA balance and the early seedling growth in pea.
1 Resumen

In the conventional plant secretory pathway, newly synthesized proteins are transported from the endoplasmic reticulum (ER) to the Golgi apparatus and to the cell surface or the vacuole. The “early secretory pathway” involves bidirectional transport between the ER and the Golgi apparatus, which is mediated by COP (COat Protein) I and COPII coated vesicles. COPII vesicles are involved in ER export and transport to the Golgi apparatus, while COPI vesicles are involved in intra-Golgi transport and in retrograde transport from the Golgi back to the ER. We have recently found that loss of function of α2-COP, a subunit of the COPI coat, caused defects in plant growth and altered morphology of the Golgi apparatus. Interestingly, the α2-cop mutant showed a strong upregulation of the COPII subunit SEC31A, but not of SEC31B. According to public microarray data, these two SEC31 isoforms are differently expressed in Arabidopsis tissues, with SEC31B expression being about 10 times higher than that of SEC31A. We have now investigated the function of these two COPII subunits in the Arabidopsis secretory pathway by using a loss-of-function approach. While the sec31A mutant did not show any obvious alterations, the sec31B mutant showed defects in plant growth and in the transport along the secretory pathway. In particular, sialyltransferase-YFP (ST-YFP), a typical marker of the Golgi apparatus, showed a partial ER accumulation in the sec31B mutant, suggesting a key role of SEC31B in ER export and transport to the Golgi apparatus. Interestingly, the vacuolar localization of a vacuolar marker, sporamin-RFP, was not significantly affected in this mutant.
C0111 COLD PLASMA AND ITS EFFECTS ON THE GROWTH AND PHYSIOLOGY OF WHEAT

Alireza Iranbakhsh\textsuperscript{1}, Mostafa Ebadi Iranbakhsh\textsuperscript{2}

\textsuperscript{1}\textit{Tehran (Tehran) Iran}
\textsuperscript{2}\textit{Islamic Azad University, Damghan Branch (Semnan) Iran}

1 Resumen

This study was carried out to reveal the possible effects of non-thermal plasma on different growth and physiology related characteristics in wheat. Short and long-term effects of nitrogen and helium derived plasma (surface power density of 0.4 W cm\textsuperscript{2}) with different exposure times (15, 30, 60, and 120 s.), repeated for 1, 2, and 4 times were assessed. The single-time manner of treatment with helium or nitrogen derived plasma significantly increased total root and shoot lengths, contrasted with four times. The obtained findings showed that root system was more sensitive than shoot. Moreover, plants were more sensitive to nitrogen-derived plasma. The modifications in various physiological characteristics were caused by plasma. the plasma generated signaling molecules (ozone, nitric oxide, and/or UV radiation) induced enhances in the activities of peroxidase and PAL and promoted protein content in leaves, especially when times and/or repetitions increased. In long-term responses to plasma, seedlings treated with the highest intensity of nitrogen derived plasma perished. Interestingly, the growth-inhibiting effects of some plasma treatment not only caught up control, but even the growth rate and biomass accumulation in were promoted. plasma technology has a considerable potency to affect plant cellular metabolism.
C0163 MANNANS AND MANNANASES IN GERMINATING BRASSICACEAE SEEDS

Néstor Carrillo Barral1, Ángel J Matilla Carro2, María del Carmen Rodríguez Gacio2, Pilar Carbonero Zalduegui3, Raquel Iglesias Fernández3

1Departamento Biología Funcional (Coruña) España
2Universidad de Santiago (Coruña) España
3Universidad Politécnica de Madrid (Madrid) España

1 Resumen
The seed germination consists of three progressive steps that exhibit a molecular and hormonal regulation: imbibition, embryo expansion and rupture of the softened seed covering layers, and embryo root emergence (Bewley et al., 2013). Upon imbibition, the endosperm is enzymatically weakened as a consequence of the hydrolysis of structural components of their cell walls (CW) (revised in Nonogaki, 2014 and Cosgrove, 2016). This softening makes more feasible the protrusion of the elongated embryonic axis through the endosperm cells surrounding the micropyle (Scheller et al., 2014). The cells of endosperm whose CW are enriched in mannans contribute to a strong mechanical resistance for the radicle protrusion. For this reason, CW-modifying proteins as endo-β-1,4-mannanases (MANs; EC. 3.2.1.78) are considered one of the key players in the radicle protrusion of germinating seeds (revised in Rodríguez-Gacio et al., 2012; Iglesias-Fernández et al., 2013; González-Calle et al., 2015). We have studied the location and degradation of mannans during the germination of two Brassicaceae seeds with (Sisymbrium officinale) and without endosperm (Brassica rapa). These seeds have two- and one-step germination, respectively. The data presented clearly conclude that mannans are preferentially localized in the mucilage layer of both seeds, whereas MAN2, MAN5, MAN6 and MAN7 transcripts are in the micropylar endosperm and radicle tip (S. officinale) and in the radicle and slightly in the vascular bundles in cotyledons (B. rapa). If the mannans are degraded during sensu-stricto germination, a unidirectional movement of MAN proteins should be contemplated in both species.
C0197 STRESS-INDUCED MICROSPORE EMBRYOGENESIS REQUIRES AUXIN BIOSYNTHESIS AND TRANSPORT WHILE THEY DECREASE DURING POLLEN DEVELOPMENT IN RAPESEED AND BARLEY

Yolanda Pérez-Pérez¹, María Teresa Solís¹, Ahmed-Abdalla El-Tantawy¹, Héctor Rodríguez-Sanz¹, Marta Pitarch⁵, Aurelio Gómez-Cadenas⁵, María-Carmen Risueño⁵, Pilar Sanchez Testillano⁵

¹Biological Research Center, CIB-CSIC (Madrid) Spain
²Dep. CC Agrarias y del Medio Natural, Univ. Jaume I (Castellón) Spain

1 Resumen
Isolated microspores can be reprogrammed in vitro by stress, they become totipotent cells, follow embryogenesis and produce doubled-haploid embryos and plants. During in vivo anther development, after meiosis, microspores develop and follow the gametophytic pathway to produce pollen grains. The involvement of auxin in these two microspore pathways is very limited.

We analyzed auxin concentration and cellular accumulation, expression of TAA1 and PIN1-like genes of auxin biosynthesis and efflux carrier during the two microspore developmental pathways in *Brassica napus* and *Hordeum vulgare*. Effects of inhibitors of auxin biosynthesis (Kynurenin), transport (N-1-naphthylphthalamic acid, NPA) and action (α-(p-Chlorophenoxy) isobutyric acid, PCIB) in microspore embryogenesis were also analyzed.

During stress-induced microspore embryogenesis TAA1 gene was up-regulated, auxin concentration increased and accumulated in early embryo cells from the first embryogenic divisions. Kynurenin treatments decreased microspore embryogenesis efficiency, indicating that de novo auxin biosynthesis was required in this microspore developmental pathway. PIN1-like gene expression was also induced with microspore embryogenesis, while NPA and PCIB inhibitors impaired embryogenesis initiation and development. These results indicated that polar auxin transport and auxin action were required for microspore embryo progression. In contrast, during gametophytic development auxin levels, TAA1 and PIN1-like expression were high at early microspore development, in tetrads and tapetum, while they progressive decreased during gametogenesis in both pollen and tapetum cells. Findings showed opposite auxin dynamics along the two microspore pathways with different fates. Endogenous auxin biosynthesis, action and polar transport are required for microspore embryogenesis initiation and progression while auxin progressively diminishes during gametophytic development, in both species.


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C0203 AUXIN AND ZINC ANTAGONISTICALLY INFLUENCE LEAF SENESCENCE

Catalina Cabot Bibiloni1, Juan Barceló Coll2, Charlotte Poschenrieder Wiens3

1Universitat de les Illes Balears Palma (Illes Balears) Espanya
2Universitat Autònoma de Barcelona (Barcelona) Espanya

1 Resumen
Leaf senescence is an active process regulated by many environmental signals and endogenous factors. Amongst the endogenous factors, the role of auxin is controversial, as both promoting and inhibiting auxin effects on leaf senescence have been reported. On the other hand, earlier findings suggest a possible role of Zn in auxin metabolism. In this work, the interaction between auxin and Zn was studied during developmental leaf senescence in A. thaliana. Several auxin and ethylene mutants were grown at 0.5 µM and 10 µM Zn concentrations until plant senescence. The following genotypes were used: Col-0, wild-type; Col(gl1), wild-type; yucca6-1D, a mutant for auxin synthesis; pin1, a mutant for IAA transport; aux1-7ein2, an auxin resistant, ethylene insensitive mutant; ein2-1, an ethylene insensitive mutant and eto1-1, an ethylene overproducer. From flowering onset to fruit maturation, plant growth, leaf chlorophyll and protein concentration and the expression of senescence gene markers was analysed by RT-PCR at different times. No significant differences in plant growth were found between Zn treatments in any genotype. Concerning the 0.5 µM Zn-treated plants, only pin1 and ein2-1 showed delayed senescence. While, save for yucca6-1D, the 10 µM Zn-treated plants had slower development and delayed leaf senescence. The high auxin yucca6-1D showed no differences in the onset of plant developmental stages between Zn treatments, which suggests a possible prevailing effect of auxin. Our results indicate that auxin and Zn might antagonistically influence developmental leaf senescence in Arabidopsis.

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Growing cereal seedlings in liquid medium helps to evidence the release of various molecules in the exudates, such as acids, oxidases, proteases, etc. It also enables to study with relative ease the root architecture according to various parameters. The link between root architecture and the liberation of protein pools, such as oxidases and proteases, is studied in a bioassay of plant interaction through the presence of chitosan, a polysaccharide that is extracted from crustacean shells. In agriculture, it is used, inter alia, to boost plants’ innate ability to defend themselves against fungal infections.

In the present work, each variety exhibits a well-defined pattern of proteases and oxidases in the bioassay, which constitutes a fingerprint. The application of chitosan enables to modulate the fingerprint, and to induce a discontinuous variation of the root architecture correlated with oxidase/protease switch with the same characteristics, while activating the appearance of 1-cys-peroxiredoxin, which is a typical seed protein. The interspecific seedling/seedling interaction amplifies the observed phenomena. In this way, each given phenotype of root architecture could be described by a mapping that includes the seedlings’ number, the interspecific varieties’ number, chitosan content, and time. This mapping helps to provide a given seedling’s variety with a well-identified architectural phenotype. Then, the study of the architectural trajectory in terms of the duration of the induced pattern or in terms of the time of resilience, i.e. the time that is necessary to recover a standard phenotype, enables us to apprehend the functional mechanisms of chitosan on the root target.

On the basis of the results that are obtained with wheat, rice and maize seedlings, a model is proposed to account for the link between chitosan and the discontinuous variation of the root architecture phenotype.
C0220 DIFFERENCES IN FRUITING EFFICIENCY AMONG CEREAL CROPS

Addy Garcia¹, Roxana Savin¹, Gustavo A. Slafer²

¹Universitat de Lleida (Lleida) España
²ICREA & Universitat de Lleida (Lleida) España

1 Resumen
Increasing yield of cereals is a major goal of breeding programs. For achieving this aim, it would be relevant to count with information on physiological determinants of yield. As the number of grains seems more relevant than their average weight, and further improvements in partitioning of pre-anthesis to the juvenile inflorescences seem unlikely, some authors have discussed the idea of focusing on fruiting efficiency (FE). There is limited literature on FE in wheat, and virtually nothing on other cereals. In this study, we quantified the FE across different cereals; identifying causes and analysing whether differences in FE brought about losses in average grains weight across modern cultivars of bread wheat, rye, 2-row and 6-row barleys, oats and triticale.

A wide range of FE variation was evidenced among different species. Wheat and triticale had the lowest FEs (c. 80 grains inflorescence⁻¹), whilst rye had the highest (c. 165 grains inflorescence⁻¹) and the two barley types and oats showed intermediate values (97-116 grains inflorescence⁻¹). Causes of the interspecific differences in FE were not always the same. Differences between triticale, wheat and rye seemed related to their differences in grain setting with no major differences in the number of primordia that reached the stage of fertile florets per unit of pre-anthesis inflorescence growth. On the other hand, differences between barley and oat seemed related to the efficiency with which the inflorescence allocates resources towards growth of florets (affecting the number of fertile florets per unit of inflorescence weight at anthesis). Overall cereals there was a significant relationship between the inflorescence dry matter partitioning to the florets (against structural parts) and the efficiency for converting inflorescence weight at anthesis into fertile florets. We found a negative trend between average grain weight and FE (cereals with lower FE tended to produce heavier grains).
C0227 GRAIN WEIGHT SENSITIVITY TO SOURCE-SINK MANIPULATIONS IN MAIZE GROWN IN THE FIELD UNDER CONTRASTING N AND TEMPERATURE REGIMES

Gustavo A. Slafer¹, Raziel A. Ordoñez², C. Mariano Cossani³, Roxana Savin³

¹ICREA & University of Lleida (Lleida) España
²Department of Agronomy, Iowa State University (Iowa) USA
³South Australian Research and Development Institute (South Australia) Australia
⁴University of Lleida (Lleida) España

1 Resumen
Maize yield depends on the interaction between the number of grains set and their weight. Physiological causes for grain weight determination are not clear. Grain weight depends on the potential size established around silking, as well as on its realization during the effective grain filling period. Controversies exist in the literature regarding the degree of source-limitation during grain filling. Most previous source-sink relationship research has been done through modifications in crop management affecting the strengths of both sources and sinks, as well as grain weight potential. We aimed to analyse likely causes of the responsiveness of grain weight to defoliation and degraining treatments (with a novel procedure: carefully opening the husks and provoking alternate rows of grains of the ear to die, spraying a broad spectrum fungicide, and finally returning the husks to their original position). Source-sink treatments were imposed 15 d after silking, and we quantified the responsiveness of grain weight to these source-sink manipulations in a large number of background environments (a wide range of N and heat stress conditions) in field experiments. Grain weight was largely unresponsive to increases in source availability but diminished by defoliations in 6 out of 7 experiments. However, this reduction weight was not hierarchical (grains from different thirds of the ear responded similarly), and was not worsened by imposing simultaneously a heat stress. Heat affected the grain growth capacity directly, and indirect effects (through reducing source strength due to accelerated senescence) were not evident. Neither defoliation increased nor degraining diminished the penalty imposed by heat, which in turn was similar for grains of different potential grain size. This study evidenced that yield in maize would be limited by sink strength during grain filling, even when the weight of the grains may respond to reductions in source-sink ratios during grain filling.
ROLE OF STORAGE PROTEINS AND GLUTATHIONE-RELATED ACTIVITIES AS BIOMARKERS FOR THE STUDY OF THE PHYSIOLOGICAL CHANGES DURING SEED GERMINATION AND SEEDLING DEVELOPMENT

Elena Lima-Cabello¹, Mohammed Mrani-Alaoui², Paula Robles-Bolívar¹, Juan D. Alché¹, Jose Carlos Jimenez-Lopez³

¹Dept. Biochemistry, Cell & Molecular Biology of Plants; Estación Experimental del Zaidín; Spanish National Research Council (CSIC) (Granada) España
²Département de Biologie, Faculté des Sciences, Université Abdelmalek Essaâdi (Tétouan) Morocco
³Jose C. Jimenez Lopez Granada España

1 Resumen

Narrow-leafed lupin (NLL) is mainly characterized by its adaptation to drought stress. However, in order for the seed to acquire a high germinability state, desiccation tolerance is an essential process that has to be achieved during seed maturation as adaptive strategy to enable seed survival during storage and to cope with severe environmental conditions. Numerous cellular and biochemical events, such as the formation of specialized protein-storage vacuoles, synthesis and accumulation of storage proteins, oligosaccharides, and activation of antioxidant defenses (capacity of scavenging ROS to avoid deleterious damage), appear associated with the acquisition of desiccation tolerance of seeds. Thus, antioxidant enzymes have been considered of particular importance for acquiring seed maturation and capacity of germination (vigor).

Despite the abundant knowledge about NLL agricultural traits, scarce information is known about molecular and cellular changes in the mature seed and germinating seedling.

In the present work, we have studied the different steps of NLL seed germination by means of microscopy and biochemical methodological approaches. A large amount of storage proteins is accumulated in protein bodies, and mobilized during germination. We have analyzed the roles of conglutin families (a, b, g, d), focusing on family b in the endosperm and cotyledon tissues during germinating stages, as well as glutathione-related activities (g-L-Glutamyl-L-cysteine synthetase, Catalase, Cu/Zn-SOD, GPx, GR, GSS, GST) in mature NLL seeds, in order to better understand the key molecular and regulatory signaling pathways underlying morphogenesis and developmental processes, and cellular redox homeostasis regulation (anti-oxidative responses capacity) of seedling germination.

The study outcomes provide evidences for the functional changes, cellular tightly regulated events occurring in the NLL seed tissues, and the antioxidant machinery involved in seeds germination capability.

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*Author for correspondence: josecarlos.jimenez@eez.csic.es
C0249 NITRIC OXIDE DETECTION IN PULVINI DURING LEAFLETS MOVEMENT OF ROBINIA PSEUDOACACIA.

Carmen Bergareche Mantuliz, Alcira Paola Angelo Barrios, Maria Luisa Moysset Agustí
U. Barcelona (Barcelona) España

1 Resumen
Nitric oxide (NO) is widely recognized as a signalling compound that acts in plant-microbe interactions, responses to abiotic stress, stomatal regulation and a range of developmental processes. Our previous studies demonstrated that NO was involved in nyctinastic movements of Albizia lophantha. This work focuses on the changes in NO levels and their cellular localization in pulvini of R.pseudoacacia during leaflet nyctinastic closure, induced at different times throughout the photoperiod. To detect endogenous NO production we proposed two different approaches: first EPR spectroscopy combined with spin-trapping technique with a lipophilic trap (Fe-DETC) and second using diaminofluorescein-2 (DAF-2) fluorescent dyes in its cell-permeable form (DAF-FM DA) for spectrofluorimetric and confocal laser microscopy. Clear three line spectrums corresponded to NO-Fe-DETC complexes were observed from pulvini extracts independently of photoperiod duration, however maximum intensities were observed from 2 h to 6 h of the photoperiod which corresponded to maximum degrees of leaflets opening. EPR signal was completely lost if pulvini were incubated with a nitrate reductase (NR) inhibitor (sodium tungstate), however pulvini treated with a nitric oxide synthase (NOS) inhibitor (L-NAME) still showed a weak EPR signal. Pulvini sampled at their maximum opening (2 h of the photoperiod) were selected for spectrofluorimetric assays. The production of the highly fluorescent triazole (DAF-2T) was related with the presence of NO, that it was maximum when pulvini were incubated with NO donors and minimum when NR and NOs inhibitors were supplied. DAF-FM DA fluorescence was asymmetrically distributed in flexor and extensor pulvini motor cells. A reduction of labelling and a homogeneous distribution of NO in extensor and flexor motor cells was detected in pulvini collected at the end of the dark period.
1 Resumen

Phenolics are responsible of wine quality because they confer sensory attributes. Most grapevine studies have focused on evaluate phenolic composition at harvest, but data concerning to phenolic accumulation during berry development are sparse. Furthermore, little is known about interactive effects among viticultural practices. Therefore, we studied grape phenolic content under two irrigation treatments (rainfed vines opposite 100% ETc irrigated vines) and two crop loads (low opposite high shoot thinning). They were combined resulting four different treatments. Content of total phenolics, anthocyanins and proanthocyanidins was analysed along berry development and ripening in three consecutive seasons (from 2014 to 2016). Berry development can be divided in two broad phenological stages: preveraison and postveraison. The accumulation of total phenolics and proanthocyanidins followed a similar change pattern: it occurred at an early stage in berry development and then decreased until veraison. During postveraison, both concentrations continued decreasing and then remained at a low level in the ripe fruit. However, anthocyanin synthesis started at veraison, increasing their concentration during ripening. In general, lower levels of total phenolic concentration were found in 100% irrigated vines throughout the stages of berry development. Furthermore, anthocyanin and proanthocyanidin concentration was also reduced under water supply along berry development. Concerning to thinning practice, a higher total phenolic and proanthocyanidin concentration was found at harvest under high thinning treatment. However, it was not clear that high thinning affected to anthocyanin content during ripening. So, treatment consisting on combination of high thinning joint to no irrigation conditions induced the highest concentration of total phenolics and proanthocyanidins at harvest, resulting on a direct positive effect on grape quality.
C0260 BRASSINOSTEROID SIGNALING AT THE STEM CELL NICHE CONTROLS CELLULAR REGENERATION UPON DNA DAMAGE

Fidel Lozano Elena¹, Ainoa Planas Riverola¹, Josep Vilarrasa Blasi², Rebecca Schwab³, Ana I. Caño Delgado¹

¹Centre de Recerca en Agrigenomica (CRAG) (Barcelona) España
²Carnegie Institution for Science - Department of Plant Biology (California) Estados Unidos
³Max Planck Institute for Developmental Biology (Tübingen) Alemania

1 Resumen

Stem cell regeneration is crucial for pluricellular organisms, supplying cells for cell turnover or tissue healing. In Arabidopsis roots, the quiescent centre (QC) is a reduced group of cells with very low mitotic activity that act as cell reservoir for the surrounding stem cells during normal growth and in response to external damage. Brassinosteroid (BR) hormones control QC division and the expression of downstream QC-specific components in charge of stem cell replenishment. Cell-specific transcriptomics in response to BRs identified BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER), a R2R3-MYB transcription factor that acts as cell-specific repressor of QC divisions³. Conversely, the ERF115 (ETHYLENE RESPONSE FACTOR 115) transcription factor is BR-activated and controls QC division and stem cell regeneration after DNA damage⁴. When there is DNA damage BRAVO is downregulated and ERF115 is upregulated, indicating that BR signalling is needed to control cellular regeneration upon DNA damage.

Despite the importance of these factors in safeguarding QC divisions locally, whether the QC function is maintained cell-autonomously or rather needs external signalling is still unknown. Moreover, it is not known if BR-mediated activation of QC divisions in the stem cell microenvironment is mediated by the BRI1 (BRASSINOSTEROID RECEPTOR INSENSITIVE 1) receptor and/or any of its homologous BRL (BRASSINOSTEROID RECEPTOR-LIKE) receptors. These questions motivated us to investigate the local regulation of quiescence and its impact on DNA damage responses. Our results will be presented at the congress.
C0319 STEROL CONTENT AND BIOSYNTHETIC GENE EXPRESSION DURING OLIVE-FLOWER OPENING AND EARLY FRUIT DEVELOPMENT

Carla Inês1, Miguel A. Paredes1, Antonio M. Cordeiro2, Maria C. Gomez-Jimenez1

1Universidad de Extremadura (Badajoz) Spain
2Instituto Nacional de Investigação Agrária e Veterinária, I.P., UEIS Biotecnologia e Recursos Genéticos (Elvas) Portugal

1 Resumen

Abstract
Sterols are lipophilic membrane components essential for diverse cellular functions and involved in various biological roles in plants, including cell and plant growth, and fertility. In olive fruit, sterols are of particular importance since they are lipids related to oil quality. However, sterol composition during early olive-fruit development remains largely unknown. In the present study, the accumulation of sterols and the expression profile of two genes involved in sterol biosynthesis, such as sterol 14α-demethylase (OeCYP51) and sterol methyltransferase 2 (OeSMT2), were examined during Olea europaea (cv. Picual) flowering and early fruit development. OeCYP51 and OeSMT2 were up-regulated by floral anthesis. The most abundant sterol in olive flower and fruit was β-sitosterol, followed by cicloartenol, but the content of the former decreased with flower opening and the content of cicloartenol did not significantly change during olive flowering. During early fruit development, OeCYP51 and OeSMT2 gene expression showed a similar pattern with a peak at 21 days post-anthesis in parallel to the sterol content increase (β-sitosterol and squalene). By contrast, the cicloartenol content was reduced during early fruit development (14-28 days post-anthesis), while the campesterol and cholesterol contents remained unaltered (14-28 days post-anthesis). A correlation was detected between the OeSMT2 accumulation transcript and β-sitosterol levels during early olive-fruit development, implying that the synthesis of 24-ethylidenelophenol might be a rate-limiting step in β-sitosterol biosynthesis. The relatively high expression levels of OeCYP51 and OeSMT2 at 21 days of fruit development could possibly be attributed to the implication of β-sitosterol in physiological processes taking place during early fruit development.
C0321 RELATION BETWEEN SALT STRESS AND FLOWERING TIME IN NATURAL POPULATIONS OF ARABIDOPSIS THALIANA

Laia Moles*, Charlotte Poschenrieder Wiens†, Mercé Llugany‡

*Plant Physiology Laboratory, Bioscience Faculty, Universitat Autònoma de Barcelona (Bellaterra) Spain
†Universitat Autonoma de Barcelona Bellaterra (Barcelona) España

1 Resumen
The floral transition is an essential process in the life of plant species that ensures seed production required for their survival. Flowering is regulated by a complex network of genetic pathways responsive to endogenous and environmental stimuli and requires the reallocation of metabolic and biochemical resources. In Arabidopsis thaliana, a long-day model plant, the flowering occurs through the action of a few floral integrator genes or transcription factors such as FT, CO, SOC1 and GI that promote flowering or FLC that inhibits it. In addition, phytohormones like gibberellic acid (GA) or abscisic acid (ABA) play an important role promoting or inhibiting flowering respectively.

Many studies suggest that both biotic and abiotic stress factors play key roles in controlling the floral transition. More specifically, salinity is an abiotic stress factor that delays flowering time in Arabidopsis thaliana through the downregulation of GA signalling causing the suppression of the expression of some flowering genes and delaying the flowering.

The aim of this study is to evaluate the relation between flowering time and salt stress in natural populations of Arabidopsis thaliana, that provide an intrinsic genetic variation to achieve the local adaptation.

To achieve this goal, we perform different experiments:

• Analysis of flowering time of the different natural accessions cultivated in soil under control and salt stress conditions.
• Same plants are used to analyse the gene expression of flowering and salinity related genes: CO, FT, SOC1, FLC, GI, AHTK1, AISOS1 and AINHX1.
• Hormone levels analysis of GA and ABA is performed with the same plants.

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C0331 UNCOVERING THE ROLE OF PIFs IN THE REGULATION OF ABA-MEDIATED DEVELOPMENTAL PROCESSES

Arnau Rovira Freixa, Pablo Leivar¹, Elena Monte²

¹Bioengineering Department, IQS School of Engineering, Barcelona, Catalonia, Spain
²Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra, Catalonia, Spain

1 Resumen

Phytochrome-interacting factors (PIFs) are members of the Arabidopsis thaliana bHLH family of transcriptional regulators that interact specifically with the active Pfr conformer of phytochrome (phy) photoreceptors. PIFs induce photomorphogenic development and are involved in regulation of different developmental processes under diurnal conditions in short-day (SD). We know that members of the PIF quartet (PIFq; PIF1, PIF3, PIF4, and PIF5) collectively contribute to the induction of growth in Arabidopsis seedlings under SD, specifically promoting elongation at dawn. In our group, we recently published a genomic analysis defining SD-regulated genes at dawn followed by identification of those SD-regulated genes whose expression depended on the presence of PIFq. Interestingly, promoter analysis showed that most PIF/SD-repressed genes were indirectly regulated by the PIFs and might be more enriched in ABA-regulated genes. Recently, significant progress has been made in defining the PIF-regulated transcriptional network, showing their interaction with a number of hormone-related genes. However, the interaction of PIF-repressed genes and ABA is still unclear. We will present recent progress aimed to unravel the relevance of this interaction.
Iron (Fe) deficiency induces the root accumulation and secretion of flavins in many dicotyledonous species when grown in hydroponics (1). Hydroponics differ significantly from natural soil environments because of differences in mechanical impedance, microbial community composition, Fe availability and interactions with plant Fe-acquisition strategies. Therefore, flavin secretion rates could differ between soil-grown and hydroponically-grown plants, as recently observed for phytosiderophores in wheat (2). Sugar beet roots accumulate and secrete riboflavin (Rbfl) and Rbfl 3’- and 5’-sulfates, helping roots to mine Fe from Fe(III)-oxides via reductive mechanisms (3). The aim of this study was to assess the root production and secretion of flavins in sugar beet grown in soils. Plants were grown up to seven weeks in two types of containers, pots and rhizoboxes, using two soils, one calcareous and one non-calcareous. In the pot experiment, rhizosphere soil solutions were collected using microsuction cups (MSCs) and exudates by placing gently cleaned roots in water with bactericide (pH 6.0). Rhizoboxes were equipped with a recently developed root exudate collector (REC; 3-4) that allows sampling of unaltered root exudates from soil-grown plants. Root extracts and exudates were analyzed for flavins using HPLC-MS. Rbfl and Rbfl 3’- and 5’-sulfates were found for the first time in root extracts and exudates from soil-grown sugar beet. Root flavin concentrations were higher in the calcareous soil than in the non-calcareous soil regardless of the plant container. Regarding root exudates, those collected in water had higher flavin concentrations in the calcareous soil than in the non-calcareous soil, whereas flavins were not detected consistently in exudates collected with REC. In soil solution, higher flavin concentrations were found in the non-calcareous soil than in the calcareous soil. The flavin secretion rate in soil-grown sugar beet seems to be determined by the root flavin concentrations and exudation medium pH.
C0091 EVIDENCE FOR THE OCCURRENCE OF STARCH DEGRADATION AND CYCLING IN ILLUMINATED ARABIDOPSIS LEAVES

Edurne Baroja Fernández, Marouane Baslam, Adriana Ricarte Bermejo, Ángela María Sánchez López, Iker Aramjuelo, Abdellatif Bahaji, Francisco José Muñoz, Goizeder Almagro, Pablo Pujol, Regina Galarza, Pilar Teixidor, Javier Pozueta Romero

1 Instituto de Agrobiotecnología - CSIC/UPNA/GN Mutilla (Navarra) Spain
2 Instituto de Agrobiotecnología (Navarra) Spain
3 SAI, Universidad Pública de Navarra (Navarra) Spain
4 Centres Científics i Tecnològics, Universitat de Barcelona (Barcelona) Spain

1 Resumen

Although there is a great wealth of data supporting the occurrence of simultaneous synthesis and breakdown of storage carbohydrate in many organisms, previous $^{13}$CO$_2$ pulse-chase based studies indicated that starch degradation does not operate in illuminated Arabidopsis leaves. Here we show that leaves of gwd, sex4, bam4, bam1/bam3 and amy3/isa3/lda starch breakdown mutants accumulate higher levels of starch than wild type (WT) leaves when cultured under continuous light (CL) conditions. We also show that leaves of CL grown dpe1 plants impaired in the plastidic disproportionating enzyme accumulate higher levels of maltotriose than WT leaves, the overall data providing evidence for the occurrence of extensive starch degradation in illuminated leaves. Moreover, we show that leaves of CL grown mex1/pglcT plants impaired in the chloroplastic maltose and glucose transporters display a severe dwarf phenotype and accumulate high levels of maltose, strongly indicating that the MEX1 and pGlcT transporters are involved in the export of starch breakdown products to the cytosol to support growth during illumination. To investigate whether starch breakdown products can be recycled back to starch during illumination through a mechanism involving ADP-glucose pyrophosphorylase (AGP) we conducted kinetic analyses of the stable isotope carbon composition ($\delta^{13}$C) in starch of leaves of $^{13}$CO$_2$ pulsed-chased WT and AGP lacking apsT plants. Notably, the rate of increase of $\delta^{13}$C in starch of apsT leaves during the pulse was exceedingly higher than that of WT leaves. Furthermore, $\delta^{13}$C decline in starch of apsT leaves during the chase was much faster than that of WT leaves, which provides strong evidence for the occurrence of AGP-mediated cycling of starch breakdown products in illuminated Arabidopsis leaves.
C0114 PHOSPHOENOLPIRUVATE CARBOXYLASE ACTIVITY AND ITS POSTTRANSLATIONAL MODIFICATIONS IN TWO SALK TDNA LINES OF GENES RELATED TO AUTOPHAGY IN STRESS CONDITIONS
Ana Mª Rodríguez Muro, Guillermo Baena Vaca, Jose A Monreal Hermoso, Sofia García-Maunúñ Ruiz-Berdejo, Ana Belén Feña Bourrellier

Dpto Biología Vegetal y Ecología, Fisiología Vegetal, Facultad de Biología (Sevilla) España

1 Resumen
Phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) is an enzyme which contributes to maintaining a positive balance of carbon in the plant. Recently a post-translational modification by mono-ubiquitination of C3-PEPC from non-photosynthetic tissues was demonstrated1. However, until today, it is not known whether this mono-ubiquitination is related to its degradation. Autophagy is the principal process of degradation and massive recycling of molecules and complete organelles. It has even a key role under stress conditions which often push the plants to intense degradation. Moreover autophagy coordinates the selective degradation of some proteins2. The proteins ATG18 and NBR1 are proteins related to autophagy3,4. Whereas ATG18 is an accessorial protein of the complex inducing the autophagosome which is expressed under saline and osmotic stress, NBR1 is a protein participating in selective degradation by picking specific mono-ubiquitinated proteins which will then be degraded by autophagy. Here we used two experimental SALK TDNA lines of Arabidopsis thaliana, Atatg18a and Atnbr1, which we exposed to oxidative and nutritional stress (i.e. lack and excess of C, lack of N and Trehalose); stresses which have been demonstrated to trigger autophagy. PEPC activity and its post-translational modifications were them study in these mutants and conditions. The results presented here show that, in such conditions of combined stresses, PEPC activity varies and its patterns of mono-ubiquitination and phosphorylation change. In addition, for the first time, a possible degradation by selective autophagy with interaction of monoubiquitinated PEPC and NBR1 in Arabidopsis is suggested.


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Catalase (CAT; EC 1.11.1.6) is a metalloenzyme considered as part of the first antioxidative barriers to cope against reactive oxygen species (ROS), due to its ability to decompose H$_2$O$_2$. CAT is one of the major enzymes located in peroxisomes, where it leads the modulation of the levels of the H$_2$O$_2$ generated in the own organelle, due to the internal metabolism, but it also participates in the removal of this ROS coming from other cell compartments. In plants, CATs have been described as homotetramers of about 220-240 kDa with subunits sizes ranging 55-57 kDa. However, using sweet pepper (Capsicum annuum L.) fruits as model, an average native molecular weight of 125 kDa was determined by non-denaturing PAGE at different acrylamide concentrations, and by gel filtration chromatography through an FPLC system. By Western blotting analysis, using an antibody raised against plant catalases, a single immunoreactive band of 55 kDa was detected. Isoelectric focusing and specific catalase activity staining provided one unique isoenzyme with an isoelectric point of 7.4, what is also atypical considering other plant CATs reported so far.

Regarding to the metabolism of pepper, it was found that in ripe red fruits the activity decreased with respect to immature green fruits, and this pattern was parallel to the catalase protein content observed after immunoblotting analysis. Besides, this behavior during ripening could be also due to post-translational modifications undergone by catalase due to nitration and nitrosylation processes promoted by reactive nitrogen species (RNS) derived from nitric oxide (NO). Thus, in vitro assays, pre-incubation of pepper samples with different chemicals including SIN-1 (a peroxynitrite donor, ONOO$^-$), and DeaNONOate and S-nitrosoglutathione (GSNO) as NO donors, provoked significant decreases of catalase activity. These results highlight the cross-talk between NO and antioxidants in pepper fruits at ripening. [Supported by Grant ALG2015-65104-P from the MINECO, Spain]
C0196 REGULATION OF NUCLEOSIDASES DURING GERMINATION AND POSTGERMINATIVE DEVELOPMENT IN COMMON BEAN

Gregorio Gálvez Valdivieso, Pedro Piedras, Elena Delgado, Manuel Pineda

Universidad de Córdoba Córdoba (Córdoba) España

1 Resumen
Nucleotides are molecules of unique importance in cell growth, development and metabolisms. They are important components involved in bio-energetic processes, are elementary units of the genetic material, play a role as cofactors, and are also components of secondary metabolites and hormones as cytokinins. Nucleotide metabolism can be divided into four processes: de novo synthesis, salvage pathways, nucleotide degradation and phosphotransfer reactions. In uricid legumes as common bean, nucleotides metabolism is particularly relevant, acting as precursors of ureides, molecules used for the transport of nitrogen from the nodules to the aerial parts of the plants. Ureides can also be used as nitrogen transport molecules in other processes that requires high nutrient mobilization as senescence and germination. Ureides are synthesized from purines that can come from the de novo purine synthesis or from the salvage of nucleic acids. The enzyme nucleosidase is involved in these pathways catalyzing the cleavage of nucleosides into ribose and nucleobase, and liberating the nitrogen trapped in the cellular pool of nucleic acids and nucleotides. We have cloned four genes encoding putative nucleosidases in common bean (PvNSH1, PvNSH2, PvNSH3 and PvNSH4). PvNSH1 has been expressed as a recombinant protein in E. coli showing that PvNSH1 can hydrolyze purine and pyrimidine nucleotides. Furthermore, we have studied the role of nucleosidases during the germination and the postgerminative development in common bean.

Acknowledgments
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C0201 ARE FATTY ACID CONTENT AND DE NOVO FATTY ACID BIOSYNTHESIS AFFECTED BY HERBICIDES INHIBITING AMINO ACID BIOSYNTHESIS?

Miriam Gil-Monreal1, Ana Zabalza1, Tagron D. Missihoun2, Peter Dörmann3, Dorothea Bartels3, Mercedes Royuela1

1Universidad Pública de Navarra Pamplona-Iruñea (Navarra) Spain
2Rutgers University (New Jersey) USA
3IMBIO (Bonn) Germany

1 Resumen

Glyphosate and imazamox are two herbicides that target different enzymes in different biosynthetic pathways, (EPSPS in the aromatic amino acid pathway and ALS in the branched-chain amino acid pathway, respectively). These herbicides provoke some common physiological effects in plants, such as induction of the pyruvate dehydrogenase (PDH) bypass, an alternative pathway to the PDH complex that produces acetyl-CoA from pyruvate. To analyse whether the acetyl-CoA produced in the PDH bypass in plants exposed to herbicides is redirected to the de novo fatty acid biosynthesis, the total fatty acid content, the relative amount of individual fatty acids and the expression pattern of different genes involved in the de novo fatty acid biosynthesis (ACC2, KASIII, KASI, KASII) were measured in leaves and roots of imazamox- or glyphosate-treated Arabidopsis thaliana plants. In leaves, a non-significant decrease in the total fatty acid content was detected after application of the herbicides and the expression of the genes was not significantly affected by the herbicides. No changes in the total fatty acid content was detected in the roots, even though the expression of the ACC2, KASIII, KASI and KASII genes decreased. The effect of herbicides on the percentage of the individual fatty acid content in leaves showed that the effect of imazamox in the leaves was minor and the effect of glyphosate stronger. In roots, the percentage of 16:0 fatty acids increased and of 18:3 decreased after glyphosate application suggesting that the synthesis of 18:3 fatty acids from the precursor 16:0 is affected. Although an increase in the acetyl-CoA pool would be expected by the concomitant activation of the PDH bypass by amino acid biosynthesis inhibiting herbicide application in plants, our results suggest that acetyl-CoA levels are limiting for fatty acid synthesis probably due to an affected PDH complex, which is the main pathway for acetyl-CoA biosynthesis.
C0229 PHOSPHORUS CONCENTRATION IN PLANT ORGANS COORDINATES RESPIRATORY BYPASSES, SYNTHESIS AND EXUDATION OF CITRATE, AND THE EXPRESSION OF HIGH-AFFINITY PHOSPHORUS TRANSPORTERS IN SOLANUM LYCOPERSICUM PLANTS

Nestor Fernandez Del Saz

Universidad De Las Islas Baleares Mallorca (Palma) España

1 Resumen

Plants exhibit respiratory bypasses, synthesis of carboxylates in leaves and roots as well as expression of high-affinity inorganic phosphorus (Pi) transporters in response to phosphorus (P) deficiency in soils which increase their capacity to take up P. In contrast, the repression of the above traits under P sufficiency suggests that AOX activity suppresses the synthesis of citrate to reduce the mobilization of Pi from soils whereas the expression of high-affinity Pi transporters is downregulated to reduce P uptake avoiding P toxicity.

This association was tested in Solanum lycopersicum plants under different scenarios of soil P availability and plant P status, by using plants grown at P-sufficient and limiting conditions, and by applying a sudden short-term (24 h) P-sufficient pulse in plants grown under P limitation. Tests were performed in plants colonized with arbuscular mycorrhizal (AM) fungi, showing an increase in plant P concentration. The in vivo activities of alternative oxidase (AOX) and cytochrome oxidase (COX) were measured by the oxygen-isotope fractionation technique together with the amount of carboxylates in leaves and roots. Moreover, P concentration in both organs as well as gene transcription of Pi transporters (LePT1 and LePT2) were studied. A coordinated response between leaf and root P concentrations with respiratory bypasses in leaves, synthesis of foliar citrate, and transcription of high-affinity Pi transporters in roots was observed.

This association indicated that a sufficient P availability in soil leads to a suppression of both AOX activity and synthesis of citrate when the transcription of high-affinity Pi transporters is repressed possibly to avoid P toxicity.
MOLECULAR ANALYSIS OF XANTHINE DEHYDROGENASE UNDER STRESS CONDITIONS SUGGESTS THAT URIC ACID PROTECTS AGAINST INHIBITORY EFFECTS OF NITRIC OXIDE IN NODULES

Inmaculada Colet Reyes, Manuel Pineda Priego, Josefa Muñoz Alamillo

Departamento de Botánica, Ecología y Fisiología Vegetal. Grupo de Fisiología Molecular y Biotecnología de Plantas. Campus de Excelencia Internacional Agroalimentario, CEIA3. Universidad de Córdoba (Córdoba) España

1 Resumen

Xanthine dehydrogenase (XDH) is an essential enzyme for the assimilation of symbiotically fixed nitrogen in ureidic legumes. Uric acid, produced in the reaction catalyzed by XDH, is the precursor of the ureides, allantoin and allantoate, which are the main N-transporting molecules in these plants. XDH and uric acid have been involved in the response to stress both in plants and animals. However, the physiological role of XDH under stressful conditions in ureidic legumes remains largely unexplored. In vitro assays showed that XDH from *Phaseolus vulgaris* can behave as a dehydrogenase or as an oxidase. Therefore, it can either protect against or increase the production of oxidative radicals. In silico analysis of the upstream genomic region of *PvXDH* gene showed the presence of several stress-related cis-regulatory elements. The *PvXDH* mRNA levels in plants treated with stress-related phytohormones, water stress and extreme temperatures showed several degrees of induction. In contrast, *PvXDH* activity was inhibited by NO in leaves, but not in nodules, probably through a mechanism mediated by peroxynitrite and iron. *PvXDH* RNA silencing indicates that uric acid produced by XDH in the nodules of this ureidic legume may serve to protect XDH, and most likely other enzymatic systems involved in nodule activity, against the inhibitory effects of nitric oxide.

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C0268 YIELD AND OVERALL FRUIT QUALITY (MORPHOLOGICAL, ORGANOLEPTIC AND NUTRITIONAL) OF TWO ORANGE CULTIVARS (CITRUS SINENSIS), ‘SALUSTIANA’ AND ‘SANGUINELLI’, CULTIVATED IN ANDALUSIA (SPAIN) GRAFTED ON FORNER-ALCAIDE Nº 5 ROOTSTOCK

Estefanía Romero-Rodríguez1, Aurea Hervalejo García1, J.M. Moreno-Rojas2, G. Pereira1, J.L. Pereira1, A.B. González-Chimeno1, Francisco José Arenas-Arenas2

1IFAPA Las Torres Tomejil (Alcalá del Río (Sevilla)) España
2IFAPA Centro Alameda del Obispo. (Córdoba) España

1 Resumen
Keywords: fruit weight, juice content, total soluble solids, titratable acidity, maturity index, firmness, total polyphenols and antioxidant activity

ABSTRACT
Two orange cultivars, ‘Salustiana’ and ‘Sanguinelli’ grafted on Forner-Alcaide nº 5 rootstock were cultivated in Andalusia (Spain) and their production characterized according to yield (kg/tree), quality parameters related to appearance: weight, diameter and external firmness; to organoleptic properties: juice content, total soluble solids (TSS), titratable acidity (TA), maturity index (MI=TSS/TA) and internal firmness; and to nutritional ones: total polyphenols and antioxidant activity. The experimental design of the plot was four randomized blocks with three trees per cultivar and block. Trees, currently entering into production, were organized on ridges with a tree spacing of 6 x 4 m. The results revealed no significant differences in yield between the studied cultivars. Regarding the morphological quality of the fruit, ‘Salustiana’ highlighted because its higher diameter and weight, but a smaller external firmness of the fruit. On the other hand, in the several parameters analyzed to determine the organoleptic quality no significant differences were found, except in the internal firmness of the fruit, recording ‘Sanguinelli’ a lower value (overall fruit acceptability). Finally, ‘Sanguinelli’ showed an increased nutritional interest due to recorded a greater concentration of total polyphenols and antioxidant capacity.
C0275 PPNAC1, A MAIN REGULATOR OF PHENYLALANINE BIOSYNTHESIS IN P. PINASTER

Maria Belen Pascual, Mª Teresa Llebrés, Rafael A. Cañas, Francisco M. Cánovas, Concepción Ávila

Universidad de Malaga. Facultad de Ciencias Malaga (Malaga) España

1 Resumen

The metabolism of phenylalanine plays a central role in the channeling of carbon from photosynthesis to the biosynthesis of phenylpropanoids duringwood formation. This crucial pathway is finely regulated primarily at the transcriptional level by MYB and NAC transcription factors. In Arabidopsis, poplar and eucalyptus, the transcriptional network controlling secondary cell wall involves NAC-domain regulators operating upstream Myb transcription factors, but in conifers functional evidence had only been obtained for MYBs. We showed that PpMYB8 is a regulator of phenylpropanoid metabolism and lignin synthesis genes (Craven-Bartle et al. 2013) and three NAC genes PpNAC1, PpNAC30 and PpNAC31 were associated to vascular development in maritime pine (Pascual et al. 2015). Of all of them, PpNAC1 is expressed in the secondary xylem and compression wood of adult trees and phylogenetic analysis classified PpNAC1 as potential candidates to be involved in a transcriptional regulatory network controlling phenylalanine metabolism in maritime pine. This NAC transcription factor has been thoroughly characterized and its role upstream the transcriptional network involving Mybs TFs will be discussed. Understanding the molecular switches controlling wood formation is of paramount importance for fundamental tree biology and has important implications in tree biotechnology.

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References:
C0277 TOMATO STEROL ACYLTRANSFERASES

Alma Burciaga Monge1, Angel Chávez Martínez1, Alejandro Lara Ayub1, Monsterrat Arró Plans2, Albert Boronat Margosa1, Teresa Altabella Artigas2, Albert Ferrer Prats3

1Centre for Research in Agricultural Genomics (Barcelona) España
2Departamento de Bioquímica y Fisiología. Facultad de Farmacia. Universidad de Barcelona (Barcelona) España
3Departamento de Bioquímica y Biomedicina Molecular, Facultad de Biología. Universidad de Barcelona (Barcelona) España

1 Resumen
Phytosterols are components of plant membranes that modulate fluidity, function and structure. Plant sterols are found in free form (FS) and conjugated as sterylesters (SE), sterylglucosides (SG) and acyl steryl glucosides (ASG). SE accumulates in cytoplasmic lipid bodies along with triglycerides, suggesting that their primary function is to maintain FS homeostasis in cell membranes. The accumulation of FS has been reported to be toxic for the plant, but plant cells have the ability of converting FS to SE to avoid toxicity. The SE constitute a storage pool of sterols and fatty acids when these are present in greater amounts than those immediately required for the cell. Despite their biological importance the enzymes responsible for sterol acylation in plants are poorly characterized. Sterol acyltransferase activity has been detected in a variety of plant tissues and it has been shown that the plant enzymes could be using several acyl donors different from those described in mammals and yeast. However, until now only two Arabidopsis sterol acyltransferases have been cloned and characterized: AtPSAT1 that encodes a phospholipid:sterol acyltransferases (PSAT) and AtASAT1, which encodes an acyl CoA:sterol acyltransferase (ASAT). By sequence homology search in databases with the Arabidopsis PSAT1 and ASAT1 protein sequences as query, we have identified a single tomato (Solanum lycopersicum cv Micro-Tom) gene encoding PSAT (SIPSAT) and eight genes encoding putative ASAT enzymes (SIASAT1-8). By functional complementation of the A. thaliana knock-out mutants psat1-1 (Banas et al. 2005) and asat1-1 (Bouvier-Navé et al., 2010) we have demonstrated that SIPSAT and SIASAT1 code for the actual tomato orthologs of Arabidopsis PSAT1 and ASAT1, respectively. Data obtained from functional and structural characterization of these enzymes will contribute to set the basis for further studies aimed at understanding the role of SE in tomato plant growth and development, fruit-ripening and their response to stress.
C0289 NITRIC OXIDE AND REACTIVE OXYGEN SPECIES RELEASED FROM POLYAMINES ARE PARTIALLY RESPONSIBLE FOR THE INHIBITION OF WHEAT ROOT ELONGATION

Laura Recalde¹, Analía Vázquez¹, María Daniela Gropppa¹, María Patricia Benavides²

¹Departamento de Química Biológica. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires (Buenos Aires) Argentina
²Cátedra de Química Biológica Vegetal. Facultad de Farmacia y Bioquímica. Universidad de Buenos Aires (Buenos Aires) Argentina

1 Resumen
Polyamines are molecules involved in plant growth and development, able to generate reactive oxygen species (ROS) and nitric oxide (NO) during their catabolism. We examined if ROS and/or NO derived from PAs are implicated in the mechanisms involved in growth inhibition in wheat roots. Triticum aestivum L. seedlings were grown in Hoagland solution containing: 0.1 mM of the NO-donor SNP, 1 mM putrescine (Put), spermidine (Spd) or spermine (Spm). A similar degree of root growth inhibition was detected in plants treated with Spm or SNP for 5 d (around 75%), while Put less inhibited root growth (about 35%). The three PAs increased nitric oxide production in the apical portion of primary roots. O$_2^-$ was greatly reduced by SNP or Spm, despite both treatments increased H$_2$O$_2$ formation in roots. SNP and Spm significantly reduced (around 50%) O$_2^-$-dependent NBT reduction, a way to measure a potential NADPH oxidase activity; only Spd strongly reduced CAT activity (to 40% of the C) whereas SOD was around 25% reduced in SNP or Spm-treated roots but 30% increased in Spd-treated roots. Spm and SNP treatments reduced lipid peroxidation in roots, whereas Put, which was the PAs that less affected growth, raised it by 75%. Putrescine content was doubled in SNP-treated roots and increased between 3 and 4 times in Put and Spd-treated roots, respectively, but increased only 25% in Spm-treated roots. Polyamine oxidase activity, that metabolize Spd or Spm producing H$_2$O$_2$, was markedly increased under Put or Spd treatments, but strongly inhibited by Spm and was not modified by SNP. These results suggest that a complex interaction exists among polyamines, ROS and NO in the inhibition of root growth, where PAs are not only a source of ROS, but could be modulating the antioxidant system, thus directly modifying O$_2^-$ or H$_2$O$_2$ levels.
C0303 STUDYING THE MECHANISM OF ADENOSINE METHYLATION IN ARABIDOPSIS
THALIANA

Evangelina Rodríguez-Alcocer, Natalia Gómez-Peral, Carlos Hernández-Cortes, Erundina Ruiz, Eduardo Burillo, Alejandro Peñín, Sara Jover-Gil, Héctor Candela

Instituto de Bioingeniería, Universidad Miguel Hernández de Elche (Alicante) Spain

1 Resumen
Recent studies have shown that N6-methyladenosine (m6A) is the most abundant internal modification present in the messenger RNA of eukaryotes. Despite this post-transcriptional modification has been known for about four decades, the study of adenosine methylation has emerged as a hot research topic only in the last few years. We are studying this type of modification using Arabidopsis thaliana as a model organism.
Currently three complexes involved are known in the methylation adenosines: (i) "writers", methyltransferases that methylate adenosine residues; (ii) "erasers", demethylases that remove methyl groups, and (iii) "readers", RNA-binding proteins that contain a YTH domain. We have selected several genes for functional studies: two encoding subunits of the methyltransferase complex, three encoding putative demethylases, and thirteen YTH proteins.
To investigate the function of these genes, we are characterizing transgenic lines that carry loss-of-function alleles (T-DNA insertion lines) and transgenic plants overexpressing their coding sequence. We are systematically performing yeast two-hybrid screens using proteins of the three functional categories as baits, which have already yielded some promising protein-protein interactions. We are also setting up a novel, high-throughput RNA tagging protocol to identify mRNA molecules targeted by proteins from the three functional categories. Our ultimate goal is to make a significant contribution to this new field by identifying the protein-protein and RNA-protein interactions that shape the m6A epitranscriptome in Arabidopsis thaliana.
**Mineral Nutrition and Water Relations**

**C0115 EFFECT OF THE BENEFICIAL FUNGUS TRICHODERMA HARZIANUM IN THE EFFICIENT USE OF NITROGEN IN WHEAT PLANTS**

Manuel Sánchez Cao, Luna Anzano, Belén Rubio, Rosa Hermosa, Enrique Monte, Carlos Nicolás Rodríguez

*Instituto Hispano Luso de Investigaciones Agrarias (CIALE) (Salamanca) España*

**1 Resumen**

Agriculture is the main source of pollution of land and global freshwater by Nitrogen. Spain has overcome the limits of sustainability and crops such as wheat (with needs close to 200 kg/ha/year but a cropping area of 2 million ha) are a suitable scenario for novel strategies to improve the NUE and therefore increase crop yields, reducing costs and keeping the environmental quality. As well as being effective biocontrol agents, strains of the fungus *Trichoderma* are beneficial to plants. They can improve the nutritional status of plants, modifying the root architecture to enhance nutrient uptake giving rise to more NUE, although the molecular mechanisms governing this process still remain hidden. We want to demonstrate that the application of *Trichoderma* strains to wheat plants, in greenhouse and open field, can reduce the needs of N fertilization without diminishing crop production.

This work was supported by grants MINECO AGL2015-70671C2-1-R and Junta de Castilla y León SA-009U16.
C0145 CACO3 A FACTOR DRIVING NATURAL DISTRIBUTION AND TOLERANCE TO CARBONATED SOILS OF ARABIDOPSIS THALIANA

Joana Terés¹, Silvia Busoms¹, Xin-Yuan Huang², Roser Tolrà¹, David E. Salt², Charlotte Poschenrieder¹

¹Plant Physiology Laboratory, Bioscience Faculty, Universitat Autònoma de Barcelona (Barcelona) Barcelona  
²Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham (Loughborough) United Kingdom

Resumen

A recent large-scale sampling throughout Catalonia located new wild populations of Arabidopsis thaliana (Busoms et al., 2015; Plant Physiol. 168: 915-929). Noteworthy, all studied populations were found on silicate substrates with neutral rhizosphere pH (7.2 ± 0.6) and low carbonate. Nonetheless, a few inland accessions grew well in slightly carbonated soils (20-30% CaCO₃) with low iron bioavailability. These A. thaliana ecotypes with naturally selected differences in sensitivity to soil carbonate provide excellent material for investigating the physiological mechanisms behind carbonate tolerance. This characterization will help to connect physiological traits for iron efficiency and carbonate tolerance with the genes governing these processes.

The aim of this study was to describe factors implicated in the tolerance to carbonated soils and test the fitness of some of the ecotypes differing in performance on carbonate soil to evaluate the existence of local adaptation to carbonated soils.

For this purpose, we performed different experiments:

- Analysis of physical and chemical properties of original soils
- Common garden experiments at two field sites with contrasting soil carbonate levels: Les Planes (high soil carbonate) and Santa Coloma de Farners (low soil carbonate).
- Same substrates were also used for laboratory experiments in which all other environmental conditions were controlled.
- Hydroponic culture with and without Fe supply to evaluate Fe-efficiency based on leaf chlorophyll concentrations.

Plants from all populations had similar fitness on low carbonate soil. However, on the carbonate-rich substrate those populations originating from sites nearby the carbonate-rich area produced more siliques than individuals collected from sites distant from carbonate-rich soils. We conclude that % of CaCO₃ in original soil is an important factor driving the natural distribution of Arabidopsis thaliana in Catalonia. Demes collected from sites nearby carbonate-rich soils are locally adapted to elevated soil carbonate, potentially through mechanisms that decrease the rhizosphere pH and improve iron efficiency.
C0146 COPPER INDUCED PHYSIOLOGICAL, BIOCHEMICAL AND BIOMOLECULAR RESPONSES IN B73 MAIZE.

Isabel Corrales Pinart1, Silvia Fornale2, Delia Spano3, David Caparrós-Ruiz2, Charlotte Poschenrieder3

1Laboratorio de Fisiología vegetal Cerdanyola del Vallès (Bellaterra) (Barcelona) España
2CRAG (Barcelona) España
3UAB (Barcelona) España

1 Resumen
Maize is one of the most agronomically important crops. It is used in the human diet and it is considered an interesting source for the production of biofuels. The study of lignification in crop species is gaining great interest due to its negative influence on their digestibility and their energetic value. Copper (Cu), among other essential functions, plays a role in lignification. The purpose of this study was to investigate the influence on root growth and lignifications of Cu concentrations ranging from deficiency to toxicity in B73 maize, whose. Maize plantlets were treated with different copper concentrations (0-10 mM) for 24, 48 and 72 h in hydroponic conditions and the stress responses were characterized by the analysis of growth and antioxidant enzyme activities. Lignification was visualized by phloroglucinol staining and the expression of the main genes involved in lignin biosynthesis was studied.

Increasing Cu concentrations produced a decrease of root elongation and - cell viability. The activity of important enzymes implicated in the defence against the Cu-induced stress, showed a significant increase. In roots, changes in isoenzyme profiles led us to consider this variation as a plant response to Cu stress. Cu-induced changes in the expression of genes related to lignification were observed; under Cu deficiency, PAL, HCT, 4CL1, CCoAOMT1, F5H, CCR1 and Lac III displayed were down-regulated, while LacIII was up-regulation in presence of 5mM Cu. The results obtained indicate that both suboptimal and excess Cu concentrations induce changes in B73 metabolism and lignification.
C0151 ROLE OF THE PLASTIDIAL NA+/H+ ANTIPORTER SLNHAD IN TOMATO SALT TOLERANCE

Espen Granum¹, M. Remedios Romero-Aranda², Benito Pineda³, Begoña García-Sogo³, Jacob R. Pérez-Tienda¹, Paloma González-Fernández³, Vicente Moreno³, Andres Belver¹

¹Department of Biochemistry, Molecular and Cellular Biology of Plants. Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Cientificas (C.S.I.C. (Granada) España
²The Institute for Mediterranean and Subtropical Horticulture "La Mayora" (IHSM-UMA-CSIC) (Algarrobo-Costa, Málaga) Spain
³Laboratory of Tissue Culture and Plant Breeding, IBMCP-UPV/CSIC (Valencia) Spain

1 Resumen

Salt stress is a widespread problem which limits crop productivity around the world. Plants have developed various strategies to cope with high salinity, and different types of ion transporters play key roles in Na⁺ and K⁺ homeostasis in plant cells and tissues. Recent work suggest that the Na⁺/H⁺ antiporter NHD1 in Arabidopsis is essential for Na⁺ export out of the chloroplast, which is important for maintaining high photosynthetic performance and productivity¹. In this study, the role of the corresponding Na⁺/H⁺ antiporter NhaD in Solanum lycopersicum was investigated. The SlNhaD encoding gene was silenced by RNAi stable gene transformation, and the phenotypes in the vegetative stage of different transgenic lines were characterized in experiments with moderate salt stress (80 mM NaCl) in a commercial-scale greenhouse. Plants grown under conditions of high salinity showed considerable accumulation of Na⁺ and moderate decreases in K⁺ contents in leaves, stems and roots, but no significant differences were found between wild type and transgenic plants. Other effects of salt stress included reductions in leaf water potential, plant growth (plant height, leaf surface area, stem diameter and root development) and stomatal density, although PSII photosynthetic efficiency (Fv/Fm) was not affected. Gene expression analyses confirmed that SlNhaD was silenced in all transgenic lines, but there were no significant changes in expressions of other important ion transporters, including HKT1;1, HKT1;2, NHX2 and NHX4.

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C0174 SUBSTRATE COMPONENTS INDUCE DIFFERENT BEHAVIOURS ON WATER RELATIONS, GAS EXCHANGE, PHOTOSYNTHETIC EFFICIENCY, AND NUTRIENTS IN WELL-WATERED AND STRESSED CISTUS ALBIDUS PLANTS

Inés Zugasti, Beatriz Lorente Pagán, María Fernanda Ortúño Gallud, Pedro Nortes Tortosa, José Antonio Hernández Cortés, Maria Jesús Sánchez Blanco

CEBAS-CSIC (Murcia) España

1 Resumen

Traditionally, residues such as urban solid wastes have been considered as non-desirable substrates. Some studies however have shown that, after composting, these organic residues can be used as growth media instead of peat with very good results. Cistus albidus responds to water deficit by developing avoidance mechanisms for regulating transpiration such as stomatal closure, a reduction in leaf area and epinasty. The objective of this work was evaluated if the use of compost manure as substrate with high Zn and Cu contents, and high water retention capacity can better withstand a water stress imposed. Changes in biomass, water relations, gas exchange, photosynthetic efficiency and nutrients were evaluated. The irrigation treatments consisted in plants well-irrigated and submitted to water stress. This experiment was carried out under controlled conditions of temperature and relative humidity. From the beginning of the experiment, the components of substrate caused an osmotic and/or toxic effect, despite being well watered. This led to a rapid stomatal closure (30 mmol m$^{-2}$ s$^{-1}$), which maintain constant leaf water potential ($\Psi_l$) values (-0.9 MPa) due to a limitation of their transpiration. Under water stress, the water retained in the substrate caused that $\Psi_l$ values remained constant, at the same levels as well-watered plants (isohydric behaviour). Well-watered and stressed compost plants accumulated less biomass than those that grew in peat, which presented usual water behaviour in both conditions: under well irrigation, the plants maintained high levels of $\Psi_l$ and $g_s$, and when water stress was imposed a significant decrease in $\Psi_l$ was noted. This leads to a decrease in gas exchange and evapotranspiration values (anisohydric behaviour). Stressed plants showed lower values of photosynthesis and biomass. Plants suffered stress in both substrates, as reflected by the lipid peroxidation values.

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C0239 DIFFERENTIAL TOLERANCE TO DROUGHT STRESS AMONG PHASEOLUS VULGARIS PLANTS CULTIVATED UNDER SYMBIOTIC AND NON-SYMBIOTIC CONDITIONS

Cristina López Vázquez, Manuel Pineda Priego, Josefa Muñoz Alamillo

Departamento de Botánica, Ecología y Fisiología Vegetal. Grupo de Fisiología Molecular y Biotecnología de Plantas. Campus de Excelencia Internacional Agroalimentario, CEIA3. Universidad de Córdoba (Córdoba) España

1 Resumen

Nitrogen derived from symbiotic nitrogen fixation is used to produce ureides in the so called ureidic legumes. Moreover, levels of ureides increase in these legumes in response to drought, despite the inhibition of symbiotic nitrogen fixation produced by water stress. Accumulation of ureides in response to the stress occurred also in non-symbiotic plants, fertilized with nitrate. Interestingly, plants grown under symbiotic conditions retained more water in their tissues than plants fertilized with inorganic nitrate, suggesting that either the symbiotic interaction or the intrinsic levels of ureides in these plants may protect them against the stress. The objective of this work is to analyse the mechanism leading to the accumulation of ureides in the plants fertilized with nitrate as well as to investigate the molecular and physiological mechanisms that govern the apparent higher tolerance of the plants grown under symbiotic conditions.

This work was supported by Grants AGL2015-69554-P (Ministerio de Economía y Competitividad, Spain) and BIO-115 (Consejería de Economía, Innovación, Ciencia, Junta de Andalucía, Spain).
**C0261 MEDICAGO TRUNCATULA COPPER TRANSPORTER 1 (MTCOPT1) PROVIDES COPPER FOR SYMBIOTIC NITROGEN FIXATION**

Marta Senovilla¹, Rosario Castro-Rodríguez¹, Isidro Abreu¹, Viviana Escudero¹, Igor Kryvoruchko², Juan Imperial³, Michael K. Udvardi², Manuel Gonzalez-Guerrero¹

¹Centro de Biotecnología y Genómica de Plantas (UPM-INIA) (Madrid) España
²The Samuel Roberts Noble Foundation (Oklahoma) USA
³Consejo Superior de Investigaciones Científicas (Madrid) Spain

1 Resumen

Copper is an essential nutrient for symbiotic nitrogen fixation. This element is delivered by the host plant to the nodule, where membrane copper transporter would introduce it into the cell to synthesize cupro-proteins. COPTfamilymembersare likely candidates to mediate copper uptake by rhizobia-infected cells. Medicago truncatula genome encodes eight COPT transporters. MtCOPT1 (Medtr4g019870) is the only nodule-specific COPT gene. Promoter:gus fusions and protein immunolocalization studies indicate that MtCOPT1 is located in the plasma membrane of cells in the differentiation, interzone and early fixation zones of the nodule. Loss of MtCOPT1 function results in a copper-mitigated reduction of biomass production when the plant obtains its nitrogen exclusively from symbiotic nitrogen fixation. Mutation of MtCOPT1 results in diminished nitrogenase activity in nodules, likely an indirect effect from the loss of a copper-dependent function, such as cytochrome oxidase activity in copt1-1 bacteroids. These data are compatible with a model in which MtCOPT1 transports copper from the apoplast into nodule cells to provide copper for essential metabolic processes associated with symbiotic nitrogen fixation.

This work was funded by ERC Starting Grant Grant (ERC-2013-StG-335284) and MINECO Grant (AGL-2012-32974). RC-R receipt a FPI fellowship from MINECO (BES-2013-062674).
C0267 MTMTP2 IS A ZINC TRANSPORTER REQUIRED FOR SYMBIOTIC NITROGEN FIXATION IN MEDICAGO TRUNCATULA NODULES

Javier Leon-Mediavilla¹, Marta Senovilla¹, Patricia Gil-Diez¹, Juan Imperial¹, Manuel Gonzalez-Guerrero¹

¹Centro de Biotecnologia y Genomica de Plantas (UPM-INIA) Pozuelo de Alarcon (Madrid) España
²Consejo Superior de Investigaciones Cientificas (Madrid) España

1 Resumen

Zinc (Zn) is an essential element in the structure and catalytic activity of a large amount of proteins (1), including many involved in Symbiotic Nitrogen Fixation (SNF) (2). Although much is known about Zn transport and delivery in plants, few studies have focused on how this is achieved in legume nodules. There are three major protein families involved in the Zn transport in plants: ZIP, P₁₁₂-ATPases and CDF transporters (3). Characterization of the Zn Medicago truncatula transcriptome showed high expression level of CDF transporter MtMTP2 in nodules. Yeast complementation tests showed that MtMTP2 exports Zn out of the cytosol. To test where MtMTP2 expression is located, ß-glucuronidase activity was determined in M. truncatula plants expressing the gus gene under the MtMTP2 promoter. GUS signal was detected in the differentiation, interzone and fixation zones. Immunolocation studies indicate that MtMTP2 was located in the plasma membrane of non-infected nodule cells and in an intracellular compartment in rhizobia-infected cells. Under symbiotic conditions, the mtp2-1 and mtp2-2 mutants exhibited reduced growth and nitrogenase activity compared to the wild type, but not when mineral nitrogen was provided in nutritive solution. These data indicate an important role of MtMTP2 in the Zn homeostasis in SNF.


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C0269 MEDICAGO TRUNCATULA FERROPORTIN2 (MTFPN2) IS A NODULE- SPECIFIC IRON EXPORTER

Viviana Escudero Welsch¹, Manuel Tejada-Jimenez², Juan Imperial², Manuel Gonzalez-Guerrero¹

¹Centro de Biotecnología y Genómica de Plantas Madrid (Madrid) España
²Consejo Superior de Investigaciones Científicas (Madrid) España

1 Resumen
Legumes are able to use atmospheric nitrogen and convert it to ammonia through a partnership with endosymbiotic rhizobia. This symbiotic nitrogen fixation (SNF) occurs in root nodules formed with rhizobia. SNF requires different transition metals, notably iron, as essential cofactors of multiple proteins involved in this process (leghemoglobin, nitrogenase…). Previous studies in our laboratory showed that iron is driven from vascular bundles to the apoplast of the infection zone of the nodule (1).

Transcriptomic studies in Medicago truncatula nodules showed that MtFPN2 is specific to the nodule. Within this organ, promoter::gus fusions and immunolocalization showed that it was located in the nodule vasculature and in some intracellular compartment in rhizobia-infected cells. Yeast complementation assays in several metal transporter mutants, indicated that MtFPN2 was able to transport iron and manganese out of the cytosol, either into an organelle or across the plasma membrane. Phenotypical characterization of two Tnt1-insertion mutant alleles (fpn2-1 and fpn2-2) revealed that MtFPN2 was important for SNF, as both lines presented a significantly lower nitrogenase activity than wild type plants.

(1) Rodríguez-Haas. et al. (2013) Metallomics 5, 1247-1253.

This work was supported by ERC Starting Grant (ERC-2013-StG-335284) and MINECO Grant (AGL-2015-65866) to M.G-G.
C0290 PHYSIOLOGICAL RESPONSES OF TOMATO PLANTS TO Fe DEFICIENCY.

Alba Tous Fandos, V. Ramon Vallejo Calzada, Carmen Begareche Mantuliz, Teresa Sauras Yera

Universitat de Barcelona (Barcelona) España

1 Resumen

As a micronutrient, Iron (Fe), has an important role in plant metabolism as it takes part of different essential processes, limiting plant growth. Furthermore, iron (Fe) deficiency causes leaf ferric chlorosis and shifts in the roots morphology. Plant nutrient uptake is controlled primarily by availability and solubility of nutrients in soil and is mainly dependent of soil pH. This is the reason why plants have strategies to solubilize and uptake Fe, such as activate FQ-R enzyme or developing lateral roots. The aim of this research is to analyze physiological and metabolic responses to Fe deficiency and simulated calcareous soil environment in Solanum lycopersicum (tomato) plants. Results show an important growth reduction in iron deficient plants with a reduction of the evapotranspiration rate and chlorophyll content. On the other hand, FC-R levels increase in plants exposed to low or lack Fe availability conditions. A higher pH induced lower levels of iron absorption, leading to cause some symptoms of iron deficiency. The pH of the growing medium is crucial to determine nutrient absorption and therefore to understand plant deficiencies.
RESUMEN

We reported the results of an isotopic study aimed at evaluating the medium to long-term effects of different water qualities (transfer water, TW, and saline reclaimed water, RW) and deficit irrigation strategies (control, C, and regulated deficit irrigation, RDI) on the water use efficiency (WUE) of mandarin trees in Murcia (Spain). Stable isotopes natural abundances ($\delta^{13}C$, $\delta^{18}O$), gas exchange parameters (photosynthesis net $-A$-, stomatal conductance $-g_s$-, transpiration $-Tr$-, etc.) and nutritional analysis at the leaf level were determined in three phenological stages (I: spring; II:summer; III: winter).

On the one hand, changes in the isotope concentrations were observed regardless treatments. The minimum $\delta^{13}C$ were measured in stage I and it was related with the lowest $A/Tr$ ratio values. $\delta^{18}O$ content was higher in stage III coinciding with the lowest $g_s$ in all treatments.

On the other hand, bearing in mind the different treatments, we observed that $\delta^{13}C$ did not vary significantly between treatments in stage II; however, a $\delta^{18}O$ increase was observed in RW-RDI, which in turn should lead to a $g_s$ decrease. According to theory, $\delta^{13}C$ is a suitable proxy of leaf-level WUE=$A/g_s$,$\delta^{13}C$ and $\delta^{18}O$ can help to separate the independent effects of A and $g_s$ on $\delta^{13}C$, since $\delta^{18}O$ shares a dependence on $g_s$ with $\delta^{13}C$, but is thought to be independent of A variation. Therefore, performing a dual analysis of $\delta^{13}C$ and $\delta^{18}O$ behavior the results indicated that the fact that $\delta^{13}C$ remained constant, necessarily implied that A decreased in RW-RDI. This was confirmed with data from gas exchange measures.

The usefulness of the dual $\delta^{13}C$ and $\delta^{18}O$ approach, in accordance with gas exchange measurements, has been demonstrated for assessing plant water use strategies.
C0298 15N STABLE ISOTOPE FOR ASSESSING THE USE OF SALINE RECLAIMED WATER AND DEFICIT IRRIGATION STRATEGIES IN MANDARIN TREES

Cristina Romero Trigueros1, Pedro Antonio Nortes Tortosa2, Jose María Bayona Gambín1, Juan Jose Alarcón Cabañero1, Emilio Nicolás Nicolás2

Centro de Edafología y Biología Aplicada del Segura. (CEBAS-CSIC) Espinardo (Murcia) España

1 Resumen
The aim of our research was to discover the effects of the long-term irrigation with saline reclaimed (RW) and transfer (TW) water and different irrigation strategies: control (C) and regulated deficit irrigation (RDI) on physiology and sustainability of mandarin trees. δ15Nstable isotope, gas exchange parameters (photosynthesis net –A–, stomatal conductance –gₕ–, etc.) and nutritional analysis at the leaf level were determined in three phenological stages (I: sprouting in 2015; II: fruit growth and RDI period in 2015; III: vegetative repose in 2016) in a commercial orchard in Murcia (Spain). Changes in δ15N concentration were observed through growth season, regardless treatments: the maximum content was found in stage II, at the same time that the highest values of nitrogen use efficiency (NUE, A/mass-based nitrogen) and leaf NO₃ concentration was measured across treatments. Moreover, taking into account the different treatments, the same pattern for δ15N in the three stages was observed: RW-C and RW-RDI treatments were more enriched than TW treatments. Although reclaimed water had higher contents of phytotoxic salts and nitrogen, both RW treatments had higher leaf Cl and Na levels than TW treatments but not leaf N concentration. Then, it is possible that an surplus of salinity caused problems in the plant N uptake. Excess N could be lost to the environment by leaching, denitrification, etc. resulting in soil enrichment in δ15N. Therefore, the higher the concentration of δ15N in RW treatments, the more inefficient the system.

Finally, a two-way ANOVA was conducted that examined the effect of phenological stages and treatments on levels of δ15N isotope and there was a statistically significant interaction for δ15N (p<0.03*). It has been demonstrated the utility of isotopic discrimination measure to predict crop sustainability in the medium to long term when using water sources of different quality combined with deficit irrigation strategies in mandarin trees.
C0325 INOCULATION WITH BRADYRHIZOBIUM SP. SEMIA 6144 INCREASES WATER DEFICIT TOLERANCE AND PROMOTES GROWTH OF ARACHIS HYPOGAEA

A Cesari1, M López Gómez2, J Hidalgo Castellanos2, M Dardanelli3

1Departamento de Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de Rio Cuarto (Córdoba), Departamento de Fisiología Vegetal, Universidad de Granada (Granada) Spain, Argentina
2Departamento de Fisiología Vegetal, Universidad de Granada (Granada) Spain
3Departamento de Biología Molecular, Facultad de Ciencias Exactas, Universidad Nacional de Rio Cuarto (Córdoba) Argentina

1 Resumen

Arachis hypogaea is one of the most economically important legumes. At present, peanut crops are subject to water deficit, which affects growth and productivity. In this work we studied the effect of water deficit on the interaction between Arachis hypogaea, Bradyrhizobium sp. SEMIA6144 and Azospirillum brasilense Az39.

Water deficit was generated by addition of PEG6000 15 mM and nitrogenase activity was determined in 30 day old plants by measuring the H2 evolution in an open-flow system (1). In addition, lipid peroxidation as oxidative marker and polyamines levels in leaves were analyzed by HPLC (2).

Water deficit and inoculation with Az39 bacterium delayed the nodulation of SEMIA6144 in A. hypogaea with a nodule/plant reduction of 40%. Nitrogen fixation/plant was reduced by 80%. Under stress, nitrogen and carbon content was increased mainly in leaves, being higher in SEMIA6144 inoculated plants. In addition, water deficit induced oxidative stress by increasing lipid peroxidation by 275%, however SEMIA6144 inoculation mitigated this effect.

Polyamines spermidine, spermine and putrescine were determined in A. hypogaea leaves with an increase in putrecine of 77% in stressed plants. In nodules, cadaverine and high amounts of homospermidine increased under stress and co-inoculation treatments.

We conclude that SEMIA6144 inoculation is the recommended treatment to mitigate the effects of water stress in A. hypogaea.

References


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Photosynthesis, Plant Productivity

C0059 EFFECT OF FOLIAR BORON SPRAY ON PHYSIOLOGICAL PERFORMANCE, YIELD AND FRUIT QUALITY OF GULUPA PLANTS (PASSIFLORA EDULIS SIMS), GROWN UNDER FIELD CONDITIONS.

Ivonne Angelica Quiroga Ramos, Luz Marina Melgarejo Muñoz, Gerhard Fischer Gebauer

Universidad Nacional de Colombia (Bogotá) Colombia

1 Resumen

Gulupa (or purple passionfruit) is one of the main exotic fruits produced in Colombia and exported to different international markets, especially United States and Europe. Crop production is affected by different problems, but the most important in Colombia is related to flowering and fruiting, due to nutritional limitations. Boron (B) is an essential micronutrient involved in different processes during plant growth, including flowering, fruit set and development. The aim of this study was to evaluate the effects of foliar boron sprays on the physiological performance, yield and fruit quality of gulupa plants, grown under field conditions. The application of B was performed at different doses (0 (control), 0.3 (T1), 0.6 (T2) and 0.9 (T3) kg ha\(^{-1}\) of boric acid (H\(_3\)BO\(_3\)) in floral bud stage. The CO\(_2\) assimilation rate (A) increased between 23 and 34% with foliar boron application compared to control (7.97 CO\(_2\) m\(^{-2}\) s\(^{-1}\)); chlorophyll content (SPAD) had similar behavior to A, nevertheless, other variables such as, potential efficiency of photosystem II (Fv/Fm), stomatal conductance (gs) and transpiration (T), did not show significant differences between treatments and control. There weren’t visual symptoms of boron deficit (control) or excess (T3), but reproductive crop phenology was negatively affected, mainly in development of productive branches, fruit diameter and fruit set. Foliar spray of B also influenced postharvest quality of fruits, where treatments with B had higher fruit weight and firmness, although, other variables such as total soluble solids (TSS) and total titratable acidity (TTA) were no affected by the application. Taking together our results, it can be concluded that the best dose to apply in gulupa plants is 0.6 kg ha\(^{-1}\), due to higher A (10.68 CO\(_2\) m\(^{-2}\) s\(^{-1}\)), fruit set (85.19%), fruit diameter (55.64 mm) and weight (69.19 g), as well as, good postharvest quality.
Differential Physiological Responses to Saline and Osmotic Stresses in Two Japonica Rice Cultivars.

Shantanu Wankhade¹, Raül Pons¹, Amparo Sanz Grau²

¹Universitat de València (Valencia) España
²Universitat de València (Dpt Biologia Vegetal) Burjassot (Valencia) España

Salinity is among the main constraints for plant growth and the single most widespread abiotic factor reducing crop yield. Physiological performance of plants subjected to saline conditions can be affected by the osmotic and ionic components of salinity. We have performed a comparative study of various physiological responses to saline and osmotic stresses at different plant developmental stages in two cultivars of japonica rice, Bahia and Bomba. Plants were grown in the greenhouse and subjected to saline (NaCl) and osmotic (sorbitol) stresses throughout the whole plant life cycle. Treatments were applied by watering the plants with the appropriate solutions. Parameters related to plant growth and development (plant height, number of leaves, plant survival), water balance (plant water content, transpiration) and photosynthesis (chlorophyll content and fluorescence, carbon assimilation rate, water use efficiency) were measured at seedling and vegetative stages. Plant survival was drastically impaired under saline stress, particularly in cv Bomba. In contrast, plants of both cvs subjected to osmotic stress completed the whole life cycle. Plant height and leaf number were also less affected by sorbitol treatments than by NaCl. Total chlorophyll content decreased in the salt stressed plants, but not under osmotic stress. Relative water content (RWC) was reduced, particularly in cv Bomba, at the seedling stage under salinity, while it was not affected by sorbitol. Significant differences were also observed between both types of stress in relation to carbon assimilation rates and other photosynthetic parameters. Our results show that the ionic component of the saline stress seems the main factor compromising plant growth and physiological performance in rice, with cv Bomba showing a higher sensitivity than cv Bahia. A better understanding of the distinct effects induced by the different components, ionic and osmotic, involved in saline stress should benefit biotechnological approaches aimed at obtaining more resistant cultivars.
C0266 INTERACTION OF M-TYPE THIOREDOXINS AND NTRC IN CHLOROPLAST REDOX REGULATION

Víctor D. Requerey, MariCruz González García, F Javier Cejudo Fernández

Instituto de Bioquímica Vegetal y Fotosíntesis (Sevilla) España

1 Resumen

Redox regulation modulates the activity of many chloroplast enzymes through a disulfide-dithiol interchange of key cysteine residues. Two different thiol redox systems exist in plant chloroplasts, the ferredoxin-thioredoxin (Trx) system, depending on photo-reduced ferredoxin thus linking redox regulation to light, and the NADPH-dependent Trx reductase C (NTRC) system, which relies on NADPH and therefore may be operative also during the night, as NADPH can be produced in darkness by the oxidative pentose phosphate pathway. Chloroplasts harbour a complex set of different types of Trxs (m1-4, f1-2, y1-2, x, z and other atypical and Trx-like proteins), which are considered to play specific roles in redox regulation of different chloroplast processes. Of these, Trxs m, the most abundant Trxs in chloroplasts, were found to be the major regulators of the Calvin-Benson cycle enzymes. However, these enzymes are also regulated by NTRC, but the relationship of Trxs m and NTRC is unknown. With the aim of studying the interaction between m-type Trxs and NTRC in chloroplast redox regulation, we generated double knockout Arabidopsis mutants combining the deficiencies of an isoform of m-type thioredoxins (Trx m1 or Trx m4) and NTRC. In order to characterize these plants, a study of their phenotypes and an analysis of photosynthetic parameters and changes in the redox status of Calvin-Benson cycle enzymes were performed. While single mutants trxm1 and trxm4 showed growth phenotypes similar to that of the wild type, the phenotype of the ntrc-trxm1 and ntrc-trxm4 double mutants was more severe than that of the ntrc mutant. Moreover, double mutants showed severely impaired redox regulation of Calvin-Benson cycle enzymes in response to light. Our results provide new and valuable knowledge of the mechanisms integrating NTRC and m-type Trxs for an optimal function of chloroplasts and how chloroplast redox homeostasis influences growth and plant development.
C0326 FLUORESCENCE OF CHLOROPHYLL IN JUVENILE "HASS" AVOCADO PLANTS IN AN ARTIFICIAL SHADE ENVIRONMENT

Fabián Giovanny Márquez Niño
Universidad Nacional De Colombia (Bogotá) Colombia

1 Resumen
Avocado leaves have been characterized as little sun-low stress according to the classification of Smith et al. (1989), so they could be considered as shade tolerant. Juvenile plants of 'Hass' avocado were subjected to three levels of luminosity: 0% (unshadow), 35% and 50% of shade, using synthetic polysombras. Using a modulated fluorometer (FMS2, Hansatech, King's Lynn, UK) fast light curves (RLC) were performed on sheets adapted to darkness, and NPQ recovery was evaluated after each curve for 10 minutes. The RLC curve was constructed by applying actinic light for 10 s at intervals followed by a pulse of saturating light increasing the intensity of PAR radiation to 2340 µmol photons m2s-1. It was found that in the leaves of plants subjected to 35% shade presented lower maximum ETR, although the initial slope α was similar in all three treatment levels. The NPQ value was higher for 35% shade (3.3) than for the other levels (1.8 and 2.3 for 0% and 50% treatments). The plants under shade of 35% are limited to photosystemic level when subjected to high levels of illumination, the other treatments do not present difference in terms of RLC curves, although unshadow plants had less NPQ.
Plant Biotechnology and Synthetic Biology

C0120 ECTOPIC EXPRESSION OF VITIS VINIFERA STILBENE SYNTHASE IN SILYBUM MARIANUM PLANT CELL CULTURES LEADS TO RESVERATROL BIOSYNTHESIS WITHOUT ALTERING PRODUCTION OF THE NATIVE FLAVONOLIGNAN SILYMARIN

Diego Hidalgo1, Ascensión Martínez-Márquez2, Rosa Cusidó1, Roque Brú2, Javier Palazón1, Purificación Corchete Sanchez3

1Laboratori de Fisiología Vegetal, Facultat de Farmacia, Universitat de Barcelona (Barcelona) España
2Plant Proteomics and Functional Genomics Group, Department of Agrochemistry and Biochemistry, Faculty of Science, University of Alicante (Alicante) España
3Depto. Botánica y Fisiología Vegetal Salamanca (Salamanca) España

1 Resumen

El creciente demanda de t-resveratrol para usos industriales hace que su producción a partir de fuentes sostenibles y renovables sea un requisito. La producción heteróloga de resveratrol en suspensores de células de plantas, a partir de la introducción de uno o dos genes, presenta el ventaja de una alta productividad de biomasa y un corto tiempo de cultivo, lo cual podría ser una opción para la producción a gran escala. En este estudio, hemos elegido Silibum marianum para probar esta estrategia. El plant es la fuente del antihepatotóxico flavonolignano silymarin y no posee el gen que codifica para resveratrol. La síntesis de fenilpropanoides en cultivos de esta especie puede ser activada por el enriquecimiento con metil jasmonato y betá-ciclodextrinas, con productos del camino (conifera lic alcohol y algunos isómeros del silymarin complejo) siendo liberados al medio. Dado que la sintasa del estilbeno comparte los mismos precursores clave involucrados en la síntesis flavonídica y/o monolignol, exploramos la posibilidad de que la transgena de estrófago 3 para producción de t-resveratrol. Los suspensores de células fueron establemente transformados con Vitis vinifera estilbeno sintasa 3 y la expresión de transgene led to extracelular t-resveratrol acumulación at the level of milligram per liter under elicitation. Resveratrol synthesis occurred at the expense of conifera lic alcohol. Production of silymarin was less affected in the transgenic cultures, since the flavonoid pathway is limiting for its synthesis, due to the preferred supply of precursors for the monolignol branch. The fact that the expressed STS gene took excessively produced precursors of non-bioactive compounds (conifera lic alcohol), while keeping the metabolic flow for target secondary compounds (i.e. silymarin) unaltered, opens a way to extend the applications of plant cell cultures for the simultaneous production of both constitutive and foreign valuable metabolites.

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C0133 THE TRANSCRIPTION FACTORS JACKDAW, BLUEJAY AND SCARECROW MAINTAIN TISSUE FORMATION IN ARABIDOPSIS THROUGH REGULATION OF THE ROOT STEM CELL NICHE ACTIVITY

Alvaro Sanchez Corrionero, Javier Silva Navas, Juan Perianez Rodriguez, Carlos del Pozo, Miguel-Angel Moreno Risueno

Centro de Biotecnología y Genómica de Plantas CBGP Pozuelo de Alarcon (Madrid) España

1 Resumen

The BIRDS transcription factors (TFs) and SCARECROW (SCR) have been shown as critical regulators of the ground tissue lineage identity as well as of patterning and formative divisions through integration of positional signals such as the mobile transcription factor SHORT-ROOT (1). We have recently found that double and triple mutant combinations of SCR and two BIRD TFs: BLUEJAY (BLJ) and JACKDAW (JKD) have severe defects in root growth. Roots of these mutants stop growth at day 3 after germination. To further understand why these TFs are critical for root growth we performed a series of experiments to determine meristem functionality and assess their possible role in differentiation. Our results show that these TFs are critical to inhibit early differentiation through maintenance of stem cell activity and number of quiescent center (QC) cells. In jkd scr and blj jkd scr mutants QC quickly disappear during growth. We traced this defect to a non-autonomous mechanism by which these TFs regulate protein abundance of SHORT-ROOT and of PLETHORA1 and 2. Unexpectedly, we also observed a reduction in the number of cell layers in the blj jkd scr root, which aggravated in the course of development. Roots of blj jkd scr were made of as little as 3 cells at the time of differentiation. We perform analyses using specific tissue markers and found that most tissue types were lost over time. The QC is capable of replacing root cells when these are lost or damaged. Next we performed experiments with bleomycin and laser ablation to remove tissue initials. We have found that in blj jkd scr roots although remaining QC cells can divide to replace lost cells, its low numbers do not appear to be able to maintain all tissue initials resulting in progressive loss of tissues. Notably, simulating this situation in wild type roots also results in loss of many tissue layers. We propose that BLJ, JKD and SCR are critical to maintain tissue growth and regeneration though a non-autonomous feedback mechanism that activates SHR, PLT and auxin signaling.
C0157 METABOLIC ENGINEERING OF TOBACCO HAIRY ROOTS FOR STILBENES PRODUCTION

Diego Alberto Hidalgo Martínez¹, Milen Georgiev², Elisabeth Moyano³, Roque Bru Martínez⁴, Javier Palazon⁵, Purificación Corchete⁶

¹Laboratori de Fisiologia Vegetal, Facultat de Farmacia, Universitat de Barcelona (Barcelona) España
²Laboratory of Applied Biotechnologies. The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, (Plovdiv) Bulgaria
³Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra (Barcelona) España
⁴Plant Proteomics and Functional Genomics Group, Department of Agrochemistry and Biochemistry, Faculty of Science, University of Alicante (Alicante) España
⁵Laboratori de Fisiología Vegetal, Facultat de Farmacia, Universitat de Barcelona, (Barcelona) España
⁶Department of Plant Physiology, Campus Miguel de Unamuno, University of Salamanca, E-37007 (Salamanca) España

1 Resumen

Resveratrol (3,5,4′-trihydroxy-trans-stilbene) (t-R), one of the most studied stilbenes, represents an excellent example of a plant secondary metabolite (PSM) with wide-ranging therapeutic potential, its biological actions including chemoprevention of melanoma, antiviral, antioxidant, anti-inflammatory, cardioprotective and platelet anti-aggregation. We reported that genetically engineered hairy roots (HR) of tobacco, carrying stilbene synthase (STS)-encoding genes from Vitis vinifera and/or the transcription factor (TF) AtMYB12 from Arabidopsis thaliana and/or artificial micro RNA for chalcone synthase (amiRNA CHS), have undergone metabolic modifications for the bioproduction of t-R. Thus, this complete study model is based on the over-expression of a TF in order to generate a holistic response in the phenylpropanoid pathway and to coordinate the up-regulation of multiple steps; amiRNA CHS will limit the normal flux through the endogenous CHS enzyme, which competes for the precursors of the imported STS enzyme used for the flux deviation.
C0160 PERFLUORODECALINS AND (Z)-3-HEXENOL ENHANCE TAXANE PRODUCTION IN TAXUS X MEDIA CELL SUSPENSION CULTURES.

Heriberto Rafael Vidal Limón1, Lorena Almagro Romero2, Mercedes Bonfill Baldrich3, María Angeles Pedreño García4, Javier Palazón Barandela3, Rosa M Cusidó Vidal2

1Facultat de Farmacia Barcelona (Cataluña) España
2Universidad de Coruña (Galicia) España
3Universidad de Barcelona (Cataluña) España
4Universidad de Murcia (Murcia) España

1 Resumen

The biotechnological taxanes production, anti-cancer agents, in plant cell cultures has been improved by the use of inductor compounds. A limiting factor in cell suspension cultures is oxygen transport. Perfluorochemicals can be used as oxygen carriers due to their high oxygen solubility, biological inertness and lack toxicity. In Nicotiana tabacum cell cultures, the addition of gassed perfluorodecalin (PFD) clearly increased cell density. Likewise, a positive effect on paclitaxel production in Taxus x media hairy root cultures was achieved by combining methyl jasmonate with degassed PFD treatment.

Volatile organic compounds (VOC) such as (Z)-3-hexenol (Hex) play a role in plant herbivore defense, resulting in stronger and faster jasmonic acid signaling. The exogenous application of Hex in maize plants activated defense genes transcription, as well as the accumulation of (Z)-3-hexenyl acetate and linalool, showing that this VOC can trigger plant defense responses.

Applying PFDs and VOCs as promising elicitor candidates, we evaluated taxane yields in two-phase Taxus x media cell cultures and studied the relative expression of key taxane biosynthetic genes by RT-qPCR. PFD-gassed, PFD-degassed, and Hex were applied with and without b-cyclodextrins (CD) + Coronatine (Cor) to check any synergic effects. In all treatments, the highest taxane production occurred at day 24. When applied with CD + Cor, PFD-gassed and Hex doubled the taxane yield of the control, while PFD-degassed tripled the production in a synergic manner, although the biomass formation decreased.

The baccatin III yield was notably increased by the addition of Hex and CD + Cor.

The expression level of genes T13OH, CoA ligase, BAPT and DBTNBT was highest in cultures supplemented by CD + Cor together with either PFD-gassed or -degassed, which correlated with production values?.

These results suggest that the addition of PFD-degassed is an effective strategy to increase taxane production, especially paclitaxel, in Taxus cell culture biofactories.
C0192 INSIGHTS IN EPIGENETIC REGULATION: AN EXPLANATION FOR THE GRADUAL LOSS OF TAXANE PRODUCTION IN TAXUS MEDIA CELL CULTURES

Raúl Sánchez Muñoz1, Irene Rosa Díaz2, Heriberto Vidal Limón2, Mercedes Bonfill2, Javier Palazon2, Elisabet Moyano1

1Universidad Pompeu Fabra Barcelona (Barcelona) España
2Universidad de Barcelona (Barcelona) España

1 Resumen
The use of plant cell cultures as biofactories could be a biosustainable and profitable alternative for the production of plant-derived compounds. They are greatly limited, however, by a gradual loss of secondary metabolism. This could be due to the strong epigenetic regulation in plants through the methylation levels in key biosynthetic genes, which are known to progressively increase in in vitro cultures.

This work is focused on the use of Taxus media cells as producers of taxanes, which are effective in the treatment of several cancers. In previous studies by our group, the same T. media line decreased its yield from a maximum of 80.8 mg/L to 11 mg/L of total taxanes while under the same growth conditions. Here we compared the methylation levels in different key genes in taxane biosynthesis in a cell line periodically subcultured for approximately 10 years with those of a recently obtained cell line. The epigenetic changes were studied by bisulfite-sequencing.

Preliminary results show that the promoter region of the BAPT gene had 40.8% of methylated cytosines in the old line and 22.4% in the recently obtained one. This increase in the methylation level was concentrated in a specific region of the promoter, which may correspond to the binding site of transcription factors.

The obtained results suggest that the gradual decline in taxane yield over time is correlated with the growing gene methylation levels in the in vitro cultures. Studying the complete sequence of BAPT and other key genes involved in taxane production and regulation in Taxus spp. could allow us to detect new zones of regulation and differential patterns of methylation in the pathway. More knowledge of secondary metabolism regulation processes may lead to an optimization of biotechnological production strategies.
C0193 BIX-01294. INHIBITOR OF HISTONE H3K9 METHYLATION, PROMOTES MICROSPORE TOTIPOTENCY AND ENHANCES EMBRYOGENESIS INDUCTION

Eduardo Berenguer Peinado, Ivett Bárány, María-Teresa Solís, María C. Risueño, Pilar S. Testillano

Pollen Biotechnology of Crop Plants lab. Biological Research Center, CIB-CSIC Madrid (Madrid) Spain

1 Resumen

Stress-induced microspore embryogenesis is a process of cell reprogramming, totipotency acquisition and embryogenesis initiation, used in plant breeding for rapid production of doubled haploids, but their regulating mechanisms are still largely unknown. We have analyzed the dynamics and possible role of histone H3K9 methylation, a major repressive modification, as well as the effects on microspore embryogenesis initiation of BIX-01294, inhibitor of histone methylation, tested for the first time in plants, in Brassica napus and Hordeum vulgare.

Results revealed that microspore reprogramming and initiation of embryogenesis involved low H3K9 methylation. With the progression of embryogenesis, methylation of H3K9 increased, correlating with gene expression profiles of BnHKMT SUVR4-like and BnLSD1-like (writer and eraser enzymes of H3K9me2). At early stages, BIX-01294 promoted cell reprogramming, totipotency and embryogenesis induction, while diminished bulk H3K9 methylation, decreased DNA methylation and reduced heterochromatin masses. In contrast, long BIX-01294 treatments hindered embryogenesis progression, indicating that H3K9 methylation is required for embryo differentiation. Findings open new possibilities to enhance microspore embryogenesis efficiency in recalcitrant species through pharmacological modulation of histone methylation by using BIX-01294.
C0211 DIFFERENTIAL EXPRESSION OF CARPOSPOROGENESIS-RELATED GENES ARE ETHYLENE-INDUCED IN THE RED MACROALGA GRATELOUPIA IMBRICATA

Montserrat Montero Fernández, Rafael Robaina Romero, Pilar García Jiménez

Universidad de Las Palmas de Gran Canaria (ULPGC) (Las Palmas) España

1 Resumen

Multiple signals and growth regulators are known to affect algal physiology. Specifically, the reproductive process and the in vitro formation of the reproductive structures, cystocarps, on red macroalgae are mainly driven by ethylene (ET), polyamines (PAs), and more recently jasmonate derivatives such as methyl jasmonate [1, 2, 3, 4]. In this sense, expression of the ornithine decarboxylase gene (ODC) in the red seaweed Grateloupia imbricata (GiODC) has also been proved to be differentially regulated by ET and correlated to PAs enzymatic activities. On one hand, GiODC expression depends on the reproductive stage of the thalli, being significantly higher on infertile thalli (no cystocarps present) and coincident with the highest level of endogenous PAs accumulation [5] and declining as cystocarp appears. On the other hand, exogenous ET treatment induces a peak on GiODC expression right after the elicitation period, which coincidently decreases as cystocarp develops [6]. As the formation of reproductive structures is the final result of a wide and complex net of metabolic and physiologic interactions, it is highly unlikely that they stand alone. However, little is known about underlying molecular signaling routes affecting it. Here, absolute quantification of expression for the main genes involved in the ET and PAs biosynthetic pathways are monitored by ddPCR for fertilized and fertile thalli after ET elicitation. In addition, stress and ROS-related genes are also investigated. Results evidence that i) genes for the same biosynthetic pathways show proximal tendencies on expression and ii) differential expression is dependent on the maturation stage of the tissue.

References
C0213 CASE STUDY: CAN BACTERIA BENEFIT A HALOPHYTE IN DIFFERENT PHENOLOGICAL SATAGE AND/OR IN CASE OF STRESS?

Jose-Maria Barcia Piedras1, Jesus-Alberto Perez Romero2, Enrique Mateos Naranjo2, Raquel Parra2, Ignacio Rodriguez Llorente6, Rosario Espuny4, Susana Redondo Gomez2, Maria Camacho5

1Dos Hermanas (Sevilla) España
2Dpt. Biología Vegetal y Ecología, University of Seville (Seville) Spain
3Dpt. Microbiología y Parasitología, University of Seville (Seville) Spain
4Dpt. Microbiología. University of Seville (Seville) Spain
5Centro IFAPA Las Torres-Tomejil (Seville) Spain

1 Resumen
We are living in an unsustainable environmental and economic situation. Within this framework, the focus has been on stopping this growth, and even decreasing, while seeking alternatives that allow the recovery of natural ecosystems and food production without being incompatible processes. The halophytes are a group of plants adapted to extreme environmental conditions such as high salinity and may be of vital importance in solving the problem presented since many of these species could to recover degraded environments, and at the same time to raise them as alternative crops in this context of changes. At the same time, these plants can be potentiated if they are associated with bacteria that inhabit their roots and allow them to develop better (promoting growth bacteria or PGP).

This work intends to serve as an example of how a PGP bacterium can act at different phenological levels of the halophyte Arthrocnemum macristachyum (A.m.), allowing a better development even in stress conditions. In germination, A.m. is sensitive to salinity and it was verified that the bacterial presence allows to maintain normal levels of germination (decreased by 20% if there is only salt). It was also observed that the phenological moment of the root colonization by the bacteria is fundamental, obtaining results more notorious when the bacterium is present from seed instead of colonizing the plant once it has been developed. In this sense the plant increases the amount of its stems significantly when they are plants that were inoculated from seed and were exposed to salt, whereas if they were inoculated adults only the salt influences vegetative growth. At the same time, it was observed how the rate of CO₂ fixation does not decrease when there is no salt in the medium and exist bacteria, a process that occurs when the plant grows without salt.
C0223 INFLUENCE OF PHOSPHATE AVAILABILITY ON PLANT MATURITY AND ACTIVATION OF CHEMICAL DEFENCES IN STINGING NETTLE (URTICA DIOICA L.)

Bárbara Simancas San Martín, Sergi Munné-Bosch, Maren Müller, Alba Cotado

Facultat de Biología. Universitat de Barcelona (Barcelona) España

1 Resumen

Dimorphic plant species can show important sex-related differences in the response to nutrient availability. How availability of inorganic phosphate (Pi) influences sexual dimorphism and whether or not male and female plants respond differently to nutrient stress is still however poorly understood. Here, we examined whether contrasting Pi availability may influence sexual differentiation in mature plants. We also evaluated to what extent males and females respond differently to varying Pi concentrations in terms of nutrient accumulation, growth, phytohormone contents, and activation of chemical defenses, with a particular emphasis on jasmonates. Results showed that reduced Pi availability delays plant maturation in both males and females, causing as well an increase in the sex ratio towards females, probably as a means of favoring population survival under stress. Furthermore, reduced Pi availability led to enhanced oxo-phytodienoic acid contents, particularly in females. This increase was, however, not accompanied by increases in other jasmonates, neither free nor conjugated jasmonic acid, thus suggesting specific defensive roles for oxo-phytodienoic acid in females. Sex biased ratios in favor of females over males and enhanced chemical defenses in the former under reduced Pi availability illustrate the great adaptive capacity of dimorphic species to reduced nutrient availability.
C0271 TEMPRANILLO REGULATES DEVELOPMENTAL TIMING IN PART THROUGH THE AGE-DEPENDENT PATHWAY

Andrea E. Aguilar Jaramillo, Esther Marín González, Luis Matías Hernández, Michela Osnato, Soraya Pelaz, Paula Suárez López
Centre de Recerca en Agrigenòmica, CSIC-IRTA-UAB (Barcelona) España

1 Resumen
The timing of developmental transitions is crucial for a coordinated development and adaptation to the environment. After germination, Arabidopsis thaliana undergoes first a juvenile-to-adult transition and later on a floral transition. The microRNA 156 (miR156) delays both transitions by down-regulating several SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes, which promote the juvenile-to-adult and the floral transition in part through up-regulation of miR172. miR156, miR156-targeted SPL genes and miR172 act therefore in an age-dependent developmental pathway. TEMPRANILLO 1 (TEM1) and TEM2 are transcriptional repressors that delay flowering. TEMs and miR156 show similar expression patterns and phenotypic effects, suggesting that they may act in a common genetic pathway. We have found that TEMs do not have a clear effect on miR156, but regulate the levels of several SPL mRNAs and miR172. miR172 is encoded by 5 genes, MIR172A to MIR172E. TEM1 binds to SPL9 and MIR172C chromatin, suggesting that the regulation of miR172 by TEM is both direct and mediated by SPL9. Transient expression experiments show TEM1-dependent transcriptional activation of MIR172C. Finally, genetic analyses show that TEMs affect the juvenile-to-adult and floral transitions partially through SPL9 and miR172. We conclude that TEMs regulate the timing of these transitions in part through the age-dependent developmental pathway.
C0287 NOVEL INSIGHTS INTO THE FUNCTIONAL ROLE OF 1-DEOXY-D-XYLULOSE-5-PHOSPHATE SYNTHASE (DXS1) DURING TOMATO PLANT DEVELOPMENT

Manuel García-Alcazar, Estela Gimenez, Benito Pineda, Carmen Capel, Begoña García-Sogo, Sibila Sanchez, Fernando J. Yuste-Lisbona, María Trinidad Angosto Trillo, Juan Capel, Vicente Moreno, Rafael Lozano

1 CIAIMBITAL-Universidad de Almería (Almería) España
2 IBMCP (UPV-CSIC) Universidad Politécnica de Valencia (Valencia) España
3 Universidad de Almería Almería (Almería) España

1 Resumen

In tomato (Solanum lycopersicum L.), the function of 1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS1) has only been studied in fruits, where it catalyses the first step of the 2-C-methyl-D-erythritol-4-Phosphate (MEP) pathway, playing an important role during fruit carotenoid biosynthesis. This study is focused on elucidating the functional role of DXS1 during plant development, which has not been described so far. Here we report the isolation and molecular characterization of the tomato white lethal seedling-2297 (wls-2297) T-DNA mutant, whose albino seedlings expanded cotyledons, but they were unable to develop true leaves from the shoot apical meristem, giving rise to premature lethality. Identification of the genomic sequence flanking the T-DNA insertion site revealed that T-DNA integration caused a 38.6 kb-deletion, which affected the DXS1 and three PEROXIDASE (POX) genes. Functional analyses of DXS1 and POX silencing lines demonstrated that a loss of function of the DXS1 gene is responsible for the albino wls-2297 phenotype. This result was further supported by means of both in vivo complementation assays with 1-Deoxy-D-xylulose-5-phosphate (DXP) and DXS1 overexpression on the wls-2297 mutant, which partially rescued the albino phenotype. Furthermore, MEP pathway genes displayed different expression profiles in the DXS1 silencing lines compared to wild-type plants, which suggested a role for DXS in transcriptional regulation of the first steps of the MEP pathway. Altogether, results prove that DXS1, in addition to playing an important role during fruit carotenoid biosynthesis, performs an essential function at early developmental stages, being it required for tomato plant development and survival.
The SnRK (Snf1-Related protein kinase) gene family plays an important role in energy sensing and various stress-adaptive responses regulation in plant systems. Despite its essential role, this family has been less studied in microalgae with only a few examples regarding nutrient deficiencies (Gonzalez-Ballester et al. 2008) and temperature stress (Valledor et al. 2013). In this study we performed comparative and gene duplication analyses, and expression patterns under several abiotic stresses of Chlamydomonas SnRK gene family. 22 sequences corresponding to SnRKs were identified and classified into two subfamilies: SnRK1 and SnRK2. Several gene duplication events explained the evolution of SnRK2 gene subfamily in this species, while conserved domains across evolution were related to specific regulatory functions present in plants. Gene expression analyses demonstrated the overregulation of this family under several abiotic or nutrient limiting stresses, specially osmotic and oxidative, being only six genes responsive to ABA. The simplicity of this family compared to other plants and the emerging evidence of the role of these genes not only in the mechanisms related to cell survival, but also to the concomitant accumulation of biomolecules of interest under stressful situations (e.g. lipids, sugars, or pigments) position this family members as interesting targets for bioengineering-based studies focused not only on increase our understanding of stress biology and ABA signaling mechanisms but also improving the biotechnological potential of microalgae.

C0306 FAST IDENTIFICATION OF TWO DEVELOPMENTAL GENES USING MAPPING-BY-SEQUENCING

Erundina Ruiz, Eva Rodríguez-Alcocer, Sara Jover-Gil, Héctor Candela Antón

Instituto de Bioingeniería, Universidad Miguel Hernández de Elche (Alicante) Spain

1 Resumen

Mapping-by-sequencing has become the strategy of choice for the rapid mapping and identification of the lesions causing a mutant phenotype of interest. This strategy has been successfully used in our laboratory to identify the genes damaged in two developmental mutants of Arabidopsis thaliana. The first mutation causes albinism and lethality at the seedling stage. The individuals from an F2 mapping population were classified and pooled according to their phenotypes (wild-type or mutant). We then purified genomic DNA from the plants of both pools, and sequenced both samples using the Illumina HiSeq 2500 mapping-by-sequencing technology. We have established efficient bioinformatic protocols to perform the downstream bioinformatic analysis. Our bioinformatic pipeline allowed us to identify a point mutation that substitutes a conserved base at the acceptor site of an intron of the At2g04030 gene in the genome of Arabidopsis thaliana. This gene encodes a plastid-localized protein of the Hsp90 family of heat-shock proteins. The fruits of the second mutant contain supernumerary valves (carpels). Using a similar strategy, we identified a non-synonymous substitution at a conserved amino acid of the ULTRAPETALA1 (At4g28190) protein.
C0328 PHOSPHOENOLPYRUVATE CARBOXYLASE KINASE FAMILY PROTEIN FROM SORGHUM BICOLOR PLANTS (SBPPCK1-3): CLONING AND BIOCHEMICAL CHARACTERIZATION

Clara de la Osa Fernández1, Francisco Pérez-Montaño2, Cristina Echevarría3, Sofía García-Mauriño3, José A Monreal3

1Facultad de Biología, Universidad de Sevilla (Sevilla) España
2Departamento de Microbiología, Universidad de Sevilla, (Sevilla) Spain
3Departamento de Biología Vegetal y Ecología, Universidad de Sevilla (Sevilla) Spain

1 Resumen
Phosphoenolpyruvate carboxylase (PEPC) (EC 4.1.1.31) is a ubiquitous cytosolic enzyme that catalyses the irreversible β-carboxylation of PEP in the presence of HCO$_3^-$ to yield oxaloacetate and Pi. PEPC plays a crucial role in C$_4$ and CAM photosynthesis, where it catalyses the initial fixation of atmospheric CO$_2$. PEPC also fulfils several important non-photosynthetic functions, in particular the anaplerotic replenishment of tricarboxylic acid-cycle intermediates. PEPC is tightly controlled by a combination of fine metabolic controls, including allosteric effectors and reversible phosphorylation by a dedicated calcium-independent kinase named PEPC-kinase (PPCK). Sorghum bicolor is an African C$_4$ grass related to sugar cane and maize, and the fifth most important cereal crop grown in the world (US Grains Council). The sequencing of sorghum genome revealed that PPCK family is composed by 3 members, Sb04g036570 (SbPPCK1), Sb04g026490 (SbPPCK2) and Sb06g022690 (SbPPCK3) (Paterson et al., 2009). SbPPCK1 is likely to be the C$_4$ protein kinase and SbPPCK2 and 3 anaplerotic proteins, although their specific physiological role remains unclear. In this work, we analyzed the pattern of expression of the 3 genes coding PPCK in different tissues and under different stress conditions. In addition, we cloned and expressed in E. coli the 3 members of the PPCK family using GST-tag approach and the gateway cloning technology (Invitrogen) (Monreal et al., 2013). These proteins were purified by affinity chromatography and biochemically characterized using a fluorescence method (ProQ, Invitrogen). Kinetic parameters for substrates PEPC, casein and histone IIIS, and ATP were calculated. Interestingly, although of unknown function, PPCK3 protein showed the higher kinase activity and the lower Km for the different substrates assayed.

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Systems Biology

C0050 TRANSFORMATION OF QUERCUS ILEX SOMATIC EMBRYOS WITH A GENE ENCODING A THAUMATIN-LIKE PROTEIN

Vanesa Cano Lázaro¹, Elena Corredoira¹, María Teresa Martínez¹, Antonio Ballester¹, Mariano Toribio², María del Carmen San José¹

¹Instituto de Investigaciones Agrobiológicas de Galicia (IIAG-CSIC) Santiago de Compostela (La Coruña) España
²IMIDRA (Madrid) España

1 Resumen
Holm oak is the dominant tree species in Mediterranean forests. However, populations of the species are being decimated by Phytophthora cinnamomi, a generalist pathogen of worldwide distribution. Overexpression of genes for resistance is not possible, as such genes have not yet been isolated. Resistant individuals are produced by genetic transformation whereby a gene expressing a pathogenesis related protein, i.e. a protein encoded by the plant genome under biotic or abiotic stress, is introduced into the plant genome. The objective of the present study was to produce holm oak somatic embryos that overexpress the chestnut thaumatin-like protein (CsTL1) gene.

In the first experiment, 2-3 proembryogenic masses (PEMs) obtained from the Q8 embryogenic line were isolated and pre-cultured for one day, one week and two weeks. In the second experiment, PEMs of three embryogenic lines (Q8, E00 and E2) were precultured for one week. In all cases, pre-cultured explants were co-cultured for 5 days with Agrobacterium tumefaciens strain EHA105 harbouring the pK7WG2D-CsTL1 binary vector. The explants were then cultured on selective medium containing kanamycin (100 mg/l) and carbenicillin (300 mg/l). After culture of the embryos for 14 weeks on selective medium, the transformation efficiency was determined on the basis of the fluorescence of surviving explants.

In relation to the preculture time, the best results (4%) were obtained with explants pre-cultured for one week. The transformation efficiency depended on the genotype, and transformation was achieved only in 2 of 3 embryogenic lines evaluated. Transgenic somatic embryos were maintained by secondary embryogenesis and subjected to molecular analysis to determine the presence and expression of the CsTL1 gene.

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C0074 PROTEOMIC APPROACH TO THE MECHANISMS OF ALUMINUM HYPER-RESISTANCE IN UROCHLOA DECUMBENS

Catalina Arroyave, Livia Chavez, Roser Tolrà, Charlotte Poschenrieder, Juan Barceló Coll

Universitat Autònoma de Barcelona (Barcelona) España

1 Resumen

Urochloa decumbens is an aluminium-hyperresistant pasture grass of high economic interest. The mechanisms of this extraordinary Al resistance are not clearly established. Full expression of phenotypic Al resistance is observed after 72 to 96 h and preceded by a sensitive phase (24–48 h), characterized by Al-induced alteration of cell wall structure and inhibition of root elongation.

Scarcely information on the U. decumbens genome hampers a molecular genetic approach to the underlying mechanisms. Here a comparative proteomic study was performed on root samples after different exposure times (0, 24 and 96 h) to control (no Al supply) and Al-stress (200 µM Al). Only 11 proteins revealed significant abundance differences in response to Al; among these 6 were clearly identified. During the transient growth inhibition (24 h) phenylalanine ammonium lyase (PAL), methionine synthase (MS), and deoxymugineic acid synthase (DMAS) decreased, while the abundance of acid phosphatase (APase) increased. Coincident with the full expression of Al resistance (96 h), PAL and MS, but not DMAS, returned to the initial levels. Carbonic anhydrase (CA) and adenylate kinase (AK) along with two unidentified proteins were much more abundant.

The results indicate that the two-phase response of U. decumbens to Al stress is related to only a few changes in protein abundance. During the alarm phase, these changes are mainly related to phosphorus mobilization, downregulation of iron acquisition and reduction of phenolic biosynthesis. After recovering, biosynthesis of both phenolics and methionine, but not Fe mobilization are restablished. Enhanced dark fixation of carbon dioxide and the increased abundance of AK indicate increased organic acid formation and better availability of ADP and Mg²⁺ to ATP synthase, respectively. Down regulation of iron acquisition mechanisms supports the view that maintenance of Fe homeostasis is a key factor in Al resistant, acid-soil tolerant plants.

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C0236 ARTIFICIAL EVOLUTION IDENTIFIES NB-LRR INTRAGENIC MUTATIONS SUPPRESSING IMMUNE-RELATED HYBRID INCOMPATIBILITY IN ARABIDOPSIS THALIANA AND THEIR EFFECT ON DISEASE RESISTANCE TO LOCAL HYALOPERONOSPORA ARABIDOPSIDIS ISOLATES.

Ruben Alcazar Hernandez

Section of Plant Physiology. Department of Biology, Healthcare and Environment. Faculty of Pharmacy. University of Barcelona. Barcelona (Barcelona) España

1 Resumen

The Arabidopsis thaliana accession Landsberg (Ler), originary from Gorzów Wielkopolski (Poland) triggers immune-related hybrid incompatibility with central asian accessions (Kashmir-2 or Kondara). The Ler incompatible locus maps to a cluster of TIR-NB-LRR genes (RPP1-like) that, in combination with Kas-2 or Kond SRF3 alleles, induce EDS1 and SA-dependent hybrid necrosis. The RPP1-like locus has been reported to be involved in the recognition of certain effectors from the natural pathogen Hyaloperonospora arabidopsidis. Incompatible hybrids that severely affect growth and reproduction are unlikely to be frequent in nature, unless transient environmental conditions enable their growth and reproduction. Adaptive mutations may also be acquired by hybrids that suppress incompatible epistasis before mating. However, such mutations cannot be identified due to the lack of a phenotypic trait in the respective parental lineages. Here, we have made use of artificial evolution to identify, by next-generation sequencing, intragenic mutations to the RPP1-like Ler locus that suppress Ler/Kas-2 incompatibility. We identify complex additive and epistatic interactions within the RPP1-like Ler locus contributing to incompatibility. We also evaluate their contribution to disease resistance to a local pathogenic oomycete as potential evolutionary driving force for the occurrence of incompatible RPP1-like alleles in nature.
C0238 NATURAL AND ARTIFICIAL MODIFIERS THAT INFLUENCE GROWTH OF TEMPERATURE-DEPENDENT HYBRID INCOMPATIBILITY IN ARABIDOPSIS THALIANA

Changxin Liu, Kostadin Evgeniev Atanasov, Rubén Alcázar

Section of Plant Physiology. Department of Biology, Healthcare and Environment. Faculty of Pharmacy. University of Barcelona (Barcelona) España

1 Resumen

Alleles of the receptor-like kinase SRF3 (Strubbelig Receptor Family 3) in Arabidopsis thaliana populations of central Asia A. thaliana cause temperature-dependent epistatic incompatibility with a Landsberg erecta RPP1 resistance locus that involves an autoimmune response. In a segregating population derived from cross between Ler and Kas-2 which contained fixed RPP1-Ler and Kas-2 SRF3 alleles, all the lines exhibited dwarfism at low temperature, but some variation was observed likely due to natural modifiers that influence growth traits. Such modifiers might enable the growth of incompatible hybrids in nature through the modulation of immune activation or developmental pathways. Because of the complexity in mapping such modifiers, we are making use residual heterozygosity in a near-isogenic line developed between Ler and Kas-2. We find segregation on chromosomes 2 and 5 that might be linked to the phenotypic variation. Interestingly, on chromosome 5 it is reported a QTL for growth in the Ler/Kas-2 recombinant inbred line population that has not yet been mapped.

In parallel to this approach, we’re making use of artificial mutations to identify modifiers of the Ler/Kas-2 incompatibility. For this, we have performed an EMS-mutagenesis screen of RPP1-like R3 Ler overexpressor lines that reproduce incompatibility in the Col-0 background. A total of thirty-five mutants have been identified that suppress to different degrees dwarfism by R3 overexpression that are being characterized.

Last, the role of SRF3 in defense is not yet established. Remarkably, this receptor is de-phosphorylated in the presence of flagellin. Here, we have developed phosphomimetic mutant lines (S452D) in srf3-2 loss-of-function background that should resemble constitutively phosphorylated SRF3 forms even in the presence of flg22. In such mutants, we have observed differential expression of PTI marker genes PROPEP3 and NHL10, thus indicating SRF3 contributes to PTI through an unknown mechanism that is being investigated.