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1. Presentation and Objectives

The formation of the Centre for Plant Biotechnology and Genomics (Centro de Biotecnología y Genómica de Plantas, CBGP) was approved in October 2005 as a new Research Centre of Universidad Politécnica de Madrid (UPM) following the proposal of faculty from the Department of Biotechnology. The creation of such a Centre had been maturing for a long time and from the beginning it had also included researchers from Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA). As a result, CBGP became a joint UPM-INIA research centre by a five-year agreement of both institutions in July 2006. This agreement has been renewed in 2011.

CBGP was created with a double goal: to do state of the art research aimed at understanding plant function, and to contribute to fulfil the needs of the economic agents within the agriculture, forestry and environment productive sectors that are potential users of this research. CBGP also has an educational role, and is a reference centre for training of both scientists and technicians in the fields of plant biotechnology and genomics.

The creation of CBGP had its origin in the perception of a two-fold necessity: to do fundamental research at a competitive level, and to respond to the demands of new products, processes and services by the economic agents related to plant biology. In the last few years, knowledge on plant biology has progressed to an unprecedented pace. A major cause for this progress is the development of innovative technologies that allow efficient and global analyses of genome structure, information and expression. These new technologies are rooted in methodological advances in high throughput sequencing of DNA and separation and identification of proteins and cellular metabolites, in the use of microchips, in the automation of many laboratory procedures, and in the development of bioinformatics tools to handle the large amounts of information generated by them.

The strategic objectives of CBGP are:

- Generation of knowledge on the genomics and biotechnology of plants and plant-interacting organisms.
- Development of new technologies and tools for functional analyses
- Development of new products and processes relevant to the productive sectors.
- Transmission of information and secondment of educational programmes for scientists and technicians.

Major goals of CBGP in the short run were to increase the relatively small number of research groups that promoted its creation, to obtain the material instruments necessary to do competitive research on plant genomics and biotechnology and to actively contribute to form specialists. These goals have been fulfilled since then. CBGP is located since 2008 at UPM’s Campus de Montegancedo, which was rated as Campus de Excelencia Internacional (CEI) by Spain’s Ministry of Education in 2009 after external evaluation of its research, development and educational activity and projects. CBGP has a 7,500 m² building specifically designed to house CBGP laboratories, offices and administration. State of the art facilities for plant growth were built, including more than 350 m² of growth chambers, and a 1,200 m² greenhouse with about one fourth of its surface authorised for P2-level confinement experiments. More recently, a P3-level containment facility has been built. Also, important infrastructure for genomics, proteomics, metabolomics and microscopy has been acquired.

A considerable fraction of CBGP’s research groups have been part of it since it became a joint UPM-INIA centre in 2006. The incorporation of new scientists has been significant since then. New research positions for group leaders were created by both institutions, and filled after open, international concourses. This has resulted in the increase of research groups from the original 15 to the present 25. Also, 13 tenured positions for junior scientists and 6 tenure-track positions were created and filled.

Finally, CBGP is quite active in training activities. It is a relevant partner in the launching of UPM degree in Biotechnology (Bachelor level), in the Master programme in Agriculture and Forestry Biotechnology, and in the Ph. D. programme in Biotechnology and Genetic Resources of Plants and Genomics.
Associated Microorganisms. CBGP is also active at organizing extension activities, such as workshops on ecological risks of transgenic crops or activities on molecular biology for high school students within the Science Week (Semana de la Ciencia) promoted by the Madrid regional government.

Within the context of Plant Biology research in Spain, CBGP has some unique traits that may confer a competitive advantage to a centre for research excellence:

- It is a joint venture between two institutions, UPM and INIA with different goals, traditions and operative systems. This will favour the collaboration among groups with complementary aims and the establishment of consortia with a critical mass sufficient for addressing ambitious research objectives and for competing in granting agencies programmes.

- CBGP is located at CEI Montegancedo. This location favours the establishment of unconventional synergies with other research centres on Campus, such as: i) Facultad de Informática de la UPM, Centro de Supercomputación y Visualización de Madrid (CESVIMA) and IMDEA Software, all for cooperation with experts in computational sciences and informatics, for developing projects using bioinformatics or computational biology approaches; ii) Centro de Domótica Integral (CedInt), for cooperation on the design of energy-efficient laboratories and plant growth facilities (Green Labs), iii) Centro de Tecnología Biomédica (CTB): complementary equipment and technology with CBGP will facilitate the development of new programmes, for instance in the fields of plant foods and human health or computational biology, and iv) Centro de Empresas: the proximity to this nursery for technology firms facilitates technology transfer, spin-offs, patents etc.

In spite of the fact that CBGP researchers shared a common physical environment at Campus de Montegancedo only since the end of 2008, the availability of new facilities and the ease with which new internal and external interactions could be established among the various research groups, have already started to shape a new working style, which is contributing to fully develop its research potential.

Research at CBGP is to be evaluated periodically by an External Advisory Council of five recognised scientists, two of which, at least, must not work in Spanish institutions. The previous CBGP external evaluation was carried out in October 2011.
2. Organisation

The organisation and operation of CBGP is managed through a specific regulation (“Reglamento de Régimen Interior”), approved in 2006. CBGP organisation is summarised in the figure below. Briefly, researchers at CBGP are organised into research groups clustered into three research areas: Plant Genetics and Development, Plant Interactions with other Organisms, and Plant Interactions with the Physical Environment.

CBGP is ruled by different organs. Collegiate organs are: i) the Executive Board (Consejo Rector), composed of UPM’s Rector and Vice-rector for Research and INIA’s Director and Subdirector for Research, plus the CBGP Director, ii) the CBGP Council (Consejo de Centro) in which CBGP scientists and staff are represented, and iii) the Scientific Advisory Board (Consejo Científico Asesor) formed by five researchers representing all three areas.

CBGP has a Director, named by the Executive Board at the proposal of the CBGP Council, two Deputy Directors and an Executive Director, named by the Executive Board at the proposal of the Director.

Research at CBGP is to be evaluated periodically by an External Advisory Council of five scientists, two of which, at least, must not work in Spanish institutions. The specific functions of all these organs are defined by CBGP’s Reglamento de Régimen Interior.

Executive Board

Guillermo Cisneros Pérez
Chancellor of Universidad Politécnica de Madrid (UPM).

Manuel Lainez Andrés
Director of the National Institute of Agricultural and Food Research and Technology (INIA).

Asunción de María Gómez Pérez
Vice-Chancellor for Research of the Technical University of Madrid (UPM).

Isabel Cañellas Rey de Viñas
General Subdirector for Research and Technology (INIA).

Antonio Molina Fernández
Director of the Research Centre for Plant Biotechnology and Genomics (CBGP).

Directorate

Antonio Molina
Director

Luis Manuel Rubio
Deputy Director of Scientific Infrastructures

Juan Carlos del Pozo
Deputy Director of Scientific Programs

María Ángeles Ayllón
Executive Director
Scientific Advisory Board

General representative of the CBGP:

Mark Wilkinson
Senior Researcher Isaac Peral Programme. UPM

Representatives of the three Research Areas:

- **Plant Development:**
  Mónica Pernas
  INIA Researcher

- **Interactions of Plants with Environment:**
  Emilia López Solanilla
  Associate Professor. UPM
  Fernando García-Arenal
  Professor. UPM

- **Biotechnology and Bioinformatics:**
  Fernando Ponz Ascaso
  INIA Researcher

External Advisory Council

**Prof. Alan Collmer**
Cornell University. Ithaca. USA.

**Prof. George Coupland**
Max Planck Institute for Plant Breeding Research. Cologne. Germany.

**Dr. Crisanto Gutiérrez**
Centro de Biología Molecular “Severo Ochoa” UAM-CSIC. Madrid. Spain.

**Dr. Salome Prat**
Centro Nacional de Biotecnología (CSIC), Madrid, Spain.

**Dr. Cyril Zipfel**
The Sainsbury Lab, Norwich, Reino Unido.

3. **Facilities**

CBGP facilities at UPM CEI Montegancedo consist of a main building, an auxiliary building, and a plant growth facility (Laboratorio de Cultivo de Plantas, LCP) with adjacent greenhouses.

**1. Main Building.** With a total surface of 7,391 m², it houses the main research laboratories and many of the ancillary facilities (meeting rooms, administrative services...):

- Research laboratories are located on floors 1 and 2, which are identical, each housing fourteen 40 m² laboratories with adjacent offices (13 m²) for Group Leaders. Three more research laboratories and adjacent offices, as well as a Bioinformatics lab (50 m²), are located on the ground floor, bringing the total number of research laboratories to 32.
- Research laboratories share common instrumentation rooms: 2 inoculation rooms, 2 cultivation rooms, 2 centrifugation rooms, 2 cold rooms, 1 darkroom, and 1 autoclave room in each of floors 1 and 2.
- Laboratories for Research Services are located on the Lower floor, and include Radioisotope, Microscopy, Electrophysiology, Genomics, Proteomics and Metabolomics laboratories, a P3-level containment facility, a Physcomitrella growth unit, and facilities for working under anaerobic conditions. Research Services laboratories occupy ca. 500 m².
- Research Auxiliary Services include: Central glassware and sterilization facility (70 m²), Stock room (135 m², including a dedicated cold room), Main building plant growth facilities (15 m²), Freezer room (110 m²), water purification (type II distributed; type I on demand), as well as compressed research gases and liquid nitrogen supplies.
• General Services include: Meeting rooms (5, 190 m² total), Seminar room (110 m²), Library (100 m²), Administration offices (200 m²), ICT and servers (50 m²) and Cafeteria (100 m²).

2. Plant Growth Facility (LCP). The 542 m² LCP facility houses plant preparation and manipulation labs (123 m²), including a plant inoculation room and a stratification room, as well as free-standing and walk-in plant growth chambers (350 m² floor area).

Adjacent to the LCP, greenhouse facilities cover a total area of 1,200 m². These include 13 general-purpose, insect-proof, remotely-controlled (temperature and radiation) 47 m² chambers, and 5 40-m² containment chambers (Plant P2) for transgenic and other containment work.

3. Auxiliary building. The ca. 700 m² auxiliary building (ALFE) houses special purpose facilities auxiliary to the main building, including: i) two additional plant growth chamber facilities, including a walk-in growth chamber for low temperatures; ii) a pilot plant facility, including a 300-l fermentor and ancillary equipment; iii) two general purpose laboratories for back-up; iv) a meeting room; and v) general office and storage areas.

Among the above, the following facilities are noteworthy:

PLANT GROWTH FACILITIES

Over a combined area of ca. 1,900 m², facilities are available for plant growth in standard incubator-type, walk-in, and greenhouse (both standard and contained) chambers. Facilities are also available for photoperiod-controlled vernalisation and freezing conditions, defined wavelength incubation, and in vitro culture.

GENOMICS FACILITIES

Two Sanger-type multicapillary sequencers, three real-time PCR machines, a High-Resolution Melting analyser, microarray hybridisation and processing devices and robotic equipment for the automation of screening and multiplying of gene banks are available.

PROTEOMICS FACILITIES

Equipment for 2-DE, spot picking and digestion, a multidimensional protein / peptide fractionator (ProteomeLab), and a MALDI-TOF instrument are available.

METABOLOMICS FACILITIES

The metabolomics facility consists of a GC/MS system (BrukerScion TQ) with triple quadrupole and robotized automatic injector; and an LC (UPLC, Ultimate 3000 and Easy nLCII) / MS /MS ESI-QTOF (microTOF-QII), all of them provided by Bruker. Instrument control, and data acquisition and processing are carried out by means of a control and data station with Bruker proprietary software.

MICROSCOPY FACILITIES

Fluorescence and optical microscopes and stereomicroscopes are available, as well as a state-of-the-art Leica TCS SP8 confocal microscope. CBGP currently offers Plant Growth, Proteomics, Metabolomics and Microscopy Services to internal researchers and to external parties.
4. **Research Report**

4.1 **Research Groups Report**
**Research Group:** Seasonal and circadian control of growth dormancy cycle in woody plants

**Group Leader**
Isabel Allona

**Postdoctorals**
Alicia Moreno
Judith Gómez

**Scientific Staff**
Ramón y Cajal: Mariano Perales

**Master Students**
Igor López
Paolo Triozzi
Julio Téllez

**Technical staff**
Tamara Hernández
Marcos Morenilla

**PhD Students**
Daniel Conde
José Manuel Ramos
Paolo Triozzi

**Visitors**
Maria Veronica Perez

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**GOALS**

- Identification of key regulators of growth-dormancy cycles in poplar.
- Understanding chromatin-mediated regulation of this process.
- Deciphering the transcriptional control of apical and lateral shoot development in response to the different seasons.
- Underpinning the disruption of circadian rhythm during winter in woody perennials.

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**GENERAL OVERVIEW**

Woody perennial found within temperate and boreal latitudes develop annual cycles of growth and dormancy in synchrony with the seasons. The study of the regulation of annual cycles of growth and dormancy has a great biotechnological importance because it would permit the optimization of the growth in addition to the adaptation to different geographic regions and to the climate change. We undertook a multidisciplinary approach to investigate the function of potential regulatory proteins involved in growth-dormancy cycles. The approach includes spatio-temporal gene expression analysis, functional studies, phenological assays, cell biology, biochemistry and genome-wide transcriptome and methylome analyses. We identified several transcription and chromatin remodeling factors that operate in response to the signals that determine the seasons, mainly day length and temperature. We established real-time gene expression methods to measure daily patterns of transcription in response to environment in poplar system. We analyzed seasonal phenology of genetic modified poplar by simulating the seasons in growth chambers. Integrating those disciplines, we found that the diurnal rhythms of HMGB activates the circadian rhythms of LHY in poplar. RAV1 transcription factor promotes axillary branching and seasonal shoot apical development, analyzing the impact of rav1-engineering on poplar biomass production in a short-rotation coppice field trial, and DEMETER like DNA demethylases control bud maturation and bud break in poplars.
RESEARCH ACTIVITY

Real-time monitoring of circadian rhythms

Precise control of gene expression is essential to synchronize plant development with the environment. In perennial plants, transcriptional regulation remains poorly understood, mainly due to the long time required to perform functional studies. Transcriptional reporters based on luciferase have been useful to study circadian and diurnal regulation of gene expression, both by transcription factors and chromatin remodelers. The High Mobility Group proteins are considered transcriptional chaperones that also modify the chromatin architecture. They have been found in several species, presenting in some cases a circadian expression of their mRNA or protein.

We generated a stable luciferase reporter poplar line based on the circadian clock gene PtaLHY2, which can be used to investigate transcriptional regulation and signal transduction pathway. Using this reporter line as a genetic background, we established a methodology to rapidly assess potential regulators of diurnal and circadian rhythms. This tool allowed us to demonstrate that PtaHMGB2/3 promotes the transcriptional activation of our reporter in a gate-dependent manner.

Moreover, we added new information about the specificity and the protein regulation of the PtaHMGB2/3 along the day. This methodology can be easily adapted to other transcription factors and reporters.

Impact of rav1-engineering on poplar biomass production: a short-rotation coppice field trial

Early branching or syllepsis has been positively correlated with high biomass yields in short-rotation coppice (SRC) poplar plantations, which could represent an important lignocellulosic feedstock for the production of second-generation bioenergy. In prior work, we generated hybrid poplars overexpressing the chestnut gene RELATED TO ABI3/VP1 1 (CsRAV1), which featured c. 80% more sylleptic branches than non-modified trees in growth chambers. Given the high plasticity of syllepsis, we established a field trial to monitor the performance of these trees under outdoor conditions and a SRC management. Under our culture conditions, CsRAV1-overexpression poplars continued developing syllepsis over two cultivation cycles. Biomass production increased on completion of the first cycle for one of the overexpression events, showing unaltered structural, chemical or combustion wood properties. On completion of the second cycle, aerial growth and biomass yields of both overexpression events were reduced as compared to the control. These findings support the potential application of CsRAV1-overexpression to increase syllepsis in commercial elite trees without changing their wood quality. However, the syllepsis triggered by the introduction of this genetic modification appeared not to be sufficient to sustain and enhance biomass production.
Chromatin-mediated regulation of growth-dormancy cycles

Plants respond to a changing environment by modifying the functional state of chromatin. Dynamics levels of 5mC methylation have been associated with postembryonic developmental transitions, yet the mechanisms underlying these processes are still scarce. We identified a DEMETER-like (CsDML) cDNA from a winter-enriched cDNA subtractive library in chestnut. Overexpression of CsDML accelerated SD-induced bud formation, specifically from stage 1 to 0. Bud acquired a red-brown coloration earlier than wild type (WT) plants, alongside with the upregulation of flavonoid biosynthesis enzymes and accumulation of flavonoids. Shoot transitions in plants.

Bud break is preceded by a reduction of genomic DNA methylation in poplar apex while PtaDML10 is induced after chilling fulfilment during ecodormancy. PtaDML10 knockdown poplars show a delayed shoot apical meristem (SAM) reactivation and growth under bud break conditions caused the induction of cell metabolism genes and key regulators of meristem development, and the downregulation of bud dormancy genes, which are prone to 5mC demethylation. These data implicate DEMETER-mediated DNA demethylation in the control of environmentally induced developmental stage transitions in plants.

Figure 3. PtaDML10 is required for bud break (A,B) Growth cessation and bud set in response to SDs, 19°C. (C) Apex stages during bud break. [UPOV,1981]. (D) Timing of bud burst was examined in response to LD and warm temperature after dormancy induction. WT, K2D and KDS plants were treated as shown in (A,B). Following a shift to SD and 4°C, plants were transfer to LD and 22°C at three different times: 1=0, t=4wk and t=8wk. Here we show apical growth reactivation monitored in the 4wk experiment. Mean bud burst scores ± SE were measured for the wild type (n = 7), K2D (n = 7) and KDS (n = 7). (E) DNA methylation level in apices of WT, K2D and KDS. DNA methylation levels (mean values +/- SE, n=4) in dormant apical meristems quantified by HPLC. Shoot apical meristems were collected at the time point close to bud burst. Each biological sample consists of a pool of n=15 dissected apices. Two independent hydrolyses were performed for each biological replicate and two HPLC analyses for each hydrolysis.

Publications and awards


Awards

PRX15/00642 Programa de estancias de movilidad de profesores e investigadores españoles en centros extranjeros. Estancia sabático para Isabel Allona en SLCU, Cambridge University, UK, MEC 2015 Duración: 4 meses

Funding

AGL2011-22625/FOR Las señales de transducción del fotoperíodo y la temperatura durante la aclimatación al frío y la dormancia invernal en especies leñosas. MICINN 2012– 2014
Acción COST FP0905 "Biosafety of forest transgenic trees: improving the scientific basis for safe tree development and implementation of EU policy directives" 2010-2014
AGL2014-53352-R Desarrollo de herramientas genéticas para modular los ciclos de dormancia-crecimiento en especies leñosas MECYT 2015-2020
Genetic variation is the basis for adaptation of the crop to any future challenge. Exploiting the phenotypic and genetic diversity may allow for successful cultivation in different climate types and provides possibilities for traditional breeding as well as, identification of target genes for marker-assisted selection.

GOALS

- The analysis of the genetic diversity and the study of the domestication process in grapevine.
- The genetic control of quality traits in grape and tolerance to abiotic stress using the grapevine natural variation.
- Origin and consequences of somatic variation in grapevine
- Development of genetic tools for genetic improvement of crops.

GENERAL OVERVIEW

How to improve production and adaptation to climate change in crops is one of the key challenges that plant scientist has to address. Our group is focused in developing new genetic and molecular tools to address this challenge. Our research program involved three main fields: 1) development of genomic tools to identify the genetic bases of the natural variation at quality traits in grape with the purpose to development molecular markers that allow to accelerate the selection in breeding programs; 2) Identify natural genotypes tolerant to abiotic stress (drought and salinity) related to climate change and evaluation of candidate genes responsible of the phenotypic variation. 3) Molecular characterization of plant genetic resources of legume collections (Vicia sativa) for breeding purposes. In order to identify the genes and the nucleotide variation that it is responsible for the fruit quality variation. We have exploited the possibilities that offer the combination of the genomic information with the different tools of genetic analysis. Elucidation of the molecular basis of these traits can allow to increase the effectiveness of the breeding programs. Other efficient tool in the identification of characters of quality is the characterization of the metaboloma of mature berries of wild accessions and clones of wine grape varieties, with the purpose of determinate biomarkers of quality for the clones and search of new metabolites in the wild accessions. Regarding the abiotic stress tolerance we will exploit the genetic diversity of the European Wild Grape (Vitis sylvestris) for the development of resilient rootstocks. In this context, the final objective is the development of molecular tools that allow to accelerate the selection in future breeding programs of wine grape.
Genetic diversity analysis in germplasm collection

Grapevine

The wild grapevine, Vitis vinifera L. ssp. sylvestris (Gmelin) Hegi, considered as the ancestor of the cultivated grapevine, is native from Eurasia. In Spain, natural populations of V. vinifera ssp. sylvestris can still be found along river banks. We have performed a wide search of wild grapevine populations in Spain and characterized the amount and distribution of their genetic diversity using 25 nuclear SSR loci. The genetic diversity of wild grapevine populations was similar than that observed in the cultivated group. The molecular analysis showed that cultivated germplasm and wild germplasm are genetically divergent with low level of introgression. We have identified four genetic groups, with two of them fundamentally represented among cultivated genotypes and two among wild accessions. The analyses of genetic relationships between wild and cultivated grapevines could suggest a genetic contribution of wild accessions from Spain to current Western cultivars. Currently, we are carrying out the phenotypic characterization of wild grapevine accessions from the Iberian Peninsula.

Common vetch

The common vetch, Vicia sativa, is one of the most important annual forage-grain legumes due to its economic and ecological advantages. INIA conserves the active Spanish germplasm collection of vetch. These plant resources from native landrace varieties are essential for the selection of characters of agronomic interest. In the current context of climate change, we have a special interest in the improvement focused on the selection and production of new varieties tolerant to drought and aridity. Our aim is the development of molecular tools (functional molecular markers) for breeding programs to select and improve drought tolerant varieties.

Natural variation at quality traits in grapevine

The color is a quality trait determined by the quantity and anthocyanin profile of the berry. The biochemical analysis of these traits in wild grape accessions showed different profiles than cultivated grapevine. In grapevine the transcriptional factors VvmybA1 and VvmybA2 are associated with this trait. We have analyzed the allelic variants present in wild grape accessions from the both end of Mediterranean basin (Iberian Peninsula and Anatolian Peninsula). Results provide evidence that variation in both transcriptional regulators has generated a new allelic series that it has been not found in cultivated grapevine. Furthermore, the allelic series showed correlation with anthocyanins content. We have performed a RNAseq analysis to evaluate their expression patterns and associate them with their phenylpropanoid profiles. Some genes of the phenylpropanoid pathway were up-regulated in wild compared with cultivated grapevine. Most noticeably, transcript levels of stilbene synthase genes showed steady increase. Transcriptional regulation of MYBA1, MYBA2, MYBF, F3’H, F3’5’H, UFGT, OMT, and FLS are in some cases correlated with the characteristic anthocyanin and flavonol profiles in wild berry skin. These results reveal a unique pattern of transcription and biosynthesis pathways regulation underlying the enological characteristics of wild grape. In addition, the expression profiles of stress-related genes showed a specific dynamic modulation during berry development in wild berries. These results yield new knowledge on the distinct chemistry and characteristics of wild berry.

**Figure 1.** Graphical representation of ancestry membership coefficients of all individuals. Each individual is shown as a vertical line divided into segments representing the estimated membership proportions in the two and four ancestral genetic clusters inferred with STRUCTURE. Individuals within each cluster are arranged according to estimated cluster membership proportions.

**Figure 2.** Heat map showing the differentially expressed genes in the full ripening stage of wild and cultivated berries. The color legend represents the abundance of transcripts. In red up-regulated and blue down-regulated.
Adaptation to adverse environment is the central survival strategy of plants. As active process, adaptation requires that resources are repartitioned that otherwise would be available for growth. When humans domesticated plants for their own purposes, they selected for high yield and rapid growth which was achieved on the cost of reduced resilience (and the lost of the genetic factors underlying this resilience). Humans compensated this by creating a highly artificial environment, where the crop is cultivated under optimal conditions and environmental challenges are compensated by agricultural means including fertilization, irrigation, weeding, and chemical plant protection. Wild ancestors of crops have to survive without relying on this artificial support and therefore represent valuable resources to mine for resilience genes or alleles. For that reason, we have analyzed wild genotypes around the Mediterranean basin and we have identified natural resilience genes or alleles. For that reason, we have analyzed wild genotypes around the Mediterranean basin and we have identified natural resilience genes or alleles.

**Figure 3.** In the top we showed the in vitro culture selection for the wild genotypes with different NaCl concentrations. In the bottom we showed the percentage of survival for different wild genotypes in different NaCl concentrations.

**Publications and awards**


**Funding**

Crop yield depends on key plant developmental transitions such as flowering or seed germination that impact in crop production. Our interest is to understand the regulatory mechanisms driving these developmental processes and contribute to secure sustainable crop production, a key challenge faced by agriculture nowadays.

GENERAL OVERVIEW

During their life cycle plants undergo a number of developmental phases. These developmental stages are characterised by specific patterns of cellular differentiation. The switch from a developmental phase to the next is under the control of spatial and temporal patterns of gene expression, so that selective activation or silencing of genes directs plant development through different phase changes. Our group is focused on understanding the molecular mechanisms involved in the regulation of plant developmental transitions. In particular we are interested in phase transitions such as flowering and germination with adaptive value for plant species as well as a significant impact on crop yield.

Chromatin remodeling plays a crucial role in the establishment and maintenance of specific gene expression patterns associated with plant developmental transitions. For that reason, one of our main interests is to understand how epigenetic changes participate in the regulation of floral initiation and seed germination, and how environmental changes influence the structural dynamics of chromatin and affect developmental gene expression patterns. To accomplish these tasks we are using a combination of genetic, molecular and functional genomics approaches directed to identify and characterise master genes and regulatory circuits involved in the control of these developmental processes.

GOALS

- Reveal the molecular mechanisms that mediate the fine control of flowering time in response to environmental and endogenous signals
- Shed light on the chromatin-mediated processes involved in the regulation of plant developmental phase transitions
- Identify novel factors required to repress germination during seed dormancy
- Unveil mechanisms responsible for the integration of developmental programs with environmental cues to mediate adaptation of Brassica crops to suboptimal conditions
RESEARCH ACTIVITY

The PHYB-HOS1-CO module integrates red-light signals into Arabidopsis flowering regulation.

In Arabidopsis, the ability to distinguish day-length is largely the result of the complex regulation of the CO gene, both at the transcriptional and posttranslational level. The coincidence of CO expression with light in LD conditions is essential for the promotion of flowering. Besides, different light qualities play specific roles in the photoperiodic response, and while blue and far-red light promote flowering, red light delays it through phyB function. It was already known that CO protein stability is controlled by various light signals during the day, but the molecular mechanism mediating the red light-induced degradation of CO that regulates photoperiodic flowering in Arabidopsis remains to be elucidated. We have revealed a key role for the E3 Ubiquitin Ligase protein HOS1/ESD6 in this mechanism. We have demonstrated that the degradation of CO under red light is prevented in the hos1 and the phyB mutants and thus HOS1 is involved in the red light-mediated degradation of CO. Besides, we have demonstrated that PhyB physically interacts in vivo with HOS1 and CO, suggesting that the mechanism by which CO is degraded under red light relies on the formation of a tripartite complex between these proteins that may be essential to modulate a correct photoperiodic response in Arabidopsis.

Arabidopsis DNA Polymerase ε recruits components of polycomb repressor complex to mediate epigenetic gene silencing of floral integrators.

We have provided an attractive model for targeting of PRC2 complex to newly synthesized DNA for reestablishment of repressive epigenetic marks after dilution by replication, which has important implications in the understanding of epigenetic inheritance in eukaryotic organisms.

We have taken advantage of viable PRC2 and DNA polymerase ε esd7 mutations in Arabidopsis to address the mechanism underlying the maintenance of the epigenetic states during replication. We have also presented sound evidence supporting a role for the catalytic subunit of DNA polymerase ε ESD7 in preserving high H3K27me3 levels at floral target loci. Using flowering time control as a subject, we have examined the function of the Pol ε in epigenetic transcriptional silencing and demonstrated the genetic and molecular interaction of this replication protein with PRC2.

Plant specific proteins EBS and SHL mediate chromatin-dependent repression of Arabidopsis master genes of flowering, reproductive development and dormancy.

We have characterized two Arabidopsis homologous genes, SHL and EBS, which encode effector proteins with functional domains specifically recognizing histone modifications essential for gene expression control in eukaryotic organisms. Both proteins are involved in the chromatin-mediated repression of flowering and have independent roles in controlling master genes of flowering time. The proteins SHL and EBS are necessary to maintain an inactive chromatin conformation of the genes that act in Arabidopsis as switches of flowering initiation. Both proteins interact with histone deacetylases, contributing to the silencing of flowering genes during vegetative growth. EBS is involved in additional processes occurring during reproductive growth, like changes in the inflorescence architecture, flower initiation and also in the control of seed dormancy.

Figure 2. ESD7 genetically interacts with the PRC1 component TFL2 and the PRC2 components CLF and EMF2. (A and C) Flowering phenotype of Col, esd7-1 introgressed in the Col background, T2-1, esd7-1 T2-1, emf2-2, emf2-2 esd7-1, Ler, esd7-1, clf-16 and clf-16 esd7-1 mutant plants grown under LD (A) or SD conditions (C). (B and D) Flowering time of these plants under LD (B) and SD conditions (D). (E and F) Analysis of the expression of FT and SOC1 floral integrator genes in WT and different mutants under LD. Total RNA was extracted from pool samples of 9-day-old (WT and mutants in Col background) and 6-day-old (WT and mutants in Ler background) seedlings grown under LDs collected 4 h after dawn. All the differences were statistically significant (P < 0.05) according to Student’s t-tests in the comparison of mutants with WT. Asterisks indicate statistically significant differences.
Histone variant H2A.Z and histone acetylation mediate different aspects of chromatin function and modulate flowering responses in Arabidopsis.

During recent years our lab has got a deeper insight into the epigenetic mechanisms that participate in the regulation of the floral transition through the molecular and genetic characterization of several Arabidopsis mutations affecting subunits of the plant SWR1 chromatin remodeling complex, which catalyzes the exchange of H2A histone by the H2A.Z variant and that regulates flowering time in plants. We have characterized the ESD1 locus, encoding ARP6, a nuclear protein similar to conventional actin, and also the AtSWC 6 locus, that encodes a zinc finger-harborin protein. Both proteins are part of the SWR1-C and mutations in the corresponding genes cause an acceleration of flowering. In addition, we have studied AtSWC4 and AtYAF9-like proteins, whose yeast orthologs are present in the SWR1-C and are shared by the HAT complex NuA4, indicating a possible functional interplay between these two complexes. Mutations in these homologues and in other NuA4-C subunits also confer alterations in flowering time and in other developmental processes.

Securing yield stability of Brassica crops in changing climate conditions

We coordinate the SYNBRACLIM project which aims at understanding the mechanisms of adaptability to abiotic stress of oilseed rape by uncovering the genetic and molecular bases of the regulation of flowering time in relation to environmental factors such as temperature and their impact on crop yield.

Figure 3. EBS Interacts with HDACs. (A) and (B) Flowering time phenotype of the double mutant ebs aetx-5 under LD (A) and SD (B) conditions. (C) in vitro pulldown assays showing the interaction of the EBS protein with HDA6 and 19. (D) BIFC assays showing the in vivo interaction between EBS and HDA6 (top panel). The EBS and HDA6 coding sequences were fused to the C-YFP (CY) and N-YFP (NY), respectively, and coexpressed in Nicotiana benthamiana cells. YFP signal (YFP), chlorophyll autofluorescence plus bright field (C-W), and overlay (C+Y-W) microscopy images are shown. (E) in planta interaction between EBS and HDA6. Samples were immunoprecipitated with HA antibody and the immunoprecipitate was probed with anti-Myc antibody. (F) Working model for the hypothetical repression mechanism of the floral integrator genes mediated by SHL and EBS, showing their interaction with HDACs.

Publications and awards


Funding

BIO2010-15589. Disección genética y molecular de mecanismos implicados en la represión de la floración.

SP3-PEOPLE 298790. Flowering Chromatin. Control of flowering time by chromatin remodeling.

ITN-SP3-PEOPLE-316965. EpTRAITs: Epigenetic regulation of economically important plant traits. EU.

BIO2013-34098-R. BrassiCHROM. Regulación mediada por cromatina de caracteres del desarrollo que afectan al rendimiento de cultivos de Brassicaceae. MINECO

BIO2016-77589-R. CHROMYIELD. Caracteres de desarrollo regulados por cromatina con influencia en el rendimiento de cultivos: diseción del tiempo de floración en Brassicaceae.

ERA46-SYBRACLIM. Securing yield stability of Brassica crops in changing climate conditions. FACCE-JPI EU.
Research Group: Stu
dying gibberellin signalling to improve seed germination and plant growth under stress

Group Leader: Luis Oñate
PhD Students: Rocío Sánchez
Master Students: Belén Rombolá, Laura Bouza Morcillo, Natalia Pozuelo

GOALS
- Characterization of gibberellin (GA) signalling mechanisms in the control of seed germination.
- Identification of new regulatory genes counteracting growth restraint under stress conditions blocking GA biosynthesis.
- Use of molecular and genetic information to improve agronomic performance.

We investigate daily and seasonal mechanism that control woody plant development. We are interested to develop new molecular markers and tools to increase lignocellulosic biomass in woody perennials.

GENERAL OVERVIEW

Gibberellins (GAs) are phytohormones required for vegetative and reproductive growth throughout the plant life cycle. GAs regulate gene expression in time and space and their levels can be altered in response to developmental and environmental cues. Several stress conditions, such as drought and salinity, are known to decrease GA biosynthesis and signalling. Although this response increases stress tolerance it reduces plant growth and productivity. We are using plant seeds as a model to study the integration of environmental signals into the decision to grow since: i) they require GAs to germinate; ii) the “decision” to germinate represents a critical stage in the life cycle of higher plants and is an important ecological and commercial trait; iii) many of the regulators found in this organ have also been recruited by other developmental stages to play similar roles. We carry out our research by using powerful and complementary approaches, some of them developed in our group (i.e.: Figure 1), and by collaborations with companies, national and international groups.
**RESEARCH ACTIVITY**

**GA signalling mechanisms in the control of seed germination**

We have found that GA-signalling in the Arabidopsis embryo epidermis along the embryonic axis is required for proper germination and uncovered the underlying molecular mechanism which is coordinating growth of the epidermis with that of inner tissues (Figure 2). This mechanism was found later to be conserved in cotton where it controls fibre cell elongation, indicating that it has been recruited by other developmental stages and suggesting that it is highly conserved in plants. Our ongoing studies, suggest that GA-signalling underlying growth of the endosperm is controlled by a set of regulatory genes different to those of the embryo. The full extent to what these mechanisms has been recruited by other developmental stages and/or plant species remains to be determined.

**GA-signalling and its interaction with stress tolerance**

In collaboration with the group of Dr. W. Dröge-Laser we have generated a seed library overexpressing Arabidopsis Transcription Factors (Weiste et al., 2007; now with more than 700 TFs). Screening this library (gain of function) increases the probability of identifying new players in the regulation of germination and GA-signalling that may have been missed by previous screening approaches with loss-of-function mutants (mainly due to genetic redundancy). We have identified mutants able to germinate faster under low GA levels similar to those produced by stress conditions. Remarkably, some of these mutants, currently under study, also have improved vegetative growth suggesting the existence of mechanisms unlinking growth and stress (Figure 3).
Using molecular and genetic information to improve agronomic performance

Living organisms synthesize a myriad of metabolites required for proper growth and development with many of them able to affect the performance of “neighbours” (i.e.: competitors, predators, symbionts). Compounds from natural sources, such as those derived from plants, are usually biodegradable and considered as more ‘environmentally friendly’ compared with many traditional herbicides or other chemicals. We have developed “sensor” plant lines and a methodology for high-throughput screening to identify agrobiological compounds (supplied by PRB S.L.) modifying seed germination properties. The identified compounds will also be excellent candidates to regulate growth in other developmental stages and plant species.

Publications and awards


Funding

2011-2014: Regulation of the hormonal balance controlling the transition between seed dormancy and germination. MICINN (BIO2010-17334) PI: Luis Oñate-Sánchez.
Research Group: Developmental study of root systems and their adaptation to soil and environmental factors: from model plants to crops

GOALS

- Complete an extensive analysis of the remarkable variability in the organization of the roots in the Brassicaceae family and their correlation with their growth in environmental stressed soils.
- Uncover the genetic and evolutionary bases of root developmental traits associated to plant adaptability to extreme environmental conditions.
- Identify new genetic tools regulating root development that can be used to improve yield security of crops, in particular Brassica crops, in the context of climate change.

GENERAL OVERVIEW

Our research group is interested in uncovering the genetic and molecular factors regulating root development in response to different soil and environmental conditions. Our research programme involved three main fields: analysis of stem cell development in the Brassicaceae Family; study of root adaptation to environmental conditions during plant evolution; and analysis of root development traits associated to plant adaptability to extreme conditions produced by climate change with special attention to Brassica crops (Brassica napus, oilseed rape). Our ultimate goal is to assist agriculture in the evaluation of the consequences of current and future adverse environmental conditions on plant growth and to help in the design of better adapted crops to face this challenge.
RESEARCH ACTIVITY

One of the key challenges that plant scientists have to address is how to improve crop production under challenging environmental conditions. Roots are in charge of delivering the nutrients and water that are usually a limiting factor in many lands. Additionally, they are also in the first line to respond to stresses produced by extreme climate conditions. The development of an efficient root system better adapted to different soil and environmental conditions is crucial for enhancing crop productivity. Our research is focused on gaining a better understanding of the genetic and molecular factors regulating this developmental process. In this context, our research program involved three main fields:

Analysis of Stem Cell Development in the Brassicaceae Family

Roots are crucial for the uptake of water and nutrients. Root growth adapts to changes in the environmental conditions through changes in their physiological and developmental programs. The stem cells are the heart of the machinery that drives the growth of the roots. Understanding the molecular mechanisms behind this plastic growth requires a better understanding of root stem cell biology. SCZ is a recently identified transcription factor that regulates stem cell development in the Arabidopsis roots. We are using SCZ gene as a molecular tool to uncover new molecular mechanisms regulating stem cell fate transitions during root development in different species of the Brassicaceae family with special attention to species of potential agronomic interest.

Root Adaptation to Soil during Plant Evolution

Our objective is to understand how roots respond to changes in soil and climate conditions and how this adaptation has been acquired during plant evolution. A preliminary analysis of the variation in root architecture in different species across the Brassicaceae family has showed that there is a remarkable diversity of root phenotypes in this family. Moreover, an initial analysis of their growth in environmental stressed soils has identified several species with roots more adapted to grow in severe habitats. We are going to use this diversity to investigate the developmental and evolutionary mechanisms underlying root adaptation in plants. A better understanding of the mechanisms regulating the adaptability of some plant species to extreme environmental conditions will provide us with the genetic tools needed to improve the efficiency of these crops to climate variability.
Root development traits associated to plant adaptability to extreme conditions produced by climate change.

Extreme climate conditions like drought, flood and heat events are predicted to be more frequent in the near future. European agriculture will require crops able to cope with variable environmental conditions without altering their productivity. Europe's premium oilseed crop, oilseed rape is one of the world's most important sources of high-quality vegetable oils for human nutrition and biofuels, and particularly in Europe is also a major contributor to vegetable protein diets for ruminant livestock. Crop yield stability is dependent on the response of key developmental programs like root development to stress conditions. Advancing our understanding of the mechanisms of how oilseed rape plants integrate developmental and growth processes in response to temperature and drought, this project will provide the basis on which more efficient production of oilseed rape can be achieved and make a significant contribution to breeding new varieties.

Publications and awards

Dissemination activities

Congresses

Publications
1. López-Gil Quereda, Mónica. Trabajo Fin de Grado. Obtención y caracterización de líneas transgénicas de Arabidopsis thaliana para el estudio de la interacción entre las proteínas SCHIZORIZA y TOPLESS. UCLM. Septiembre 2014.

Funding
Research Group: Plant hormonal regulatory networks

GOALS

- Functional characterization of AMIDASE1 from Arabidopsis thaliana
- Understanding the cellular regulation of auxin homeostasis
- Disclosing the intimate jasmonate/auxin crosstalk in biotic stress responses
- Identification of molecular targets to improve saccharification traits
- Determination of the regulatory role of indolic compounds during seed maturation

GENERAL OVERVIEW

Our group aims to understand how plant hormones orchestrate plant stress responses at the molecular level through their complex interconnection. Plant hormones are small signaling molecules that act at micromolar concentrations and are capable of re-programming the transcriptional portfolio of a responding cell or tissue. Through their interaction they can integrate diverse internal and external stimuli, e.g. developmental processes, temperature, light, mechanical forces, as well as biotic stresses, and translate those cues into the adequate adjustment of the transcriptome, which ultimately leads to changes in the growth program or chemical responses of the plant. Herein, our particular interest is with jasmonate/auxin crosstalk, which has been shown to affect not only root growth, but also the enforcement of cell walls in tissues adjacent to a wound site. We identified two auxin biosynthesis-related genes, YUC8 and YUC9 that are specifically induced by wounding through the COI1-JAZ-MYC module. Over-expression of the two genes led to substantial changes in the phenotype of the plants, including strongly lignified stems. Further analysis revealed that the jasmonate/auxin-mediated increased lignification also involves an increased production of ethylene. Ultimately, this hormone cascade drives local enforcement of secondary cell walls, which represents a physical barrier to prevent spreading of pathogen infections or re-enforcement of cell layers close to a wounding site, in order to maintain structural integrity. On the other hand, we found a novel molecular mechanism by which seed maturation is seemingly controlled. Very much like seed germination, which is controlled by the interplay of gibberellin and abscisic acid, we think that the rapid cell expansion of embryo cells and seed filling is controlled by the ratio between indole-3-acetamide (IAM) and indole-3-acetic acid (IAA). In this regard, we were able to identify a small number of potassium channels that are under the differential control of IAM and IAA. T-DNA insertion lines of these target genes show a significant embryo phenotype, which highlights their contribution to cell expansion during the course of the seed maturation phase.
RESEARCH ACTIVITY

Indole-3-acetamide dependent auxin biosynthesis

The biosynthesis of auxin, the most prominent plant growth promoting substance is mainly realized by the activity of a two-step metabolic pathway referred to as the IPA route. In this metabolic pathway, tryptophan aminotransferases and Flavin containing monoxygenases convert L-tryptophan into indole-3-pyruvic acid (IPA), which is subsequently converted into the major auxin, IAA. However, apart of this major pathway, a small number of alternative routes for the biosynthesis of auxin are discussed. One of them is assumed to proceed via the intermediate IAM, which is finally referred to as the IPA route. In this metabolic pathway, tryptophan is mainly realized by the activity of a two-step metabolic pathway.

Over the last couple of years, we functionally characterized AMI1 in planta. Today, we know that AMI1 indeed contributes to auxin biosynthesis in vivo. However, to our own surprise, we found that the main function of the enzyme is possibly not the production of IAA, but rather the control of the IAM pool, as evidenced by multiple assays on the transcriptional regulation of AMI1. Whole-genome DNA microarrays led to the finding that AMI1 likely acts as a molecular hub that is involved in the integration of multiple abiotic and biotic stress responses. In particular the mutant root phenotype that can be rescued by the addition of sugar led to the connection of stress responses with the energy status of the plant through the transcriptional control of AMI1 levels in the plant. Confirming our hypothesis, ami1-2 mutant plants showed significantly increased JA levels and were shown to be more resistant to bacterial and fungal pathogens. On the contrary, inducible AMI1 lines appeared to be more susceptible to pathogen attacks. Furthermore, we were able to demonstrate that the cellular IAM content in embryos is an important determinant of embryo cell expansion and seed filling. Accumulation of IAM resulted in strongly impaired embryos.

Fig 1: A) Root branching phenotype of three ami1 mutant alleles relative to wild-type Arabidopsis (WT). On the left side, seedlings were raised on plates containing 0.1% sucrose, whereas on the right, the seedlings were grown on plates containing 1.5% sucrose. Apparently, the addition of higher sucrose contents was sufficient to rescue the root branching phenotype of ami1-1 and ami1-3. B) Confirming our microarray data, ami1-1 mutant seedlings accumulate significantly more jasmonic acid, which should render them more resistant to pathogen attack. C) In order to provide further empirical evidence for our working hypothesis, ami1-2 knockout and AMI1 inducible overexpression mutants were treated with the virulent hemibiotrophic bacterium Pseudomonas syringae pv. Tomate DC3000. AMI1 mutant performance was compared to wild type (Col-0) Arabidopsis and either susceptible (fts2) or more resistant (cpr5) Arabidopsis mutants.

Optimized lignocellulose exploitation from potato canopy

Over the last years, our lab also pursued the goal to improve the usage of agricultural plant residues for bio-combustible production. Herein, we were particularly focusing on the optimization of the extractability of glucose from potato (Solanum tuberosum) areal tissues, which are generally discarded due to their slight toxicity. In this coordinated project, we took a systematic approach, down-regulating the expression of key genes related with the regulation or biosynthesis of lignin by the generation of corresponding RNA-interference (RNAi) lines. In total, a number of 16 target genes were selected on the basis of shared synteny, which involved the query for conserved genetic blocks of order between the chromosome sets of Arabidopsis thaliana, potato and tomato. Among the studied molecular targets we identified two gene loci, SICAD2 and SICCR1, which significantly improved the saccharification of potato leaf material when knocked-down by corresponding RNAi constructs. The cellulose to glucose conversion in these lines was enhanced by 2- to 8 fold, respectively. Since the extractability of lignocellulosic biomass is not supposed to be realized on the expense of reduced biomass and, therewith tightly connected, plant performance, we also investigated numerous basic parameters, including tuber yield, fresh and dry weight, as well as more complex traits, such as total lignin contents, monolignol composition, and the global architecture of the stem and extracellular polysaccharides. In contrast to most lines, e.g. SHCT and SICoAMT that showed a dwarfish phenotype or no impact on glucose extractability, the two identified lines showed a significant shift of S to H and S to G-lignin, respectively, which is most likely the molecular reason for the improved glucose extractability. Notably, the identified RNAi lines showed no considerable trade-offs relative to wild type.

Fig 2: A) Transgenic potato plants grown in the greenhouse. CAD2 and CCR1-RNAi lines are examples of plants showing a normal phenotypes compared to Desirée wild-type potato plants. B) Mass spectrometric analysis of internode 3 from three selected CAD2-RNAi and CCR1-RNAi lines in comparison to Desirée wild-type potato plants. The enzymatic cellulose to glucose conversion is normalized to the glucose extractability observed in Desirée WT plants. The amount of glucose extracted per mg cell wall material was set to 1 and all other values calculated accordingly.
Different levels of jasmonate/auxin crosstalk

Over recent years, mounting evidence led to the widely accepted concept that plant hormone action is not the read-out of linear signaling pathways, but rather determined by the extensive combinatorial activity of the signaling molecules and the integration of their signaling pathways, both in terms of regulating growth and development and in adapting to external stimuli. Further complicating the scenario, the triggered physiological processes are not only dependent on the perceived stimulus, but also on the specific properties of the responding tissue in terms of sensitivity and responsiveness to a given signaling molecule class. In this context, the aim of our lab is to shed light on the mechanistic and conceptual crosstalk between auxin and oxylipins, in particular between IAA and JA. In previous studies, taking a transcriptomics approach, we identified two YUCCA genes, YUC8 and YUC9, to respond differentially to a treatment with MeJA. The observed induction of the two IAA biosynthesis genes involves the COI1-JAZMYC signaling module. In-depth analysis of the YUC8 and YUC9 over-expression lines revealed an auxin contribution to the induction of secondary growth in areal tissues of Arabidopsis plants, which ultimately results in a strong lignification of the responding tissue. From this, we conclude that the JA-mediated induction of local IAA biosynthesis serves the purpose of physically reinforcing tissues that were affected by either herbivore or pathogen attacks. With the objective of corroborating this notion, we exposed YUC8 and YUC9 loss- and gain-of-function mutants to both spider mites and Pseudomonas syringae pv. tomato DC3000. As expected, the YUC8 and YUC9 over-expressing, more lignified plants appeared significantly more resistant to the applied biotic stress conditions.

Fig. 3: A) Histochemical analysis of Arabidopsis stems. 100 μm thick cross-sections of inflorescence stems (20 cm above the rosette) of WT (Col-0), YUCBox and YUCBox plants were stained either with (ac) calcifluor or (d) congo red. The position of the xylem (Xy) and interfascicular fibers (IF) are indicated. Scale bar = 100 μm. B) Plant damage assay after spider mite (Tetranychus urticae) herbivory. The left side shows leaves from wild-type and mutant plants after 4 days of T.urticae feeding. The right side of the figure depicts the quantification of total plant damage area in WT and YUC8/9 mutants. Data are means ± SE. Five biological replicates per genotype were assessed. Statistical analysis was performed using Student’s t-test comparing between the WT plants and each mutant genotype. Asterisks indicate significant difference at ** P < 0.01. Scale bar = 1 mm.

Publications and awards


Patents
S. Pollmann, J. Vicente-Carbajosa, J. Medina, J. Kehr “Solanum tuberosum plants for biofuel production” International application number: PCT/EP2016/053541; Priority country: International; Priority date: 20/02/2015; Owner: REPSOL S.A.
J.C.del Pozo Benito, C. Manzano Fernandez, P. Ho “Use of compounds to regulate vegetal growth” Application Number: P201630412; Priority country: Spain; Priority Date: 05/08/2016; Owner: INIA

Funding
01/2012 – 09/2015 Individual MICINN Grant, Project identification BFU2011-25925
03/2012 – 02/2016 European Commission, Marie Curie Career Integration Grant, Project Identification FP7-PEOPLE-CIG-2011 303744
01/2012 – 09/2015 Project OPTISOL in REPSOL – UPM INSPIRE program financed by REPSOL S.A., Spain. Coordinator S. Pollmann
01/2015 – 12/2017 Coordinated MINECO Grant, Project identification BFU2015-55575-R. Coordinator S. Pollmann
We will need to develop a Real Sustainable Agriculture Program to maintain plant production in the future, balancing this production with conservation of the environment. We will have to make a more rationale use of inputs, including water and fertilizers.

We are interested in understanding how root system architecture (RSA) is determined in response to external clues or stresses. Roots are essential organs to absorbed water and nutrients. The majority of root biology and responses to stresses has been studied under root illumination conditions, at least in the model plant Arabidopsis. As roots are underground organs, we hypothesized that this light stimulus might alter root responses. Currently, using a new cultivation system, D-Root, which has been engineered in our lab, we have characterized the root response to phosphate (Pi) and nitrogen starvation (N) or the excess of salt in the medium. We found that responses to these stresses in dark-grown roots are different than in illuminated roots. The efficiency of this absorption under deficiency conditions (low level of water or nutrients) will be one of the primary factor that limits plant production. We have undertaken genetic, genomic and metabolomic approaches to understand how roots respond to phosphate deficiency. For this studies, using the D-Root system, we have identified several novel genes that are specifically induced by Pi starvation in roots. Genetic and pharmacological analyses showed that these genes plays important roles in the response to low phosphate.

In addition, we screened for mutants that showed altered root system. One these mutants, fip1-1, is affected in the alternative polyadenylation of mRNAs. We are carrying out genomic analyses to identify new polyadenylation sites in response to abiotic stresses. These studies revealed that FIP1 is important to sustain alternative polyadenylation, selecting proximal polyA sites. The second characterized mutant, lrg1, is involved in protein N-glycosylation.
Root responses to phosphate starvation

Phosphate is a major nutrient that is essential for plant growth. Pi deficiency compromise plant growth and productivity. Previous studies have shown that, under Pi starvation, roots stopped growing, developed more lateral roots (LRs) and formation of more and longer root hairs. However, this Pi starvation response in dark-grown roots (DGR) is different. These roots do not stop growing and LRs developed at regular intervals. Using this conditions, we have analyzed the transcriptome of DGR with or without Pi in the medium. We have identified a large number of genes that have not been identified in previous transcriptomics analyses in Pi starved roots. We have started the molecular characterization of some of these genes. One of these genes seems to be a regulator of S Adenosyl biosynthesis and metabolism pathway. In addition, using the D-Root we have identified an Arabidopsis ecotype that accumulates up to 5 times more phosphate than Columbia. Currently, we are initiating the generation of F2 and F3 crosses to identify QTLs.

Light is an stress for root development

Roots grow in darkness, but they may be exposed to light under certain circumstances, showing negative phototropism. After perceiving light, roots bend to escape from light (root light avoidance), and reduce their growth. How negative growth responses of roots to light are regulated is not well understood. We show that flavonols regulate root growth responses to light through repression of cell proliferation and promotion of cell differentiation. Flavonol levels are low when roots grow in darkness, but they strongly increase upon illumination. This higher content of flavonols reduces auxin signaling and superoxide radical content, and subsequently reduces root growth. Unilateral illumination of roots induces accumulation of flavonols at the meristem side closer to light, promoting local cell differentiation and growth re-orientation to avoid light. We show that flavonol accumulation in the root transition zone is triggered by pathways promoting differentiation (cytokinin and hydrogen peroxide), and that these pathways are required for root light avoidance. Flavonols function as positional signals, integrating hormonal and ROS to regulate organ growth in response to light.

OBP4 regulates cell reprogramming

Plant growth and development requires a continuous balance between cell division and differentiation. In root meristems, meristematic cells divide continuously to sustain root growth. Later, these cells differentiate, acquiring specialized functions, but losing their mitotic potential. Some plant cells types, such as pericycle cells, have a remarkable plasticity to be reprogrammed and to regenerate new organs. A functional screening of transcription factors, using the Spanish collection TRANSPLANTA, identifies Arabidopsis OBP4 as a novel regulator of root growth by reducing cell elongation and cell differentiation. Overexpression of OBP4 regulates the levels of a large number of transcripts in roots; many of them involved in hormonal signaling and in the formation of callus, a mass of proliferative cells derived from reprogrammed pericycle cells. OBP4 does not induce cell division in the root meristem but it promotes pericycle cell proliferation, forming callus-like structures at the root tip as shown by expression of stem cells markers. Callus formation is enhanced by ectopic expression of OBP4 in wild type or in alf4-1.

Figure 1: Root response to phosphate starvation is different in illuminated or dark grown roots. Using our D-Root device, we grew Arabidopsis plants with roots in presence of light (LGR) or in darkness (DGR). In presence of light, Pi-starved roots stopped growing and increase the density of lateral roots and root hairs. In darkness, Pi starvation does not affects so dramatically to root growth. In addition, the density of lateral roots is not increased.

Figure 2: A) Flavonols accumulate in light grown roots (LGR) to higher levels than dark-grown roots (DGR). B) DGR were illuminated with a lateral light to stimulate the light avoidance response. Flavonol accumulation was preferentially detected in the illuminated side. D) TT4 gene, which is essential for flavonol biosynthesis, is induced in the illuminated side during the light avoidance response. E) Lower light avoidance response in tt4 is due to less cell elongation in the transition zone.
Alternative polyadenylation regulates plant development

We have identified that the sbrel52 mutation affects to FIP1, a subunit of the polyadenylation machinery forms part of the in CPSF complex: Cleavage and Polyadenylation Specificity Factor. Recently, in mammals, it has been shown that Fip1 regulates mRNA alternative polyadenylation (APA) to promote stem cell selfrenewal and somatic cell reprogramming. APA plays a critical role in posttranscriptional gene control, regulating alternative splicing, RNA stability or protein translation. We have found that *fip1-1* shows a different response to several biotic stresses, indicating that APA is important to respond to a large number of stresses (Fig. 4). We have also carried out a detailed study of the fip1-1 root morphology. We found that *fip1-1* roots grows more in stress conditions such as illumination, nitrogen deficiency or salt. Recently, we have carried out, in collaboration with Dr. Hunt, an polyA site and alternative polyadenylation in fip1-1 nad control plant in normal condition and during the response to salt and nitrogen deprivation

Figure 3: A) Root tip phenotype of control and OBP4 overexpressing plants. Cell division and elongation was reduced in the OBP4-ox. B) OBP4 is induced during callus formation. C) Overexpression of OBP4 promotes de formation of callus in a callusinducing. D) Fluorescence and bright field photographs of roots of Ei-OBP4/J0121 or Col-0/J0121x grown in CIM with estradiol. Overexpression of OBP4 increased the activation of pericycle cell and callus formation in these pericycle cells

Publications and awards

5. Garrido-Arandia, M.; Silva-Navas, J; Ramírez-Castillejo, C; Cubells-Baeza, N; Gómez-Casado, C; Barber, D; Pozo, J; Melendi, PG; Pacios, LF; Diaz-Perales, A. (2016). “Characterisation of a flavonoid ligand of the fungal protein Alt a 1”. Scientific Reports. DOI: 10.1038/srep33468
12. 12-15 See also publication from Miguel Moreno-Risueño group

Patents:
“Use of compounds to regulate vegetal growth” Application Number: P201630412 Priority country: Spain Priority Date: 5-8-2016 Owner: INIA
J.C.del Pozo Benito, C. Manzano Fernandez, P. Hoyos Vidal, M. J. Hernaíz, S. Pollmann
Award: Fulbright Fellowship for a sabbatical stay in the Universidad of California San Diego.

Funding
BIO2011-28184-C02-01. Conexión de las Auxinas y el Ciclo Celular a través del complejo SCF-SKP2. Desarrollo de las Raíces Laterales II. MICINN
Regulation of translation during Abiotic stress conditions in plants

**GOALS**

- Characterization of the molecular mechanisms involved in the regulation of translation during specific plant developmental programs and in response to environmental stresses.
- Identification of plant mRNAs regulated at translational level.
- Identification and characterization of new proteins involved in plant adaptation to stress.

**GENERAL OVERVIEW**

Most plants complete their life cycle in a single location and, therefore, their development and reproduction depend on the environmental conditions they are exposed to. Environmental stresses such as drought, high and low temperatures, saline soils, etc., seriously affect plant development and growth.

To cope with these challenges, plants have evolved genetically based physiological, biochemical and molecular strategies that allow them to survive under such adverse conditions. One of the earliest plant responses to environmental stresses is global inhibition of protein synthesis, allowing the specific translation some mRNAs involved in the response to stress. Despite the importance of this response, the knowledge about the mechanisms involved in this translational regulation is very poor in plants.

Our lab is mainly focused on characterising the mechanisms involved in this translational regulation and, based on this information, identifying new regulators of the stress response in plants.

Our final goal is to contribute to understand how plants respond to stress in order to select better environmentally adapted crops, and in such a way improving crop security and production.
RESEARCH ACTIVITY

Identification of new regulators involved in the adaptation to stress in plants

In order to identify new regulators of the heat stress response in plants, we firstly carried out a high-throughput translatome analysis in Arabidopsis seedlings subjected to a moderate heat stress (Yangüez et al., 2013). From this study we identified different mRNAs that were highly translated under a strong general translational inhibition. In addition, and with the same objective, we have carried out a comparative proteomics analysis of plants grown under control conditions, during a heat acclimation event (38°C for 1 h) and at the early stages of the recovery periods that selectively lead plants to either survival or death (Figure 1). This quantitative proteomics analysis allowed us to: (1) get a deeper insight of the proteins regulating the acclimation process, (2) unravel the common and specific proteins involved in each of the cited response to heat and (3) identify pivotal proteins involved specifically in the commitment to plant survival or death in response to heat (Echevarría-Zomeño et al., 2016).

These two studies granted the selection of new potential regulators of plant adaptation to stress for further studies. One of the proteins identified as a potential heat stress regulator in both studies was the cytosolic cochaperone HOP3.

HOP3 a new regulator of the ER stress response in plants

HOPs (HSP70-HSP90 organizing proteins) are a highly conserved family of cytosolic cochaperones, whose role in adaptation to stress was not previously evaluated in plants. In a recent study by Fernández-Bautista et al., we showed that HOP3, one member of the HOP family in Arabidopsis, plays an essential role during ER stress in plants. HOP3 interacts in vivo with cytosolic HSP90 and HSP70, indicating that it is a functional member of the HOP family in Arabidopsis. Interestingly, we showed that HOP3 interacts specifically with BiP, a major ER chaperone. BiP belongs to the Hsp70 family, however, it lacks the conserved octapeptide sequence that has been involved in the interaction between HSP70 and HOP in other species, making this interaction completely unexpected. Despite the lack of this conserved sequence, interaction analyses indicate that HOP3 binds to the nucleotide binding domain of BiP, demonstrating a noncanonical HOP binding to this major ER resident protein. Consistent with the interaction with BiP, HOP3 is partly localized at the endoplasmic reticulum (ER). Moreover, HOP3 is induced both at transcript and protein levels by UPR inducer agents. Finally, hop3 loss-of-function mutants show a hypersensitive phenotype in the presence of the ER stress inducer agents, demonstrating that this protein plays an essential role during the ER stress response. In line with this observation, the hop3-1 mutant shows a reduction in pollen germination, a developmental process especially vulnerable to disturbances in ER protein homeostasis. These findings open the exciting possibility that HOP3, through its role in the alleviation of ER stress, could play an important function during specific developmental programs and in response to different environmental stresses.
Characterization of the molecular mechanisms that regulate translation initiation under environmental and developmental cues

One of the earliest responses of plants to stress is the regulation of protein synthesis. This regulation takes place mainly at the initiation step of protein translation. In mammals, one of the main mechanisms for translation regulation are the control of eIF4E activity by interaction with other proteins (4E-BPs and 4E-binding partners in Figure 3). Despite the broad function of these eIF4E interacting proteins in the regulation of general and specific inhibition of translation in other eukaryotes, no orthologs have been found in plants. In our lab, we are interested in characterising the mechanisms involved in the regulation of translation initiation under different stress conditions and during specific developmental programs in plants. Our data suggest that, in contrast to the general view, the regulation of translation initiation is not so conserved in plants as in other eukaryotes, highlighting the extraordinary contrast to the general view, the regulation of translation initiation is not so conserved among eukaryotes, no orthologs of the cited eIF4E interacting proteins have been identified in plants, rendering the knowledge about how plant eIF4E regulates mRNA translation completely unexplored.

Fig 3: Mechanisms for inhibition of general and specific mRNA translation through the interaction of different proteins with eIF4E in mammals. Under control conditions eIF4E interacts with the 7mGpppN cap structure of the mRNA and with eIF4G, allowing mRNA initiation of translation. However, under different stress conditions and developmental programs, different proteins (4E-BPs and 4E-binding partners) interact with eIF4E leading to a general and selective inhibition of mRNA translation, respectively. Although, the main players in the initiation of translation are conserved among eukaryotes, no orthologs of the cited eIF4E interacting proteins have been identified in plants, rendering the knowledge about how plant eIF4E regulates mRNA translation completely unexplored.

Publications and awards


Awards:
The Project N-cycle is awarded as one of the best business plans of the XII Entrepreneurship Competition UPM (2015). IP: Dra. Mar Berrocal
Participant: Dra. Mar Castellano
Associate editor of Frontiers in Plant Science within the research topic: “Relevance of Translational Regulation on Plant Growth and Environmental Responses” (2016).

Funding
S2013/ABI-2734. Tradaptación. “Regulación traduccional y abordaje de su potencial biotecnológico para mejorar la adaptación de las plantas a estreses bióticos y abióticos”. CAM.
RTA2013-00027-00-00. “Evaluación del potencial biotecnológico de la proteína 3P para aumentar la tolerancia de las plantas a altas temperaturas y a patógenos”. INIA.
STG2014-68. “Plant cIRES Biotech”. “Functional characterization of plant cIRES and their use as biotechnological tools”. ERC.
BIO2010-15751. Estudio de la regulación del factor de iniciación de la traducción IF4E en respuesta a estrés abiótico en plantas. MCINN.
We try to decipher the molecular strategies triggered by plants in response to different stressors or stress combinations, to identify mechanisms and discover novel molecules useful for crop improvement in the face of climate change.

GENERAL OVERVIEW

Plant-pest relationships are intricate interactions encompassing complex networks of molecules, signalling pathways and strategies to overcome defenses developed by each other. The induction of plant defence genes is initiated when specific receptors recognize either the presence of a pest (phytophagous insect or acari), or the damage incurred by them, or even the existence of volatiles emitted as plant-plant cues. The success of plants to withstand biotic stresses depends on their fast response by triggering a wide-range of specific genes and compounds with defensive properties. We are very interested in deciphering the molecular mechanisms involved in this process and to identify molecules and pathways that allow plants discriminate among species of insects and mites and activate specific responses.

In addition, protein breakdown and mobilization are some of the major metabolic features associated with leaf senescence mediated by stresses. Their consequences are essential for nutrient recycling and plant surviving. The massive net degradation of proteins related to this process involves broad metabolic networks, different subcellular compartments, and several types of proteases and regulators. We have focused our attention in deciphering the roles of barley C1A cysteine-proteases, the most abundant enzymes responsible for the proteolytic activity during leaf senescence, as well as in their specific inhibitors, the cystatins, in the control of proteolysis.

The goal is the use of plants with altered proteolytic regulation in crop improvement in the face of climate change.
Plant defense responses to spider mite feeding.

The two-spotted spider mite *Tetranychus urticae* is a cosmopolitan agricultural acari pest feeding on over 1,100 different plants, 150 of them of agricultural importance, causing damages approaching 1 billion dollars worldwide. In addition, another spider mite species extremely invasive is *T. evansi* which only feed Solanaceae species, a pest of recent introduction from South America in the EU agriculture. Due to high fecundity, inbreeding and short generation time *Tetranychidae* populations develop resistance to acaricides after one to four years of use. Our research tries to understand the differential ability of mites to feed on a wide or narrow plant host, to decode the plant responses (genes and signaling pathways) that confer resistance to mites and to apply the knowledge gained through basic science as a potential new avenue for acari-pest control. The effect of changing climate on plant and spider mite physiology and plant-spider mite interaction is still unknown. To determine factors that contribute most to crop success/failure within the changing agricultural ecosystem is essential to develop strategies to mitigate pest damage and increase plant performance. The project gained at the FACCE-ERA-NET call will allow our team, as member of the GAP-M consortium (Genomics in Agriculture Pest Management), the identification and analysis of the critical parameters, and the development of new tools for efficient and environmentally-friendly spider mite pest management under drought. Our group is characterizing some of these genes that participate in the intracellular signaling cascade to trigger host resistance to spider mites.

Protein degradation/mobilization events during barley leaf senescence

High nutrient mobilization efficiency is an intrinsic feature of plant senescence. This process involves a well-orchestrated activation of genes encoding catabolic enzymes to remobilize nutrients, in particular nitrogen, from the senescing organs to still growing plant parts and developing grains. Each senescing-promoting factor (abiotic/biotic) up-regulates a subset of senescence-associated genes (SAGs), many of them encoding proteases with a crucial role in nutrient recycling. We study the roles of barley C1A cysteine-proteases, the most abundant enzymes responsible for the proteolytic activity during leaf senescence as well as the role of cystatins, their specific modulators that exert a complex regulatory function in this physiological process. Our results have demonstrated that, besides senescence-mediated by abiotic stresses such nitrogen starvation, darkness or drought, the spider mite *T. urticae* feeding has a potential impact and accelerates barley leaf senescence. Currently, we are studying the leaf senescence behavior of transgenic barley lines over-expressing or silencing protease or cystatin genes, under abiotic (drought) and biotic (spider mite feeding) conditions. First results have shown that modifications on cysteine proteases and cystatin genes may produce alterations on proteolytic events and specific senescence phenotypes (delayed or accelerated), as well as differential plant behaviour in response to abiotic/biotic stresses. Besides, these data corroborate the role of proteolytic process in nutrient recycling and support the potential of biotechnology to modulate proteolysis in order to obtain higher grain yield.

Figure 1. Symptoms and silk production derived from spider mites (*Tetranychus*) feeding in pepper, *arabidopsis*, bean and tomato plants.

Figure 2. Natural senescence phenotypes of 10 week-old barley wild-type (WT) plants and over-expressing (OE Pap1) and silencing (KD Pap1) barley lines, corresponding to the cathepsin F-like cysteine protease encoding gene (HvPap-1). KD Pap1 plants show a delayed-senescence phenotype.
The main objective of this line is to know the origin and evolution of different gene families involved in plant defense mechanisms and in pest attack. Comparative genomics analyses of protein families are based in current bioinformatics tools and genomic databases. These analyses are performed to determine the existence and the number of members of each family in different clades. With these sequences, phylogenetic analyses are made in order to identify species-specific proteins involved in plant resistance and pest attack, and to determine the sequence variations that could explain their specific roles.

Figure 3. A. Plant databases for comparative genomics. B. Schematic phylogenetic tree showing the evolution of the phytocystatin family. C. In silico modelization of the interaction between the mature barley cathepsin B and its propeptide.

Publications and awards


Funding

Viruses are the major group of emergent pathogens of animals and plants. Emergence is a complex of ecological and evolutionary processes, requiring virus-host encounters and virus adaptation to the new host. Understanding emergence is central to its anticipation and prevention.

GENERAL OVERVIEW
The long term research goal of the group is understanding the emergence of new viral diseases. Plant viral diseases affect food security, crop and forest productivity, and the composition and dynamics of natural ecosystems. The highest socio-economic impact of infectious diseases is often caused by emerging diseases, i.e., those whose incidence is increasing in a new host population. Major factors favouring disease emergence are changes in host ecology and environment and genetic change in pathogen and host populations. Hence, the research in the group is organised around the evolutionary ecology of plant-virus interactions. We focus on plant-virus co-evolution and on the factors that disrupt co-evolutionary dynamics. The specific questions currently addressed include:

i) The relationship between biodiversity, infection risk, and plant virus host range, as related to the effects of ecosystem simplification by human activities on virus host range, virulence and emergence.

ii) The effects of plant community structure on adaptation of viruses to hosts and host range evolution along the specialist-generalist continuum.

iii) The genetic mechanisms generating across-host fitness trade-offs that will limit host range expansion through virus adaptation to new hosts species or genotypes.

iv) The evolution of dominant and recessive resistance genes in in plant host populations, as conditioned by virus infection and by the environment.

v) The molecular genetic mechanisms underlying tolerance of plants to virus infection, and their relationship with phenotypic plasticity of the plant-virus interaction along the antagonism-conditional mutualism axis.

vi) The estimation of relevant evolutionary parameters in order to develop realistic models of virus emergence.

These questions are approached using different host-virus systems, including crops, model species and wild plants in natural ecosystems.
RESEARCH ACTIVITY

Modulators of the evolution of virus virulence.

A goal of our group is to understand which factors modulate the evolution of virulence, i.e., the harmful effect on host fitness of pathogen infection. One such factor is rate and mode of transmission. Our earlier model analyses indicated that the emergence of highly virulent genotypes of Cucumber mosaic virus (CMV) causing necrosis of tomato plants required high transmission rates. We tested this prediction by passaging CMV at low or high aphid vector densities in tomato plant populations. As predicted, only under high aphid densities did highly virulent genotypes invade the CMV population. The role of the mode of transmission, vertical or horizontal, on virulence was analysed by serially passaging CMV in Arabidopsis under vertical or horizontal transmission. Vertical passages led to higher vertical transmission, lower virus multiplication and lower virulence. Horizontal passages did not modify virus traits. We observed also reciprocal host adaptation during vertical passages. Thus, we showed the key role that both mode of transmission and plant-virus co-evolution have in determining virulence.

The modulation of virulence by the environment was examined in the CMV-Arabidopsis interaction under different temperature and lightintensity conditions. Virulence differed between host genotypes and environments, the effect of infection varying from detrimental to beneficial for the host. These important results demonstrate that plant viruses can be either antagonists or mutualists, depending on the environment.

Overcoming host resistance in gene-for-gene plant-virus interactions is an instance of host range expansion, which can be hindered by across-host fitness trade-offs. Identifying such trade-offs and their causes is relevant, as the use of genetic resistance is a major strategy for controlling crop viral diseases in crops. We had shown that overcoming of L-gene resistance in pepper by tobamoviruses was associated with severe withinhost multiplication penalties. Now we introduced mutations determining the overcoming of L3 and L4 alleles in cDNA clones of Pepper mild mottle virus. Assays in susceptible host genotypes in single and in mixed infections showed that resistance-breaking (RB) mutations had pleiotropic effects on virus multiplication that, according to the specific mutation, the host genotype, and the type of infection, single or mixed with other virus genotypes, were antagonistic or positive. Thus, there are fitness trade-offs both across hosts and across types of infection, and the emergence of RB mutants will depend on the genetic structure of the host population and on the frequency of mixed infections. L-gene RB mutations occur in the virus coat protein, and could affect particle stability and virus survival in the soil after harvest. We showed that soil survival differed among virus pathotypedepending on differential effects of RB mutations on particle stability, and that RB costs in survival add to costs in multiplication. These results also show that plant resistance can select for altered survival, which may condition RB evolution.

Host range evolution and the overcoming of host resistance

Fig 1. Evolution of CMV seed transmission and virulence across passages of vertical transmission. Seed transmission rate was estimated from the number of infected seedlings out of 100 seeds per plant. Virulence is represented as one minus the ratio of seed weight in infected to mockinoculated plants: 1-(SWI/SWm). Data are plotted as mean ± standard error of the five independent lineages in each passage for Fny CMV (blue squares), De72-CMV (red triangles) and LS-CMV (green diamonds). The black line in each panel represents the fitted regression line for lineages of all three strains combined. Modified from Pagán et al. 2014. PLoS Pathogens

Fig 2. Reaction of susceptibility or resistance of pepper (Capsicum) accessions carrying different alleles at the resistance locus L to various Pepper mild mottle virus coat-protein mutants. From Moreno-Pérez et al. 2016. Journal of Virology.
**RESEARCH ACTIVITY**

Environmental heterogeneity and plant-virus interactions

Ecosystem simplification due to human activities has been linked to virus emergence. To address this important question we have studied virus infection in the wild pepper or chiltepin, which grows in Mexico in differently modified habitats, from wild to cultivated populations. We have shown in the past that human management of the chiltepin habitat results in increased infection risk by two begomoviruses. We have more recently analysed the relationship between habitat anthropisation and genetic diversity, mutation fixation and recombination in these viruses. Further, we have shown by genetic and phenotypic characterization of the alleles of a recessive resistance gene, that anthropisation results in altered selection for resistance of chiltepin to potyviruses. Currently we are addressing the relationship between ecosystem simplification and virus infection risk, host range and adaptation to host by means of NGS-determination of the viruses infecting plants in different habitats in Central Spain.

**Fig 3. Phylogeny of pvr2 resistance gene haplotypes and functional analysis of the pvr2-Vpg:PVY interactions. The phylogeny was constructed using pvr2, coding sequences. Bootstrap values (1000 replicates) are indicated on the nodes. A to Q are pvr2/eIF4E1 haplotypes identified in chiltepin populations pvr1+ and pvr2+ to pvr225 denote pvr2/eIF4E1 alleles deduced from the pvr2/eIF4E1 coding sequences; pot1+: Polivirus susceptibility allele from tomato. pvr2-Vpg:PVY interactions were evaluated by yeast two-hybrid assays. Yeast growth (%) indicates the percentage of the yeast growth on the selective medium (LWH) compared to the reference yeast colonies co-transformed with pGADT7::pvr2+ and pGBK7::Vpg-PVY. From Poulicard et al. 2016. PLoS Genetics**

**Publications and awards**


**Funding**

BFU2015-64018-R. Evolución de la gama de huéspedes y de la virulencia en la emergencia de virus de plantas. MINECO

Era Net_ARIMNET 2-618127. Emergent viruses and virus vectors in Mediterranean basin crops (EMERAMB). MINECO

RTA2013-00079-C03-02. Diagnóstico, caracterización y control del agente causal de una nueva enfermedad observada en cultivares de ajo, puerro y cebolla. INIA

CGL2013-44852-R. Entender la evolución de la gama de huéspedes de los virus de plantas para su control sostenible en un panorama de cambio global. MINECO

Realización de estudios de identificación de plantas de ajo resistentes a la infección por distintas especies de virus de aliciaceas. COPAMAN SCL
GOALS

- To determine how metals are exchanged at the plant-microbe interface.
- To determine how plants have optimized metal exchange with its symbionts to thrive in metal-limiting environments.

GENERAL OVERVIEW

Some transition metals (iron, copper, zinc, molybdenum,…) are essential nutrients for life. In plants they are involved in a plethora of processes, from photosynthesis to the immune response. However, there is a prevalent low metal bioavailability in most agricultural soils. This has a profound impact on crop production and human nutrition. Our research efforts are directed towards understanding how metal homeostasis is maintained in model legume *Medicago truncatula*. Legumes are one of the most important food and staple crops worldwide, the main vegetable protein source, and key elements in sustainable agriculture strategies due to their ability to fix atmospheric nitrogen in symbiosis with certain soil bacteria (rhizobia). This symbiotic nitrogen fixation requires relatively vast amounts of metals and, consequently, exerts a heavy toll on the host plant metal homeostasis. This is in part ameliorated by another endosymbiont, arbuscular mycorrhizal fungi that improve nutrient uptake, including essential metal oligonutrients, when they are lacking in the surrounding soil. Consequently, within this framework of *M. truncatula* metal homeostasis, we are paying closer attention to the metal exchange mechanisms between this legume and its two main associated endosymbionts, rhizobia and arbuscular mycorrhizal fungi. 

*Understanding how plant-associated microorganisms affect transition metal uptake and allocation in their host plant will lead to developing novel ways of improving plant growth, crop yields, and nutritional value.*
Transition metal exchange in plant-microbe interactions

In this period we have focused on studying metal delivery to legume nodules using as a model the system *Medicago truncatula*-Sinorhizobium *meliloti*. Transcriptional analyses of these nodules have allowed us to identify 20 plant genes involved in metal transport upregulated in nodules. Promoter:GUS fusion studies and immunolocalization studies have shown that MtNramp1, MtCOPT1, MtMOT1.3, and MtZIP6 are metal transporters located in the plasma membrane of rhizobia-infected cells. Expression in a wide range of yeast strains has shown that they are involved in iron, copper, molybdate, and zinc uptake, respectively. Affecting the expression levels of these genes results in a loss of nitrogen fixation capabilities, while no phenotype has been detected when nitrogen fixation is not required, or when the mutated gene is reintroduced.

Adaptation to metal-limited environments

We have studied the symbiotic phenotype of 100 *M. truncatula* ecotypes when growing in low-iron conditions vs the standard conditions. We have selected the top three nitrogen fixers and the bottom two. The expression levels on the metal transporters identified in the nodule transcriptomic analyses have been determined, unveiling two metal transporters whose expression correlates with higher nitrogen fixation rates in low-iron conditions.

Figure 1: Localization of HA-labelled MtNramp1 (A), MtMOT1.3 (B), MtCOPT1 (C), and MtZIP6 (D) in *M. truncatula* nodules. Blue indicates DAPI-stained DNA, green shows the S. melliloti constitutively expressing GFP, and red is the position of the Alexa594-conjugated antibody used to tag the HA-labelled transporters. Phenotype of the metal transport mutants for MtNramp1 (E), MtCOPT1 (F), and MtMOT1.3 (G), and RNAi silenced MtZIP6 (H) is shown compared to wild type plants (left) and, the mutant transformed with a wild-type copy of the mutated gene (right).

Publications and awards


Funding

AGL-2015-65866-P. Diverting metals to *Medicago truncatula* nodules. MINECO

ERC-2013-SIG-335284. Metal homeostasis in the tripartite symbioses arbuscular mycorrhizal fungi-legume-rhizobia. European Research Council

AGL-2012-32974. Metal transport to *Medicago truncatula* nodules. MINECO
No plant is an island. Plants live in community with microorganisms that thrive around, on and within them. These microorganisms play important roles on plant growth, nutrition and health, and are key to the development of sustainable agriculture practices.

**GOALS**

- Characterization of plant microbiomes from model and crop plants (monocots, such as sugarcane, and legumes)
- Genomic and biological characterization of microbiome isolates
- Characterization of new symbiotic and non-symbiotic rhizobia present in plant rhizospheres and endospheres
- Characterization of new legume-rhizobial symbioses
- Genomic bases of plant-host preference in the legume-Rhizobium symbiosis

**GENERAL OVERVIEW**

Our group uses omic technologies to study to the microbial component of two different, but related, plant-microorganisms associations.

1. **Plant microbiomes.** The use of omic technologies is allowing the characterization of complex microbial ecosystems without the need to previously isolate its components. Among such ecosystems, the plant microbiome stands out as an important determinant of plant growth, nutrition and health. Contrary to previous beliefs, plants harbor a rich and diverse microbiome, and microbiome components can play roles in growth (eg. through plant hormone production), nutrition (eg. Through facilitation of water or nutrient [N, P, …] acquisition), and health (eg. by antagonizing plant pathogens). At the same time, thorough genomic and physiologic characterization of isolated components from the plant microbiome opens the way to designing new, improved plant inoculants that will contribute to more sustainable agricultural practices, and is an important part of our mid-term objectives. Among the components of plant microbiomes, an unexpectedly large number of rhizobia is being revealed. These rhizobia do not usually carry determinants to establish the classical legume-rhizobial symbiosis, and have been isolated from a wide variety of plants, plant rhizospheres, and agricultural soils. The characterization of this hitherto unsuspected diversity is also part of our research objectives.

2. **Legume-rhizobial symbioses.** We continue to study legume-rhizobial symbioses, often in collaboration with CBGP groups, on the characterization of hitherto undescribed symbioses (such as in the Genisteae, with Lab 251, Palacios group) or processes (metal homeostasis, with Lab 279, Gonzalez-Guerrero group). Finally, we use omics techniques to study the determinants of plant-host preference in the legume-R. leguminosarum symbiosis.
Genomics of microsymbiont selection by the plant host in the legume-Rhizobium leguminosarum bv. viciae symbioses

*R. leguminosarum* bv. *viciae* establishes efficient symbioses with members of the Tribe Fabeeae (*Pisum, Lens, Lathyrus, Vicia*). Although all bacterial isolates can nodulate all of the Fabeeae, it had long been suggested that, when given a choice, plants select specific rhizobial genotypes among those present. We have adapted population genomic technologies developed for diploid organisms to genomically study this problem (Jorrin & Imperial 2015a) and have been able to show that plants do select preferred genomic variants, although they may not be abundant in the soil (Jorrin and Imperial 2015b). Our ongoing studies show strong correlation between specific polymorphisms in the nod region and plant host preference, suggesting possible molecular explanations for this observation.

The Sugarcane microbiome

Sugarcane is one of the most photosynthetically efficient crops, and when given enough nutrients, light and water, can give yields of ca. 150 Tm biomass/ha. Despite these high yields, it has been continuously grown in some areas of Brazil (state of São Paulo) with very little fertilization, particularly regarding nitrogen, and with no apparent decrease in growth yields. On the basis of these observations, Brazilian microbiologist Joanna Döbereiner suggested that microorganisms in association with sugarcane fix atmospheric nitrogen that accounts for the high yields even with little fertilization. Although a good number of new and interesting sugarcane diazotrophic endophytes have been isolated, and although nitrogen fixation has been demonstrated at the field level, the nature and identity of the key diazotrophs has not been established. In collaboration with the Arruda group (CBMEG, Unicamp, Brazil) and with funding from Repsol we have undertaken the characterization of the sugarcane microbiome with the ultimate goal to identify and characterize the sugarcane-associated microorganisms that contribute to the crop’s sustainability. We have used amplicon metagenomics to characterize prokaryotic (16S rDNA) and fungal (ITS) components of the sugarcane microbiome. Our results (de Souza et al. 2016) show that different plant compartments (rhizosphere, endosphere, epiphytic culm and leaves, endophytic culm and leaves) harbor an unexpectedly rich and diverse microbiome, with over 23,000 bacterial OTUs and over 11,000 fungal OTUs in total. This microbiome is reacquired with every planting, is established early in the plant’s development, and maintained throughout the vegetative growth. Interestingly, many of the previously isolated and characterized sugarcane endophytes, did not appear to be major components of the microbiome. *Glucanacetobacter diazotrophicus*, in particular, which had been proposed to be the main endophytic sugarcane diazotroph, appeared to be a very minor component of the microbiome. A sugarcane microbiome community-based culture collection has been established that, contrary to classical methodologies, does not require pure cultures to be isolated. In this way, possible nutritional interactions that would potentially hamper pure culture isolation are maintained (Armanhi et al. 2016). A high throughput multiplex methodology for full 16S rDNA sequencing of community cultures using the Pac Bio RSII platform has also been established (Armanhi et al. 2016). The ca. 5,000 OTU community collection retrieved up to 90% of the bacterial component of the core sugarcane microbiome, and contains a large number of not previously described bacteria whose role on the crop’s biology is currently under study.
In collaboration with CBGP’s Lab 251 (Palacios group) we have continued the characterization of novel bradyrhizobial microsymbionts isolated from members of the Genisteeae (Lupinus, Retama, Genista, Cytisus, ... ) in the Iberian Peninsula, in Northern Africa, and in South America, a research line initiated by Emeritus Professor T. Ruiz-Argüeso, as follows:
1. Lupinus mariae-josephae, an endemism described in the Valencia region in Eastern Spain. Our work has led to the description of novel bradyrhizobia (Duran et al. 2014b, c), and to the development of bacterial inoculants to ensure propagation of the plant and, as a result, ensuring that it is no longer endangered (Navarro et al. 2014).
2. Lupinus micranthus from the Iberian Peninsula and from Northern Algeria (Bourebaba et al. 2016).
3. Phaseolus lunatus from Peru (Duran et al. 2014a).

Publications and awards

Funding
**GOALS**

- Identification of the expression program under different light conditions.
- Targeting of photoreceptor function in pathogenesis.
- Definition of the chemoreceptor/ligand profile involved in the infection process.
- Targeting of chemoreceptors involved in perception driving the entry process to the plant apoplast.
- Development of bioinformatics tools for the study of the distribution of perception domains among phytopathogenic bacteria genomes.

**GENERAL OVERVIEW**

Woody perennial found within temperate and boreal latitudes develop annual cycles of growth and dormancy in synchrony with the seasons. The study of the regulation of annual cycles of growth and dormancy has a great biotechnological importance because it would permit the optimization of the growth in addition to the adaptation to different geographic regions and to the climate change. We undertook a multidisciplinary approach to investigate the function of potential regulatory proteins involved in growth-dormancy cycles. The approach includes spatio-temporal gene expression analysis, functional studies, phenological assays, cell biology, biochemistry and genome-wide transcriptome and methylome analyses. We identified several transcription and chromatin remodeling factors that operate in response to the signals that determine the seasons, mainly day length and temperature. We established real-time gene expression methods to measure daily patterns of transcription in response to environment in poplar system. We analyzed seasonal phenology of genetic modified poplar by simulating the seasons in growth chambers. Integrating those disciplines, we found that the diurnal rhythms of HMGB activates the circadian rhythms of LHY in poplar, RAV1 transcription factor promotes axillary branching and seasonal shoot apical development, analyzing the impact of rav1-engineering on poplar biomass production in a short-rotation coppice field trial, and DEMETER like DNA demethylases control bud maturation and bud break in poplars.
Light as a signal controlling pathogenic behaviour

Bacteria inhabit the phyllosphere, where they are highly exposed to light, among other environmental factors. Light and circadian cycle regulate plant defence mechanisms. Plant pathogens might have evolved to sense light conditions associated with different levels of plant resistance. We have analyzed the presence of both blue and red light photoreceptors in a group of Pseudomonas. In addition, we have studied the effect of white, blue and red light on PsPto features related to epiphytic fitness. While white and blue light inhibit motility, bacterial attachment to plant leaves is promoted. Moreover, these phenotypes are altered in a blue-light receptor mutant. These light-controlled changes during the epiphytic stage cause a reduction in virulence, highlighting the relevance of motility during the entry process to the plant apoplast. Moreover, the analysis of the transcriptional changes driven by white light has been carried out through microarray hybridization experiments. The results highlight the relevance of this signal for the control of the expression of traits linked to pathogenesis, besides motility, as it is the case of genes involved in the regulation of the type III secretion system. Therefore, light is as a key signal used by plant pathogens to regulate “life style decisions” which, in turn, could determine the establishment of the disease. The study in phyllosphere bacteria of the effects of white and monochromatic lights on gene expression, come up as an important approach to understand this phenomenon.

Chemoperception during the infection process

Chemotaxis enables bacteria to move towards an optimal environment in response to chemical signals. In the case of plant pathogenic bacteria, chemotaxis allows pathogens to explore the plant surface for potential entry sites with the ultimate aim to prosper inside plant tissues and to cause disease.

The contribution of motility and chemotaxis to the pathogenicity of Dickeya dandantii 3937 has been studied previously in our laboratory. Their capacity to mediate chemotraction and chemorepellance in response to compounds such as sugars, amino acids and plant hormones, such as jasmonic acid (JA) has been assessed. In order to identify candidate chemoreceptors sensing wound-derived plant compounds, we carried out a bioinformatics search for candidate chemoreceptors in the genome of Dd3937. The study of the chemotactic response to several compounds and the analysis of the entry process to Arabidopsis leaves of 10 selected mutants in chemoreceptors allowed us to determine the implications of at least two of them (ABF-0020167 and ABF-0046680) in the chemotaxis-driven entry process through plant wounds. Our data suggest that ABF-0020167 and ABF-0046680 may be candidate receptors of jasmonic acid and xylose, respectively. We have also analyzed the chemotactic

Figure 1: Light treatment inhibits flagellum biosynthesis and disease (A) PAGE electrophoresis of extracellular and membrane proteins from bacterial cultures exposed to darkness (lane 1) or white light (lane 2) (B) Western blot analysis of these proteins reacted with a flagellin antiserum (C) Bacteria stained with simplified Leifson method are shown. (D) Bacterial population per unit area (bars) and number of lesions (grey points) on tomato plants were determined at 6 days post inoculation (dpi) with cells pretreated for 10 min with white light or maintained under darkness. (E) Pto population in A. thaliana Col-0 leaves at 6 dpi with Pto cells. The bacterial suspension was pretreated for 10 min with white light (70 μE/m2s) (WL) or maintained under darkness (D). The bacterial suspension treated with light was subsequently subjected to a 100-min dark treatment (WLD), and treated again with 10 min of white light (WL-D-WL). After each treatment, an aliquot of the inoculum was used to challenge plants.

Figure 2: (A) Schematic representation of the distribution of Dd3937 LBRs among bacteria according their ecological niche. Path. stands for pathogenic and environ stands for environmental. (B) Entry of Dd3937 WT and mutant strains in A. thaliana leaves. (C) Entry of Dd3937 WT and complemented strains in A. thaliana leaves. (D) Entry-dependent colonization assay in A. thaliana leaves. (E) Non-entrydependent pathogenicity assay in A. thaliana leaves.
response to a collection of plant derived compounds in Pseudomonas syringae pv tomato DC3000. Altogether, the obtained results point to the hypothesis that the chemoperception is not a bi-univocal process and more than one chemoreceptor could be involved in the perception of one compound, as well as when a certain compound could be perceived by more than one MCP.

**Bioinformatics approach to the study of bacterial pathogenicity in plants**

In parallel, we are trying to underpin the concept of bacterial pathogenicity towards plants at the genomic level, using a machine learning approach. To do so, we have developed a bioinformatics tool which identifies known pathogenicity factors from a bacterial genome. This tool, named PIFAR, is available on-line and can be applied to any number of novel genomes. Using the results provided by PIFAR we have trained a supervised machine-learning classifier that allows the identification of plant-associated bacteria with a precision of ~93%. The application of our method to approximately 9500 genomes predicted several unknown interactions between well-known human pathogens and plants, and it also confirmed several cases for which evidence has been reported. We observed that factors involved in adhesion, the construction of the plant cell wall and detoxifying activities were highlighted as the most predictive features. The application of our strategy to sequenced strains that are involved in food poisoning, for example, can be used as a primary screening tool to determine the possible causes of contaminations. Moreover we work in the development of an object-oriented programming tool to study and classify MCP chemoreceptors and light receptors from bacterial genomes. The ultimate objective is to understand the relations between these traits and the plant-pathogenic life style.

**Publications and awards**


**Funding**

AGL2012-32516. Papel de las señales de la planta y del ambiente en el establecimiento de la enfermedad causada por bacterias en plantas. MINECO.

AGL2015-6381R. La luz y las señales derivadas de la planta como moduladores delestilo de vida en bacterias.
Understanding the interaction between plants and necrotrophic fungi: deciphering the complexity of plant disease resistance responses and of fungal lifestyle determinants

**GOALS**

- SignWALLing: cell wall integrity and cell wall-derived signals regulating plant immune responses..
- Functional genomics of the necrotrophic fungus Plectosphaerella cucumerina: deciphering fungal lifestyle determinants.

**GENERAL OVERVIEW**

The group interest is the characterization of the molecular and genetic bases of plant resistance to necrotrophic fungi, a group of pathogens causing devastating diseases in crops. We use as model patho-system the interaction between Arabidopsis thaliana and the ascomycete fungus Plectosphaerella cucumerina, a pathogen that colonizes Arabidopsis plants in their natural habitats. The main goal of our group is to understand how plants sense this type of pathogens and activate immune responses to confer enhanced disease resistance. Among these immune responses are those activated by signals derived from plant cell walls. The characterization of cell wall integrity contribution to plant disease resistance and to the modulation of defensive responses are also studied by our group. In parallel, we are deciphering the molecular mechanisms determining the different lifestyle in Arabidopsis (e.g. pathogenic, non-pathogenic and endophytic) of several P. cucumerina isolates, whose genomes have been sequenced and annotated. Using comparative and evolutionary genomic studies we have identified fungal determinants explaining the differential interactions of these fungal isolates with Arabidopsis plants. The group also studies how plants shape their fungal endophytic microbiota in natural habitats and whether this type of fungi positively regulates plant physiology and fitness (http://www.cbgp.upm.es/en/natural_endophytes.php).
Fungal recognition and immune responses regulation.

Arabidopsis resistance to necrotrophic fungi is complex and depends on the interplay of different signaling pathways (Figure 1), such as those mediated by the defensive hormones (Llorente et al., 2008; Herrnández-Blanco et al., 2007; Berrocal-Lobo et al., 2002, 2008; Sanchez-Vallet et al., 2012; Denance et al., 2013). Also, non-host resistance, secondary metabolites derived from tryptophan and antimicrobial peptides play key roles in resistance to necrotrophs (Figure 1; Lipka et al., 2005; Stein et al., 2006; Bednarek et al., 2009; Sanchez-Vallet et al., 2010; Frerigmann et al., 2016; Harris et al., 2014; Yeung et al., 2016).

The group is specially focused in deciphering how plants recognize P. cucumerina and activate immune responses. Several molecular components have been identified, such as the receptor protein ERECTA (ER) and the heterotrimeric G protein (Llorente et al., 2005; Sanchez-Rodriguez et al., 2009; Figure 1). ER also regulates different developmental processes, such as stomata patterning by interacting with the ER family (ERI) paralogs, ER-like 1 (ERL1) and ERL2, TOO MANY MOUTHS (TMM) and SERKs receptors (including BAK1). We have demonstrated that this multiproteic receptosome also modulates Arabidopsis thaliana resistance to PcBMM (Figure 2) Remarkably, the secretion Epidermal Pattern Factor peptides (EPF1 and EPF2), which are perceived by ERF members, do not regulate ER-mediated immune responses further suggesting that the cues underlying ERI/TMM/BAK1-mediated immune responses are distinct (Jorda et al., 2016). We have identified a MAP3K functioning downstream ER that is a key regulator of a novel, non-canonical immune pathway whose constitutive activation (CA-MAP3K) results in broad-spectrum disease resistance to fungi, oomycetes and bacteria with different life styles (Sopena et al., submitted). CA-MAP3K plants constitutively express defense-associated genes and show alteration in their cell wall composition/structure. Heterotrimeric G protein, particularly the β-subunit (AGB1) and the two g-subunits (AGG1 and AGG2), has been recognized as important mediator of Arabidopsis thaliana immunity to different pathogens (Delgado-Cerezo et al., 2012; Jiang et al., 2012). These regulatory proteins interact with additional genetic components that modulate different plant responses, such as cell wall integrity/structure (Klopfleisch et al., 2011; Jiang et al., 2012; Delgado et al., 2013) or ROS production (Torres et al., 2013; Morales et al., 2016). We have identified novel components (sgb10:sgb13) of AGB1-mediated signaling pathway in a suppressor screen of agb1 susceptibility to P. cucumerina. The functional characterization of these sgb mutants indicates that AGB1 regulates a complex regulatory network and that novel immune responses are activated in the sgb plants.

SignWALLing: cell wall integrity and cell wall-derived signals regulating plant immune responses.

Plant cell wall is a complex structure constantly subjected to dynamic remodelling in response to internal cues and external constraints. Wall adaptation to these signals is regulated by a dedicated plant cell wall integrity (CWI) monitoring system that initiate compensatory responses to restore wall integrity. This CWI system consists of a set of wall sensors/receptors that specifically bind wall-derived ligands, so-called Damage-Associated Molecular Patterns (DAMPs), that are released upon alteration of CWI. This plant monitoring system also functions during pathogen infection, since microbes modify wall composition to favour colonization. The perception of wall DAMPs and microbial molecules (so-called Pathogen-Associated Molecular Patterns PAMPs) by specific plant Pattern Recognition Receptors (PRRs) triggers immune responses (Miedes et al., 2013; Sanchez- Rodriguez et al., 2010).
In this biased screening, a significant number of cell wall mutants (cwm) showed altered susceptibility/resistance to one or more pathogens (Miedes et al., unpublished data). Resistance and developmental phenotypes from the cwm collection and their biochemical cell wall compositions have been mathematically integrated to build a predictive model correlating specific changes in cell wall epitopes (composition) with resistance/growth phenotypes. This model indicates that modulation of CWI might be an efficient strategy to obtain crop varieties with improved resistance to biotic/abiotic stresses (Miedes et al., 2013). Novel cell wall derived DAMPs have been isolated from the cwm walls that modulate immune responses (Bacete et al., 2017 and unpublished data).

Functional genomics of the necrotrophic fungus Plectosphaerella cucumerina

We have established the patho-system P. cucumerina-Arabidopsis as a model to study the genetic and molecular bases of necrotrophic fungi pathogenicity (Ramos et al., 2013, 2015). Three P. cucumerina isolates that differ in their lifestyle and interaction with Arabidopsis have been characterized (Figure 3): PcBMM is a fully pathogenic isolate; Pc2127 does not cause necrosis in wild-type Arabidopsis plants, but infects immune deficient Arabidopsis mutants (e.g. cyp79b2cyp79b3); and Pc0831, is an endophytic isolate identified in natural Arabidopsis populations that causes not harm to the plant (Sanchez-Vallet et al., 2010; Ramos et al., 2013). The genomes of these three isolates have been sequenced and annotated, and comparative and evolutionary genomic analyses have been performed that led to the identification of specific genomic and transcriptomic features explaining the different interactions between these isolates and Arabidopsis (Munoz-Barrios et al., unpublished data). Similar studies have been performed in the interaction between the fungal endophyte Colletotrichum tofieldiae and Arabidopsis, revealing specific features related to the beneficial responses of this mutualistic fungus (Hacquard et al., 2016; Hiruma et al., 2016). Three P. cucumerina isolates that differ in their lifestyle and interaction with Arabidopsis have been characterized (Figure 3): PcBMM is a fully pathogenic isolate; Pc2127 does not cause necrosis in wild-type Arabidopsis plants, but infects immune deficient Arabidopsis mutants (e.g. cyp79b2cyp79b3); and Pc0831, is an endophytic isolate identified in natural Arabidopsis populations that causes not harm to the plant (Sanchez-Vallet et al., 2010; Ramos et al., 2013). The genomes of these isolates and Arabidopsis (Munoz-Barrios et al., unpublished data). Similar studies have been performed in the interaction between the fungal endophyte Colletotrichum tofieldiae and Arabidopsis, revealing specific features related to the beneficial responses of this mutualistic fungus (Hacquard et al., 2016; Hiruma et al., 2016). Both Colletotrichum and Plectosphaerella endophytic isolates have been obtained from Arabidopsis natural populations (see Associated Research line http://www.cbgp.upm.es/en/natural_endophytes.php)

Figure 3. A. Disease symptoms in wild-type plants (Col-0) and the immune deficient mutant cyp79b2cyp79b3 at 7 days post inoculation with either a pathogenic (PcBMM), a non-pathogenic (Pc2127) or and endophytic (Pc0831) isolate of P. cucumerina fungus. The control plants (Mock) were treated with water. B. Confocal microscopy images of wild-type plants (Col-0) and the immune deficient mutant agt1-1 at 24 hours after inoculation with PcBMM-GFP and Pc2127-GFP transformants constitutively expressing FP.

Publications and awards


Funding
- Respostas de inmunidad reguladas por el sistema de percepcion de integridad de la pared celular vegetal (WALLSENSE; BIO2015-64077-R)
- Ministerio de Economia y Competitividad (MINECO) (2015, Retos call). 327.000 €
- FunChI (PCIN-2015-142) MINECO (ERA-IB2, APCIN call).150.000 €
- SignWALL: Plant immunity regulated by cell wall integrity (FP7-PEOPLE-2013-IEF #624721). 173,370,60 €
- Resistencia de Arabidopsis thaliana a patógenos regulada por la pared celular (BIO2012-32910). MINECO (2012 call). 327.000 €
Research Group: Bacteria-Plant symbiotic associations

**GOALS**
- Mechanisms of metalloenzyme biosynthesis in endosymbiotic bacteria
- Role of protein secretion systems in the symbiosis
- Potential of novel Rhizobium-legume symbiotic systems
- Adaptations of rhizobia to specific conditions within the root nodule

**GENERAL OVERVIEW**

The Rhizobium-legume symbiosis is a non-polluting source of nitrogen for legume crops. Symbiotic nitrogen fixation is carried out by the nitrogenase enzymatic complex synthesized by endosymbiotic Rhizobium cells. A sophisticated exchange of signals is required for specific recognition and metabolic adaptation of both partners. Our lab aims at the characterization of rhizobial traits that may contribute to an improved nitrogen fixation by this system. We have characterized key steps on the biosynthetic pathway of [NiFe] hydrogenase, a metalloenzyme that increases the energy efficiency of the nitrogen fixation process. Also, we have elucidated mechanisms for the provision of nickel, an essential element for hydrogenase biosynthesis. We are also studying the role of protein secretion systems in the symbiosis through comparative and functional analysis of gene clusters encoding these kind of nanosyringes and potential effectors translocated through them. Research has also been conducted on the potential of novel symbiotic combinations based on poorly known endemic legumes that might provide new applications such as dune stabilization and soil formation in arid areas. An opening area of research at the lab is the study of traits involved in the metabolic adaptation of rhizobia to host-specific conditions within the nodule. The habitat provided by indeterminate nodules such as those form alfalfa, pea, and vetch is a tough environment in which NCR peptides from the plant contribute to turn the microsymbiont into a "metabolic slave" dedicated to fix nitrogen. Proteomic analysis is revealing novel host-specific bacterial proteins that might show new mechanisms used by rhizobia to adapt to these conditions.
RESEARCH ACTIVITY

Hydrogenase: a tool to increase nitrogen fixation efficiency

The synthesis of a hydrogen-uptake [NiFe] hydrogenase allows many diazotrophs to recycle hydrogen evolved by nitrogenase, thus leading to more efficient nitrogen fixation. Analysis of specific deletion mutants and “in silico” studies carried out in collaboration with Dr. Fernandez-Pacios have allowed the elucidation of structure-to-function relationships in *Rhizobium leguminosarum* hydrogenase-related proteins acting as enzyme subunits, chaperones, scaffolding, and oxygen-protective proteins. The combined action of these proteins allows the synthesis and assembly of metal cofactors and enzyme subunits into a mature enzyme in the presence of oxygen. Nickel is a key component in the active centre of the enzyme.

Given the low availability of this element in the environment, the cell requires efficient nickel uptake systems. Functional analysis and characterization of site-specific mutants has been carried out in collaboration with Drs. Mandrand-Berthelot and Rodrigue have led to topological, kinetic and functional characterization of the nickel transporter HupE as a energy-independent diffusion facilitator that provides nickel for hydrogenase synthesis.

Role of protein secretion systems in the symbiosis

Similarly to many bacterial pathogens, rhizobia are able to inject proteins, called effectors, into the plant cells by means of different protein secretion systems. Genomic analysis of different rhizobia (*Bradyrhizobium* sp. and *Rhizobium etli*) has allowed the identification of type III secretion systems (T3SS), structurally related to bacterial flagella in these endosymbiotic bacteria. Also we have found gene clusters encoding the components of the recently described type VI (T6SS), similar to the tail spike of the T4 phage. Using specific mutants in T3SS and T6SS we have demonstrated that these secretion systems have relevant roles on the establishment of effective symbiosis and on the definition of host range. Immunological detection with specific antisera raised against a T6SS component is allowing us to study the regulation of this system in free-living cultures and symbiotic cells of *R. etli*.

Potential of novel Rhizobium-legume systems

Analysis of new symbiotic combinations based on poorly known endemic legumes might provide new applications for the Rhizobium-legume symbiosis. A research line focused on the characterization of diazotrophic symbiosis of wild and cultivated lupin species and other legume shrubs (i.e. Retama raetam) has been established in collaboration with the group of Dr. Imperial at CBGP and with research groups from Argelia and Tunisia. This work has unveiled a huge diversity of previously unknown endosymbiotic soil bacteria. Along with host legumes, these bacteria play a key function in the reduction of soil degradation and in the prevention of desert progression. These applied objectives are combined with more basic research on the genomics of these endosymbiotic bacteria. A novel species, *Bradyrhizobium valentinum*, has been described as symbiont of the recently discovered legume *Lupinus maria-josephae*.
Rhizobial traits involved in adaptation to legume host

Using a proteomics approach we have identified a set of rhizobial proteins (including stress-responsive proteins, one transcriptional regulator and one amino acid transaminase) expressed in bacteroids in a host-specific manner. The specific role that these proteins might have in the symbiosis is being approached through generation and analysis of specific mutants. Also, the repertoire of plant-derived NCR (Nodule-specific Cysteine-Rich) peptides present in bacteroids induced by the same *R. leguminosarum* strain in pea and lentil plants has been obtained through a combination of transcriptomic and proteomic analysis. The data obtained indicate the existence of both host-specific and common components of this family of peptides, likely contributing to the different response observed in the endosymbiont. These data indicate the existence of additional levels of plant-dependent control on the bacterial metabolism inside the nodule.

![Partial heatmap of gene products differentially detected in Rlv UPM791 bacteroids from pea/lentil (top/bottom) plants.](image)

**Publications and awards**


4. Ahnia, H; Bouilla, F; Bouilla, A; Boucheffa, K; Durán, D; Bourebaba, Y; Salmi, A; Imperial, J; Ruiz-Argüeso, T; Rey, L. 2014. “Cytisus villosus from Northeastern Algeria is nodulated by genetically diverse Bradyrhizobium strains”. Antonie Van Leeuwenhoek. DOI: 10.1007/s10482-014-0173-9.

5. Durán, D; Rey, L; Mayo, J; Zúñiga-Dávila, D; Imperial, J; Ruiz-Argüeso, T; Martinez-Romero, E; Ormeño-Orrillo, E. 2014. "Bradyrhizobium paxllaeri sp. nov. and Bradyrhizobium icense sp. nov., nitrogen-fixing rhizobial symbionts of Lima bean (Phaseolus lunatus L.) in Peru". International Journal of Systematic and Evolutionary Microbiology. DOI: 10.1099/ijs.0.060426-0.

6. Durán, D; Rey, L; Navarro, A; Busquets, A; Imperial, J; Ruiz-Argüeso, T. 2014. "Bradyrhizobium valentinum sp. nov., isolated from effective nodules of Lupinus mariae-josephae, a lupine endemic of basic-lime soils in Eastern Spain". Systematic and Applied Microbiology. DOI: 10.1016/j.syapm.2014.05.002.


**Funding**

RHIZOMETAL (2011-2014), MICINN BIO2010-15301
UPM AL16-PID-05
Research Group: Biochemistry of Nitrogen Fixation

Group Leader
Rubio Herrero, Luis Manuel

Postdoctorals
Barahona Martin, Emma
Burén, Nils Stefan
Echavarri Erasun, Carlos
López Torrejón, Gema

PhD Students
Eseverri Sabaté, Álvaro
Navarro Rodríguez, Mónica
Payá Tormo, Lucía
Pérez González, Ana

Scientific Staff
Caro Bernat, Elena

Former:
Arragain, Simon
Jiménez Vicente, Emilio
Kniewel, Ryan
Verma, Hemant Kumar

Visitors
Curatti, Leonardo
Hernández, Jose Angel
Ortiz Márquez, Juan
César
Regan, John M.

GOALS
 To engineer active nitrogenase in eukaryotic organisms, with special focus on cereal crops
 To understand the biosynthesis of the iron-molybdenum cofactor of nitrogenase
 Improve biological hydrogen production by using nitrogenase directed evolution

The generation of cereal plants able to express nitrogenase, and thus of assimilating atmospheric nitrogen, has the potential of changing agriculture systems worldwide.

GENERAL OVERVIEW

Cereal crop yields are generally increased by addition of chemically synthesized nitrogen fertilizers, which are inaccessible to many smallholder farmers in Sub-Saharan Africa. Our long-term goal is to engineer varieties of cereals that require little or no nitrogen input and deliver higher and more resilient yields. This goal will be achieved by making the plants acquire nitrogen from the atmosphere rather than from synthetic nitrogen fertilizers. The strategy is to transfer to the plant the bacterial genes needed for the biogenesis of nitrogenase, the protein complex that performs biological nitrogen fixation. The direct transfer of nitrogen fixation genes to the plant has the advantage of developing technology that is in the seeds. We aim to reconstitute the nitrogenase biosynthetic pathway, delivering concepts, knowledge, tools and methodologies for engineering nitrogen fixation in staple cereal crops. Synthetic biology, plant and yeast cell cultures, combinatorial genetic transformation of cereals and model plants, and biochemical complementation assays of resulting nitrogen fixation proteins are the tools being used. In addition, my laboratory tries to understand the unique reactions taking place in the biosynthesis of the iron-molybdenum cofactor (FeMo-co) of nitrogenase. Finally, we contribute knowledge and technology to the other widely recognized biotechnological application of nitrogenase, which is the biological production of H2, a clean energy vector.
Nitrogenase Engineering in Eukaryotes

Only a small group of bacteria and archaea are capable of biological nitrogen fixation (BNF), a process by which the inert N2 is reduced to NH3 and thus is made available to all organisms. Our vision is to engineer BNF in plants by direct transfer of bacterial nitrogen fixation (nif) genes in order to express a functional “eukaryotic” nitrogenase. The most immediate obstacles to engineer BNF in plants are the sensitivity of nitrogenase towards O2 and the complexity of nitrogenase, which requires a large number of genetic parts to function optimally. We lead a consortium of researchers that are engineering BNF components in cereals and assessing their functionality. Model plants (tobacco and common bean) and yeast systems are also being used to gain relevant biochemical and physiological information to guide cereal engineering. BNF-Cereals Phase I (2011-2016) was an early stage proof-of-concept. Its main outcome was the expression and maturation of one functional, O2-labile nitrogenase component in mitochondria of aerobically grown yeast (Figure 1). By breaking through the limitation imposed by O2 in the production of nitrogenase within a eukaryotic cell, we delivered enabling technology that is instrumental to engineer nitrogen-fixing cereals. BNF-Cereals Phase II aims to engineer BNF components in higher plant organelles and to assess their functionality. Our goals in this research line are: (1) to provide concepts, tools and methodologies for engineering BNF in cereal crops; (2) to establish the functionality of all individual components of the nitrogenase biosynthetic pathway in eukaryotic cells.

Optimization of hydrogen production by nitrogenase

In the process of reducing N2 to NH3, the nitrogenase enzyme evolves H2. This old observation has encouraged researchers to investigate the applications of nitrogenase as catalyst for biological H2 production. However, major barriers to re-engineer and optimize H2 production by nitrogenase became apparent, mostly due to nitrogenase complexity and low catalytic efficiency. We have recently developed an efficient high-throughput screening to select H2 overproducers in libraries containing millions of nitrogenase variants (Figure 2). This system is being used to generate new nitrogenase (and hydrogenase) variants by in vitro protein evolution techniques. Our goal is to provide tools to help overcome these barriers and improve their use as potential catalysts for direct biophotolysis.

Understanding the biosynthesis of nitrogenase and its cofactors

Due to its great agronomical and ecological significance, nitrogenase has been subject of extensive biochemical, genetic, and structural analyses. The iron molybdenum cofactor (FeMo-co) of nitrogenase, located at the active site of the nitrogenase enzyme is ultimately responsible for BNF activity. Understanding the details of FeMo-co biosynthesis and nitrogenase assembly will improve agronomical applications of biological N2 fixation. FeMo-co is a complex metallocluster that serves as paradigm to understand the biosynthesis of simpler [Fe-S] clusters, which are ubiquitous in nature and perform basic functions in all forms of life. We aim to characterize the enzymes and metal clusters needed for nitrogenase to function efficiently. We hypothesize that the complex machinery required for FeMo-co synthesis could...
be a limiting factor to nitrogenase activity (Figure 3). We use a multidisciplinary approach to study FeMo-co synthesis. By understanding the molecular mechanisms, underlying interactions, and activities of enzymes involved in this process, we aim to establish a foundation for rational metabolic engineering of nitrogenase. Our specific research lines in this topic are: (1) to understand the biosynthesis of the central Fe-S core of FeMo-co by the SAM-radical protein NifB; and (2) to find and investigate simpler metabolic pathways for nitrogenase biosynthesis.

Figure 3. Model for the biosynthesis of nitrogenase FeMo-cofactor (Jiménez-Vicente et al. 2015).

**Publications and awards**


**Funding**

Bill & Melinda Gates Foundation OPP1143172. BNF-Cereals Phase II (2016-2020). $5,000,000.


ERC Starting Grant 205442. Towards optimization of hydrogen production by nitrogenase (2008-2014). 1,968,000 €
Molecular mechanisms regulating fungal pathogenesis in *Magnaporthe oryzae*-rice interaction

**Research Group:** Fungal pathogenesis

**Group Leader:** Ane Sesma

**Postdoctorals**
- Marie Demuez
- Marta Pérez Martín
- Julio Rodríguez-Romero

**PhD Students**
- Adriana Illana Otero
- Víctor Ortega Campayo
- Marco Marconi
- Alejandro Rodríguez Iglesias

**Technical Staff**
- Cristina Arribas de la Rosa

**Master Students**
- Fco. Borja Cuevas Fernández

**GOALS**
- Characterisation of new fungal genes required for fungal infection.
- Identification of genome-wide polyadenylation sites to understand posttranscriptional fungal networks involved in plant colonisation.
- Improve our understanding of M. *oryzae* infection process, which is an essential requirement for the development of effective and durable strategies against this devastating disease of rice and wheat.

**GENERAL OVERVIEW**

Research in our lab is focused on the molecular mechanisms underpinning *Magnaporthe oryzae*-rice interaction, particularly how *M. oryzae* can infect both aerial and underground plant tissues. The recognition of plant cell surfaces (leaves/roots) leads to the development of different infection structures (appressoria or hyphopodia) before fungal penetration. The screening of a *M. oryzae* T-DNA library on rice plants allowed us to identify organ-specific and general pathogenicity genes. Our results supported the hypothesis that hyphopodia represent primitive appressoria. Thus, the hyphopodium is likely to be an intermediate developmental step that takes place during the formation of a fully mature appressorium. The evolutionary history of the *Magnaporthaceae* family - whether the ability for root or aerial colonization came first - can give insights into the evolutionary potential of numerous important cereal pathogens. Currently, we are characterizing in more detail three genes required for *M. oryzae* plant colonisation: the karyopherin Exp5, the fungal-specific transcription factor Tpc1 and the RNA-binding protein Rbp35. We have undertaken a systems biology approach that includes transcriptomics, bioinformatics, cell biology and biochemistry to expand our knowledge on the transcriptional and post-transcriptional networks that regulate *M. oryzae* plant infection.
**RESEARCH ACTIVITY**

The Zn(II)2Cys6 transcriptional regulator Tpc1 is required for polarized growth and virulence in the rice blast fungus

Cellular polarity is an intrinsic feature linked to filamentous growth. The establishment of polarity is a critical process in pathogenic fungi, mediating infection-related morphogenesis and host tissue invasion. The onset of transcriptional events that control polar growth during plant infection are poorly understood. We have characterised the gene TPC1 (Transcription factor for Polarity Control 1) in M. oryzae, a putative Zn(II)2Cys6 transcription factor exclusive to filamentous fungi. TPC1-deficient mutants showed severe defects infection-associated autophagy, glycogen metabolism and plant tissue colonisation. By tracking actin-binding proteins, septin-5 and autophagosome components, we showed that TPC1 regulates cytoskeletal dynamics and infection-associated autophagy during appressorium-mediated plant penetration. We found that Tpc1 regulates NoxD, the p22phox sub-unit of the fungal NADPH oxidase complex. Thus, Tpc1 controls the spatial and temporal regulation of cortical F-actin through regulation of the NADPH oxidase complex during appressorium re-polarisation. Mechanistically, we provide evidence that Tpc1 is a core developmental regulator in filamentous fungi, linking the regulated synthesis of reactive oxygen species with polarity control during host invasion.

**Figure 1. Blast disease on rice and M. oryzae development on distinct plant tissues.** (A) Panicle blast symptoms in a rice field; (B) noninfected (left) and infected (right) rice panicle with necrotic lesions on (a) neck, (b) leaf blade and (c) collar; (C) typical diamond-shaped lesions with brown margins on 3-weeks-old rice leaves; (D) scanning electron micrograph showing a M. oryzae conidium (CO) that has produced an appressorium (AP) on a rice leaf surface; (E) necrotic symptoms produced by 3-weeks-old seedlings on roots; (F) confocal image of a M. oryzae conidium producing an hyphopodium (HY) during rice root colonization.

**Multilayer regulatory mechanisms control cleavage factor I proteins.**

Cleavage factor I (CFI) proteins are core components of the polyadenylation machinery that regulate alternative polyadenylation. Despite their relevant role, studies on the significance of their own regulation are scarce. Filamentous fungi contain two CFI protein classes: Rbp35/CfI25 complex and Hrp1. Proteolytic processing generates two Rbp35 isoforms, Rbp35A and Rbp35B, in M. oryzae. We have dissected in detail Rbp35 regulation and characterize two additional CFI proteins, CfI25 and Hrp1. Mutational studies reveal that Rbp35 processing occurs after its Arg-Gly-Gly domain, and together with the Met-Asn-Gly-rich region control Rbp35 localisation and function. Truncated proteins of Rbp35 are about ten-fold up-regulated, which highlights an autoregulatory role of its C-terminal domain. In correlation with this, a proteomic approach reveals that Rbp35 contributes to control protein levels of a subset of genes. Carbon depletion induces the transcription of two polyadenylated transcripts (uORF1 and uORF2) that derive from RBP35 5'UTR. Strikingly, uORF1 regulates TOR-dependent fungal growth on minimal medium. Additional characterization of other M. oryzae CFI proteins, Hrp1 and CfI25, reveals their involvement in fungal development and plant pathogenicity. Our data suggest that CFI proteins exhibit complex regulatory mechanisms, and open new perspectives in how regulation of CFI proteins, and consequently alternative polyadenylation, fine-tunes fungal gene expression in response to environmental stress.

**Figure 1. RBP35 is an RRM protein involved in fungal virulence.** (A) Δrpb35 strains show reduced disease symptoms on leaves and roots. (B) Nuclear localization signals a relocated at the C-terminus of Rbp35. Confocal images of Δrpb35 conidia complemented with different Rbp35:mRFP constructs. Nuclei are indicated by white arrowheads. DIC, differential interference contrast images. Full-length mRFP fusion construct (Rbp35-Ct) shows a nuclear localization as previously described. Mutations in the RGG module and truncations in Rbp35 C-terminal end contribute to its mislocalisation.
Using a genome-wide sequencing approach we have identified a total of 9,589 poly(A) sites in the WT strain and 10,118 in the Δrbp35 mutant. In the WT, 94% of the poly(A) sites are located in annotated 3’ UTRs, and alternative polyadenylation (APA) has been found in 1,643 genes (21.6% of all mapped genes). A nucleotide profile surrounding the poly(A) sites has identified recognition motifs that differ slightly from yeast. Interestingly, the average 3’ UTR length of Rbp35-dependent genes is significantly shorter (~250 nucleotides) in Δrbp35 compared to WT (~300 nucleotides). Our mapping analysis suggests that the recognition of the UGUA motif located 50 nucleotides upstream of the cut site by the fungal Rbp35/CfI25 complex is essential for a proper cleavage reaction. In addition, 457 genes that are alternatively polyadenylated in WT have lost one or more poly(A) sites in Δrbp35. Significantly, Rbp35 is required to avoid an inappropriate processing of pre-mRNAs containing a specific U-rich motif found 7 nucleotides upstream of the poly(A) site. In summary, this genome-wide comparative analysis has helped us to identify i) the nucleotide context surrounding poly(A) sites in fungal pre-mRNAs; ii) the potential motif recognised by Rbp35/CFI25; iii) the involvement of Rbp35/CFI25 in APA; and iv) the dual function of the Rbp35/CFI complex in the 3’ end processing of pre-mRNAs in M. oryzae.
To understand the role of the ligands of plant allergens in the induction of allergy.

GENERAL OVERVIEW

Allergies are immunological diseases that develop in two phases. In the first one (sensitization phase) the allergen is processed by antigen-presenting cells inducing an allergic profile and the phase ends with production of specific immunoglobulin E (IgE) antibodies. In the second, effector phase these specific IgE antibodies are bound to the surface of effector cells inducing the release of mediators (histamine) that produce the symptoms after contact with the allergen. While most studies on allergy have focused on the presence of specific IgE antibodies, much less is known about the sensitization phase before antibody production. Our main interest is to elucidate the molecular processes underlying the sensitization phase. The reasons why a specific protein becomes an allergen are as yet not well understood and despite considerable efforts over the years to find common features in allergenic proteins, no conclusive results have been reached. Given that the sequences recognized by T cells and IgE’s (epitopes) are within highly conserved regions and that most of the members in a protein family lack allergenic activity, other determinant factors had to play a major role in the induction of allergies. We have undertaken a dual methodological approach to investigate these determinants. By combining experimental techniques and methods from Molecular Biology, Biochemistry, and Immunology with computational methods that include Molecular Mechanics and Molecular Dynamics calculations, we are gaining insight into the decisive role played by ligands carried by allergens in the sensitization phase of allergic disease.
RESEARCH ACTIVITY

Role of allergen proteins in plant

Pru p 3 and Alt a 1 are proteins used as model systems to study allergens. Pru p 3 is the major allergen from peach and a representative member of the lipid transfer protein (LTP) family. The role of Pru p 3 in plant had remained largely unknown. We found that peach LTP is located on the stigma of pollinated flower so that its localization and timing expression suggest a role in flowering linked to pollination. The protein is also expressed in a limited period of time if peel fruit and is primarily localized in trichome secretory cells during fruit development. These results support the hypothesis that Pru p 3 might be involved in processes to inhibit second pollination in flower and herbivore feeding until seed is completely developed. Alt a 1 is a strongly allergenic protein associated with chronic asthma from the fungus Alternaria alternata and has a unique architecture with no direct structural relation. We were able to determine that the protein is firstly localized inside ungerminated fungal spores. When Alternaria germinates, the infected plant expresses plant defense proteins such as pathogenesis-related PR5 proteins and produces reactive oxygen species (ROS) as a defense response. Monomeric Alt a 1 interacts with PR5 proteins blocking their defense action and the free flavonol ligand is able to reduce ROS levels to facilitate infection.

Molecules transported by protein allergens are essential for allergenicity.

The reasons why a specific protein may become an allergen are not yet well understood. Only about 1% of known protein families include allergens and indeed not all the members of those families are allergenic. Lipidic ligands carried by allergens, or exposed together, might influence the host response to the specific allergen. Accordingly, immune-regulatory lipids could contribute in a significant manner to the allergenicity of proteins.

We have identified the ligands of both Pru p 3 and Alt a 1 proteins. The ligand of the major allergen from peach (Pru p 3) was characterized as a derivative of the alkaloid camptothecin bound to a long hydrophobic tail that corresponds to phytosphingosine. The ligand of the fungal allergen from Alternaria (Alt a 1) was identified as a catechol-containing methylated flavonol closely related to quercetin, the well-known flavonoid model compound. Complementing our in vitro work intended to elucidate the nature of these ligands, our in silico approach has allowed us to model the structures of the complexes and explore protein-ligand interactions. Structures and interactions are essential to elucidate the molecular mechanisms of the sensitization phase as well as the onset of allergic response.
Our results suggest that the lipidic segment of the ligand of Pru p 3 could act as an adjuvant with the ability to modulate the immune system towards a response characteristic of allergy. The protein could release its lipidic ligand to CD1d receptors in epithelial and dendritic cells, and the complex Cd1d-lipidic response characteristic of allergy. The protein could release its lipidic ligand to act as an adjuvant with the ability to modulate the immune system towards a mechanism of activation of immune cells.

In the case of Alt a 1, the combination of in vitro experimental and in silico computational results adds valuable information to elucidate the molecular events that occur when spores from fungi of Alternaria genus arrive at bronchial epithelium in humans. Different oligomerization states together with the effects of variations of pH on one side and the absence/presence of the flavonol ligand on the other side, are pieces of evidence that support the participation of Alt a 1 into recognition pathways leading ultimately to activation of immune cells.

**Fig. 3**

A. Structural formula of the ligand of Pru p 3: 10-hydroxy-camptothecin (red) bonded to phytophosphosine (blue) though an amide group. B. Molecular surface of the modeled Pru p 3-ligand complex. The polar end of camptothecin is exposed to the solvent while the phytophosphosine tail is buried into the hydrophobic pocket of Pru p 3. C. Structural formula of the flavonol ligand of Alt a 1 with chemical composition C20H20O7 and a quercetin moiety. Six positional isomers for methyl groups in the A-ring are compatible with this structure. Our data point to the isomer with R5 = OCH3, R6 = CH3, R7 = OCH3 and R8 = CH3 as the most likely candidate. D. Structure of tetrameric Alt a 1 protein with its flavanol ligand in the inter-subunit binding site.

**Publications and awards**


**PATENTS**


**Funding**

P090210106. Análisis de la alergenicidad y reactividad cruzada de alérgenos pertenecientes a la familia de proteínas de transferencia de lípidos. ALK-Abelló, SA.

BIO2013-41403-R. Efectos de los componentes asociados a alérgenos en el desarrollo de alergias. MICINN.

RIRAAF RD12/0013/0014 (RETICS). Red de investigación de reacciones adversas a alérgenos y fármacos. Instituto de Salud Carlos III
We use a combination of biochemistry, molecular biology, structural biology, and plant culture technology to address applied problems. Our work is mainly focused on the environmental sector and the forest sector.

**GOALS**

- Phytoremediation of persistent organic pollutants in the field (trees)
- Functional genomics investigation of pollutant metabolism in plants (trees)
- Improving tree biomass production, from management to molecular technology
- Defining novel mechanisms for heat- and water-stress tolerance in forest plantations

**GENERAL OVERVIEW**

We are interested in applied problems where trees play significant roles. One major line of research targets the natural ability of trees to clean up soil pollution (phytoremediation). We focus mainly on persistent organic pollutants, such as polychlorinated biphenyls, pentachlorophenol or hydrocarbons derived from fossil fuels. Molecular technologies are applied here to uncover novel metabolic pathways and define the roles of relevant components. We also apply these technologies under field conditions, to study natural responses in heavily polluted sites. The second line of research aims at improving yields in forest plantations, especially for bioenergy (biomass production). Our studies range from culture management to molecular analysis, both under controlled conditions and in commercial settings. As before, the goal is to identify key components and evaluate their applied interest using a biotechnological approach.
RESEARCH ACTIVITY

Tree biomass and stress tolerance

Environmental stress causes vast losses in the forest sector, with drought and heat episodes being the foremost drivers. We use different approaches to dissect natural tolerance mechanisms in species and hybrids of economic relevance, mostly poplar, walnut and chestnut. Over the last years we have identified some key genes and evaluated their applied potential through a biotechnological scheme. It covers from genetic studies in model species to research under field conditions. Our results bolster the feasibility of improving valuable genotypes for plantation forestry, an increasingly important field where in vitro recalcitrance, long breeding cycles and other practical factors constrain conventional breeding.

For example, we have obtained the first poplar lines with a substantial and durable increase in thermotolerance. Specifically, these lines accumulate a major cytosolic sHSP with convenient features. Experimental evidence was obtained linking the unique biochemical activity of such protein with protective effects under stressful conditions. Moreover, significant positive correlations were found between phenotype strength and protein accumulation. The remarkable sHSP baseline levels of our poplar lines have not been previously reported, even in model plants stressed under controlled conditions. They are not matched either in poplar populations growing under field conditions, as judged by quantitative real-time PCR and immunodetection analysis. It is noteworthy that no pleiotropic effects were found in our lines that might decrease yields in commercial plantations. Such lines also outperformed controls under other stressful conditions, including in vitro micropropagation, i.e., shoot production and ex vitro survival. The applied interest of these findings was highlighted in the On the inside section of the journal Plant Physiology.

Drought tolerance provides another good example. By combining proteomics, genetic analysis and controlled stress treatments, we have identified major responsive components in poplar trees. Besides well-known protective proteins, such as dehydrins, molecular chaperones or enzymes that attenuate oxidative stress (SOD, APX, GR and others), novel components have been pinpointed (unpublished results). Detailed characterization of several enzymes is under way. Whereas their families are widely distributed in plants and other organisms, no previous connections have been reported with drought tolerance. Our efforts are now focused on their biochemical activity and regulation under different stresses and hormone treatments as well as under field conditions. The integration of functional and phylogenetic studies suggests that at least some of these enzymes have suffered a recent process of diversification and neofunctionalization.

Phytoremediation and metabolic engineering

Environmental pollution is a first-rate problem and traditional cleanup methods are too expensive and inefficient. Phytoremediation, the use of plants and their associated microbiota, has recently emerged as an ecological and cost-effective alternative. Arguably, trees are the ideal organisms for soil and water clean-up. Overexpression to increase syllepsis in commercial elite trees without changing their wood quality. However, the syllepsis triggered by the introduction of this genetic modification appeared not to be sufficient to sustain and enhance biomass production.

Overview of the polluted area where our research group has planted over two thousand trees as part of the LIFE11 ENV/ES/000505 project. Several tanks for warship refueling appear in the background. Once the plantation was established (picture), samples were periodically taken for detailed molecular characterization: qRT-PCR analysis, enzymatic assays, RNAseq-based transcriptomic studies and metagenomic studies. Location: Naval Force Base at San Fernando, Cádiz.

We are currently interested in exploring the potential of commercial...
poplar varieties (Populus spp.) for in situ degradation of relevant pollutants, especially aromatic and aliphatic hydrocarbons derived from the fuel industry. We are also interested in halogenated compounds listed in the Stockholm’s Convention on Persistent Organic Pollutants, such as polychlorinated biphenyls, pentachlorophenol and dioxins. Our group has received several grants to conduct these studies, including recent European LIFE+ funding to evaluate different remediation technologies at field scale. Among other partners, this project involved Spain’s Department of Defense, which provided a heavily-polluted petrol plant in the naval base of San Fernando, Cádiz. We have planted there over two thousand trees, from previously-selected local species, to analyze their influence on the evolution of the pollution plume. At the same time, we have used molecular technology to analyze their natural response to various treatments and levels of petrol-derived hydrocarbons under field conditions. Besides qRT-PCR and biochemical studies, different omics approaches are under way in collaboration with the University of Malaga and its Supercomputing and Bioinformatics Center. These include (1) transcriptomic analyses of relevant genes involved in the uptake, accumulation and/or degradation of hydrocarbons and (2) metagenomic analysis of the root-associated microbiota during the remediation process.

![Image of active site of novel poplar oxidorreductase](image_url)

The nicotinamide coenzyme appears in the upper part (yellow). The figure also highlights the side chains of two active site residues essential for the catalytic mechanism (Lys, pink; Tyr, cyan). We have successfully over-expressed the enzyme from poplar in Escherichia coli BL21(DE3).

Publications and awards


Funding


BIO2013-46076-R. Estudio de la señalización por giberelinas para mejorar la germinación de las semillas y la resistencia al estrés. MINECO-Retos.

BIO2016-77840-R. Genes que regulan la germinación de las semillas y el crecimiento de Arabidopsis como herramientas para mejorar la producción de biomasa y el rendimiento de las cosechas. MINECO-Retos.
The group tries to exploit the formidable multilevel potential that plant viruses have for biotechnological developments.

GOALS

- Get further insights into the developmental trait flower stalk elongation, differentially affected by similar virus strains
- Exploitation of TuMV-derived nanoparticles for industrial and diagnostic purposes
- New biotechnological tools for the study of plant/virus interactions

GENERAL OVERVIEW

Viruses offer a myriad of opportunities to be targets or tools of plant biotechnology. On one hand, viruses are intracellular pathogens impossible to combat chemically, hence fighting against them requires different approaches frequently involving biotechnological components. A clear example is the exploitation of molecular-based technologies for virus detection, diagnosis, and/or typing. Deployment of natural or transgenic resistances to viruses also demands a wide range of molecular technologies, useful for structural genomic characterizations of plant varieties in this regard, too. On the other hand, plant viruses are organisms developed to interact with plant components. These interactions often lead to host physiological or developmental alterations, yet they have the built-in advantage of being a useful lead to understanding and potentially modifying both the interaction itself (infection symptoms), and plant physiology or development with different purposes. Finally, viruses can be efficient vehicles for heterologous gene expression in plants exploited as biofactories, and its encapsidated forms (virions) are but highly complex and stable nanostructures, the basis for development of multiple nanobiotechnological applications.
RESEARCH ACTIVITY

Infections caused by closely related viruses differentially alter plant developmental pattern

Viruses often cause symptoms in infected plants. Yellows, mosaics, or dwarfisms are well known traits associated with virus infections. But viruses are also able to alter significantly the plant developmental pattern, an effect not so well studied so far. In this work the Group of Plant Virus Biotechnology of the CBGP coordinated an international study showing how two different, but closely related viruses (strains of the same virus species), alter host plant development in almost opposite directions. A single viral protein was identified as the main responsible for these alterations in each of the strains, in addition to the plant genes altered in both processes and their position within a global plant protein interaction map. These results show the possibility of externally altering the plant developmental pattern without a strict dependence on the availability of mutants or plants more drastically modified genetically. Although this application is still not near, the results of this work show that it is possible.

Turnip mosaic virus (TuMV) is a potyvirus able to infect Arabidopsis. Different strains of TuMV differentially alter Arabidopsis traits upon infection, most plant organs being affected by the global alteration. Two TuMV strains have been studied in this regard. The cover shows effects on flower development and pollen release. The radish-infecting JPN 1 isolate is a representative of the strain deeply altering the developmental pattern, but not impairing totally, stamen growth and pollen production (right upper panel). On the contrary, Arabidopsis infection by the brassica-infecting isolate UK 1 significantly shortens stamens and completely precludes pollen release (lower panel). The left upper panel shows an uninfected flower for comparison (Sánchez et al. DOI: 10.1094/MPMI-05-15-0111-R).

Immobilization of an industrial enzyme on nanonets derived from a plant virus

Enzyme immobilization is a biotechnological practice widely exploited in different industrial areas. Recently, several nanoscaffolds are being developed for their use in enzyme nanoimmobilization. These developments derive from the interesting advantages of nanoimmobilization over more traditional immobilization approaches. In this development the lipase B from Candida antarctica was covalently immobilized on a scaffold formed by viral nanoparticles derived from a potyvirus. The specific activity of the nanoimmobilized enzyme was several times higher than the commercial enzyme's one.

An immunological marker derived from a development using plant-derived nanoparticles, applied to the diagnosis of a liver disease

A peptide from Vascular Endothelial Growth Factor Receptor-3 (VEGFR-3) was applied to uncover a significant rise of autoantibodies against the protein in serum levels, under cholestasis conditions and fibrosis in chronic cholestatic liver diseases. This rise was identified as a new marker of liver diseases. The finding is a direct consequence of a previous development by the Group.
An infectious clone of a Turnip mosaic virus isolate belonging to a radish-infecting specialized strain

Plant virus infectious clones are useful tools in the study of viral gene functions and plant/virus interactions. CBGP’s Plant Virus Biotechnology group in collaboration with the IBMCP’s Plant Virus Biotechnology group developed an infectious clone of Turnip mosaic virus JPN 1 isolate. Turnip mosaic virus (TuMV) belongs to the Potyvirus group, which includes viruses causing big economic loses in horticultural crops. No isolate of the strain including isolate JPN 1 had been successfully copied into an infectious clone so far, which limited the study of this important viral strain.

Publications and awards


Funding

RTA2013-00079-C03-01. Diagnóstico, caracterización y control del agente causal de una nueva enfermedad observada en cultivos de ajo, puerro y cebolla. INIA.
Research Group: Gene Networks in the Seed

Seeds are a model system for studies in development and stress responses and the major biotechnology target for human food supply

GROUP LEADER
Jesus Carbajosa

POSTDOCTORALS
Amir Hossain (MSCA COFUND)

POSTDOCTORALS
Vicente

PHD STUDENTS
Marcela Gomez Paez

VISITORS
Anna Tackiewicz

Scientific Staff
Jan Zouhar (Ramon & Cajal Programme)
Raquel Fernandez (Assistant Prof)
Pilar Carbonero (Emeritus Prof)

Technical Staff
Mar Gonzalez Ceballos
Marcos Morenilla Alonso

Visitors
Raquel Iglesias
Pilar Carbonero (Emeritus Prof)

GOALS
- Characterization of transcription factors responsible for seed development and seed-specific expression
- Identification of novel components of the endoplasmic reticulum (ER) stress signaling pathway
- Implementation of new methods to construct gene networks and gene function prediction

GENERAL OVERVIEW

Research activities in our group relate to the study of seed gene regulation. We have identified transcription factors (TFs) that control the expression of genes associated to maturation and germination phases in seed development. Functional characterization of conserved cis-regulatory elements present in the promoters of maturation and germination specific genes, and of TFs interacting with them allowed to establish regulatory networks. The origin and evolution of these networks is currently investigated in non-seed plants like Physcomitrella patens, whereby they might retain an ancestral function related to plant land conquer and desiccation tolerance. We are also implementing a new research line relative to the characterization of TFs involved in stress responses associated to late developmental stages of seed maturation (i.e. dehydration, hypoxia, endoplasmic reticulum (ER) stress, etc.) and their connection to abiotic stress responses in vegetative tissues.
RESEARCH ACTIVITY

Gene Regulatory Networks in Seed Development

Transcription factors (TFs) are regulatory proteins with a key role in evolution and a great biotechnological potential. They are crucial in regulating gene expression, acting in a combinatorial way and have been important targets in domestication and plant breeding. The identification of key regulatory transcription factors (TFs) involved in the maturation and germination of seeds from crops (barley) and from model plants (Arabidopsis thaliana and Brachypodium distachyon) has been a goal of our group in the past years. A number of TF genes belonging to several families, such as bZIP, DOF and MYB have been demonstrated to be important in both processes. However, it is still unclear to what extent these TF genes are conserved in monocot- and dicotyledonous seeds. The AFL sub-family of B3 TFs (VP1/ABI3, FUS3, LEC2) has been recently studied and its origin, through the course of evolution, traced back to non-vascular plants such as Physcomitrella patens. The first member of this family to be described was the maize Viviparous-1 (Vp-1) as a central player of Pre-Harvest Sprouting. Its ortholog from barley (HvVP1) has been demonstrated to play essential roles in the regulation of genes upon maturation and germination through interaction with GAMYB, BPBF and BLZ2. The study of HvFUS3 from barley unveiled a common transcriptional regulation of seed specific genes between cereals and Arabidopsis (AtFUS3).

Endoplasmic Reticulum (ER) Stress in Plants

Unfavorable environmental and developmental conditions, like seed formation with hyperaccumulation of storage proteins, may cause disturbances in protein folding in the endoplasmic reticulum (ER) that are recognized and counteracted by components of the Unfolded Protein Response (UPR) signaling pathways. The early cellular responses include transcriptional changes to increase the folding and processing capacity of the ER. We are currently investigating the following aspects of the ER-stress in plants: (a) Identification and characterization of plant components homologous to the ones described in other eukaryotic systems, (b) search for new pathway components using strategies based on gene network analyses and the use of genomic platforms available for Arabidopsis thaliana, and (c) characterization of the identified components in relation to physiological processes of agronomic and biotechnological interest, like growth under nutrientlimited conditions, senescence and seed germination. Respect to strategy (b), we systematically screened a collection of inducible transgenic Arabidopsis plants expressing a library of transcription factors for resistance toward UPR-inducing chemicals. We identified 23 candidate genes that may function as novel regulators of the UPR and of which only three genes (bZIP10, TBF1, and NFYB3) were previously associated with the UPR. The putative role of identified candidate genes in the UPR signaling is supported by favorable expression patterns in both developmental and stress transcriptional analyses.
Developmentally induced stress in the seed as its connection to abiotic stress responses in vegetative tissues

Seed development imposes particular stress conditions like dehydration or hypoxia in the late maturation phase. We have investigated the function of a gene induced during seed formation and under precise abiotic stress conditions in vegetative tissues of Arabidopsis. Transgenic plants harbouring promoter-GUS fusions were analysed to confirm the expression patterns under different stresses. Interestingly, expression of the SUBDOF gene was induced by flooding and under hypoxia treatment. Generation and phenotypic assays of Arabidopsis plants with gain and loss of function of the SUBDOF gene support its participation in hypoxia tolerance.

Publications and awards


Patents


Awards

Salvador de Madariga Fellowship as a Research visitor to Hasebe's Laboratory (NIBB, Japan)

Funding

BFU2013-49665-EXP. Boosting energy and carbon in plant systems. MINECO
BIO2014-53181-R. Endoplasmic-reticulum stress in plants: responses and effects. MINECO
OPTISOL.Optimized lignocellulose exploitation from Solanaceae Canopy. REPSOL.
PUC1566-2016-2017. Internationalization Program PUC, (Pontificia Universidad Católica de Chile).
New strategies for the improvement of crops under conditions of climate change: Identification of new genes involved in the assimilation of micro-nutrients
We make it easier for researchers to find, integrate, and rigorously analyze data - both global and local - and publish their results transparently and reproducibly, adhering to contemporary standards for excellence in scholarly activity.

**Research Group:** Biological Informatics

**Group Leader**
- Mark D Wilkinson

**Postdoctorals**
- Beatriz García Jiménez
- Alejandro Rodríguez González
- Marco Marconi

**PhD Students**
- Alejandro Rodríguez Iglesias
- Mikel Egaña Aranguren
- Adrian García
- Mario Prieto

**GOALS**
- Define the Findable, Accessible Interoperable, and Reusable (FAIR) Data Principles
- Explore automated FAIR data transformations, and their required metadata
- Explore technologies transforming narrative publications into FAIR statements
- Define and test algorithms that generate microbiome engineering workflows
- Explore the evolution of RNA Processing complexes in non- & pathogenic fungi

**GENERAL OVERVIEW**

Our laboratory follows two primary lines of investigation that span pure information sciences, and more organism-oriented bioinformatics. The FAIR Principles - Findability, Accessibility, Interoperability, and Reusability - for scholarly data publishing have been adopted by the European Commission, the American NIH, and the leaders of the G20 nations. We explore automated FAIR transformations of scientific data, and how to add "intelligence" to software such that it can discover, and accurately and autonomously use a world full of FAIR Data. Among our biological interests, there is evidence that the pathogenicity of plant fungal infections is dramatically reduced by modifying polyadenylation site selection. We therefore investigate all aspects of fungal RNA processing from an evolutionary perspective, examining the protein/domain composition and binding-sequence profiles of RNA binding proteins and complexes across the fungal kingdom. Finally, our metagenomics studies detect and predict microbial community changes over time, associated with known perturbations, with the goal of microbiome engineering.
**RESEARCH ACTIVITY**

**FAIR Data**

To fulfill the expectations of scholarly transparency and reproducibility, a broad group of stakeholders in scientific data publishing recently published the FAIR Principles to guide contemporary data representation and sharing - principles of Findability, Accessibility, Interoperability, and Reusability. Our laboratory were lead authors of this publication, which appeared in 2016 in the Nature Publishing Group journal Scientific Data (Fig. 1). The FAIR principles have been globally acknowledged, and are already becoming part of policy for research governing bodies and funders worldwide, including the European Open Science Cloud, national research cloud initiatives such as those in Australia and Africa, Horizon 2020, the NIH, and even the leaders of the G7 nations. Our laboratory is also the EU lead for the "FAIR Data Skunkworks" rapid-prototyping team, where we produce innovative software that simplifies the process of publishing and using FAIR data for both researchers and repository hosts. Through the Skunkworks process we evolved two key components:

* The FAIR Accessor - a standard, multi-layered approach to providing metadata about a repository and/or its data contents, as well as a standardized approach to describing how to access those contents.
* The FAIR Projector - a lightweight way of making traditional, non-interoperable data (such as those in spreadsheets and other 'opaque' formats) FAIR, such that it may participate in our envisioned "Internet of FAIR Data and Services".

Two prototype implementations of FAIR Accessors and FAIR Projectors have been published in peer-reviewed journals. In the first-of-its-kind prototype, we created a novel semantic description of the interactions between pathogens and hosts - the Plant Pathogen Interaction Ontology (PPIO). We then used this to transform the content of the Rothamsted/BBSRC (UK) Pathogen Host Interaction Database, and re-publish it according to the FAIR Data Principles. In parallel, we generated an extensive dataset describing the constitution and evolution of the polyadenylation machinery in the fungal kingdom, and published this in a popular public general-purpose repository, Zenodo, following the FAIR Principles. We then demonstrated that we could discover data in both of these resources, and cross-query over them using a standard query language. Finally, we are investigating how FAIR representations of scholarly assertions can be automatically extracted from the scientific literature and evaluated for their likelihood of validity. This will be accomplished through automatic detection of the "certainty" being expressed by the researcher in their narrative text. Chains of fact-citations can then be automatically reconstructed to generate a full history of each scientific claim, its associated evidence, and any points in the citation-history where the claim increased in certainty.

**Evolution of RNA Processing in Fungi**

RNA Processing occurs at many levels, involving numerous proteins, complexes, and functions spanning transcription, splicing, transport, translation, silencing, and more. Previous work by our CBGP collaborator Dr. Ane Sesma demonstrated that the pathogenicity of Magnaporthe oryzae is altered by changes in RNA Processing activities. As such, we undertook a comprehensive analysis of the evolution of RNA Processing mechanisms being available in a format that is machine-accessible; and an explicit, attached description of the conditions under which it may be reused, and how it should be cited.

![Figure 1. The FAIR Data Principles, as published in the Nature Publishing Group journal Scientific Data, describe the qualities and behaviors that should be expected from contemporary scholarly data resources. These include: having a globally unique identifier; having a clear protocol that describes how to access the data; having extensive high-quality metadata describing the content of the data, how it was generated;](http://www.nature.com/sdata/)

![Figure 2. Heatmap showing the presence/absence of specific subunits of the mRNA Splicing protein complex over a broad range of fungi spanning the majority of significant clades in the kingdom. Black regions reveal how certain components are absent from groups of related species, sometimes even spanning a widely diverse set of species. Such diagrams allow us to focus on the set of proteins that represent the "core" of that functionality, and that are shared in all species; we then contrast this with those that appear to be disposable, and attempt to interpret this in light of the lifestyle, habit, or pathogenicity of the organism.](http://www.nature.com/sdata/)
throughout the fungal kingdom, attempting to identify core components of these activities, novel components that exist in only a limited number of clades, and components that may have been lost from certain groups of species, possibly hinting at relationships between RNA Processing and the organisms' habits, ecological niche, and/or pathogenicity.

Clustering microbiome states, and predicting microbial population dynamics

We have created algorithms capable of identifying, and predicting changes to, the "state" of any microbiome as it is exposed to perturbations such as chemicals, drugs, or environmental influences (Fig.3). The novelty is that these algorithms can be applied to longitudinal (e.g. time-course) microbiome data, identifying the set of "states" (species diversity and quantity), and more importantly, the possible paths of transition from one state to another in response to perturbations. Once defined, our second algorithm can formulate a "prescription" to achieve a desired end-state (e.g. from sickness to health) - the sequence of perturbations that will drive the microbiome through a set of state transitions to the desired goal.

Figure 3. A map of the 5 possible states of the human vaginal microbiome (colors), and the transitions from state-to-state (arrows) in response to the activity of "digital penetration". In this diagram, we note that the red, blue, light green, and orange states are extremely stable (few, thin lines point from one state to another); in contrast, the thick arrows emerging from the green sector show that, given digital penetration activity, this state is particularly susceptible to changes, and there is a high likelihood of transitioning to either the red state, or the orange state. These kinds of well-defined state transitions reveal that there are a limited number of paths from one state to another - for example, an unhealthy to a healthy state - and that we can generate a probabilistic model defining the sequence of interventions that must occur that provide the optimal chance of reaching the goal state.

Publications and awards


Funding

Isaac PerellóMaríe Curie cofund Universidad Politécnica de Madrid Ministerio de Economía y Competitividad grant number TIN2014-55993-RM
Contract between Universidad Politécnica de Madrid, and the Dutch TechCenter for Life Sciences, Netherlands. Agriculture and Agrifoods Research Council of Canada, "Microbial profiling of the epiphytic microbiome using cpn60 and mPUMA"
4.2 Associated Research Lines
GOALS

Our research is addressed to elucidate phytopathogenic fungi-mycoviruses interactions, focusing the studies on Botrytis cinerea.

- Biological and molecular characterization of mycoviruses infecting the fungus B. cinerea
- Analysis of mycoviral effect on fungal gene expression and virulence
- Study the RNA silencing function as an antiviral defense mechanism in B. cinerea
- Study the origin and evolution of mycoviruses

GENERAL OVERVIEW

The grey mold fungus Botrytis cinerea (teleomorph Botryotinia fuckeliana) is a necrotrophic plant pathogenic fungus that causes enormous economic losses worldwide in pre- and postharvest crops as important as horticultural, ornamental and fruit trees, so it has turned into model of study of necrotrophic fungi with a very wide host range. B. paeoniae, together with B. cinerea, cause the grey mold disease in peonies. Fungal viruses or mycoviruses are widespread in all major groups of phytopathogenic fungi and oomycetes, and, in general, are associated with non-symptomatic infections of their hosts, but some of them decrease the virulence of the infected fungus. The discovery of mycoviruses and its use as a tool to increase the knowledge about fungi biology, as well as its possible application in biological control strategies, has stimulated its study in B. cinerea. The main goal of our research is to gain insight in the molecular mechanisms of the interaction between mycoviruses and B. cinerea, and in the implications of this interaction with the plant, in order to develop more efficient control approaches.

Using Next Generation Sequencing we have discovered and molecular characterized mycoviruses of different genera infecting B. cinerea and B. paeoniae field isolates. We have also performed the biological characterization of several them, using conventional techniques, to analyze the effect of mycoviruses on the morphology and virulence of the fungus. We have also analyzed and demonstrated that the mechanism of RNA silencing in B. cinerea and B. paeoniae acts as a
RESEARCH ACTIVITY

Discovery of new mycoviruses infecting fungi

We have analyzed two collections of B. cinerea isolates, one obtained from horticultural crops and other isolated from grapevine from the South and Center Spain, respectively, and found mycoviruses in more than 50% of the isolates examined. One of the mycoviruses found in these collections was Botrytis cinerea mitovirus 1 (BcMV1). We have performed the sequence analysis of a 376 nt region from BcMV1 obtained from 27 Spanish B. cinerea isolates and we have found high identity at nucleotide (95.5 to 100%) and at amino acid level (96.4 to 100%) in this RNA dependent RNA polymerase region, within Spanish BcMV1 population. Therefore, based in the identity values between sequences, we have considered that there are different BcMV1 isolates or variants infecting the Spanish B. cinerea isolates analyzed. The Electron Microscopy studies of partial purifications of several isolates of the fungus showed the presence of viral-like particles, indicating that some of the mycoviruses are encapsidated. We have found also some fibrillar structures in lacunae close to nuclei in some isolates. Mitochondria were hypertrophied and less electron-dense, and we also observed vesicles inside mitochondria as have been found in plants infected with viruses where the infection causes membranous inclusion bodies and an excess of lipids. In plants, virus induced vesicles are formed from cellular organelles and they have been associated with viral RNA replication sites. We have also determined the effect of some mycoviruses on the virulence and phenotype of the fungus. We have transferred a mix of mitoviruses, a genus of mycoviruses difficult to eliminate from the fungus, from infected isolates to isolates free of mycoviruses via hyphal anastomosis and we have observed similar phenotype but some modifications in the virulence of the infecte fungus. We are obtaining new fungal isolates infected with a single mitovirus to analyze the effect of each one independently.

Molecular characterization of new mycoviruses

Several groups, including ours, have reported the presence of mycoviruses in several species of the phytopathogenic fungus Botrytis, but only a few of them have been sequenced and assigned as new virus species. NGS experiments have been previously applied to search for mycoviruses using purified dsRNA as template for cDNA library construction and high-throughput sequencing. This methodology allows the detection of mycoviruses with dsRNA genome as well as dsRNA intermediates originated during replication of ssRNA mycoviruses. Since the level of dsRNA intermediates could be in some cases undetectable, we have used direct sequencing of total RNA, an approach that also permits the detection of mycoviruses with dsRNA and DNA genomes. A great advantage of this strategy is that also allows the in-parallel sequencing of host transcripts and hence, a transcriptomic analysis of fungal mRNAs during mycoviral infections. We have identified several new mycovirus from field isolate of Botrytis species infecting grapevine. One of the new mycovirus infecting B. paeoniae, Botrytis ourmia-like virus (BOLV), is phylogenetically close to plant ourmiaviruses, results that provides a new insight into the relationship between fungal and plant viruses. We have also molecular characterized a novel negative single-stranded RNA virus infecting B. cinerea.

Comparison of the sequence of Botrytis cinerea negative-stranded RNA virus 1

![Figure 1. A. Electron micrograph of a long flexuous rod shaped virus like particle in partially purified preparations from mycelium of a B. cinerea isolate. B. Fibrillar structures related with nuclear lacunae and cytoplasm vesicles. C. Hypertrophied and less electron-dense mitochondria. D. Vesicles in mitochondria.](image1)

![Figure 2. Sequence properties of Botrytis ourmia-like virus (BOLV). (A) Northern blot hybridization analysis of BOLV-specific RNA using a negative-stranded riboprobe for detecting the 3’ terminal region of the genome. Position of each band of the Millennium RNA marker (Ambion) is shown on the left. Ethidium bromide staining of the gel prior to transfer is shown as loading control. (B) Schematic representation of BOLV RNA genome and Ourmia melon virus (OuMV) RNA1 showing location of ORFs.](image2)
(BcNSRV-1) showed a strong identity with RNA dependent RNA polymerases (RdRps) of plant pathogenic emaraviruses and tospoviruses. We have also found all the molecular signatures present in the RdRp of the genus Emaravirus and in other genera of family Bunyaviridae. An ancestral state reconstruction using the conserved RdRp motifs of type members of each family of (-)ssRNA viruses indicated that BcNSRV-1 could possibly derive from an invertebrate and vertebrate-infecting virus.

RNA silencing as an antiviral defense mechanism in B. cinerea

RNA silencing is an ancient regulatory mechanism operating in all eukaryotic cells. In fungi it was first discovered in Neurospora crassa, although its potential as defense mechanism against mycoviruses was first reported in Cryphonectria parasitica, and later on in several fungal species. There is little evidence about the antiviral potential of RNA silencing in the phytopathogenic species of the fungal genus Botrytis. Moreover, little is known about the RNA silencing components in these fungi, although the analysis of public genome databases identified two dicer-like genes in B. cinerea, as in most of the ascomycetes sequenced until now. We have used deep sequencing to study the viral small RNA (vsiRNA) populations from different mycoviruses infecting field isolates of Botrytis spp. The mycoviruses under study belong to different genera and species and have different types of genome (dsRNA, (+)ssRNA, and (-)ssRNA). In general, vsiRNAs derived from mycoviruses are mostly of 21, 20 and 22 nucleotides in length, possess sense or antisense orientation either in a similar ratio or with a predominance of sense polarity depending on the virus species, have predominantly U at their 5' end, and are unevenly distributed along the viral genome showing conspicuous hotspots of vsiRNA accumulation. These characteristics reveal striking similarities with vsiRNAs produced by plant viruses suggesting similar pathways of viral targeting in plants and fungi. We have shown that the fungal RNA silencing machinery acts against the mycoviruses used in this work in a similar manner independently of their viral or fungal origin.

Publications and awards

2 - Donaire L, Pagán I, Ayllón MA. (2016). "Characterization of Botrytis cinerea negative-stranded RNA virus 1, a new mycovirus related to plant viruses, and a reconstruction of host pattern evolution in negative-sense ssRNA viruses". Virology 499: 212-218. DOI: 10.1016/j.virol.2016.09.017
3 - Donaire L, Rozas J, Ayllón MA. (2016). "Molecular characterization of Botrytis urmia-like virus, a mycovirus close to the plant pathogenic genus Ourmiavirus". Virology 489: 158-164. DOI: 10.1016/j.virol.2015.11.027

Funding

AGL2009-11778-Interacción virus-hongo-planta: virus de Botrytis cinerea, mecanismos de patogénesis y silenciamiento del RNA. MICINN
AGL2014-62178-EXP- Origen y evolución de mycovirus, Proyecto Explora Ciencia- MINECO
Under the general objective of our group aimed at identifying and characterizing the main transporters involved in K⁺ mineral nutrition and Na⁺ transport in fungi and plants, two specific objectives have recently been developed:

- Characterization of molecular mechanisms involved in Na⁺ uptake in plant roots
- Identification of transporters involved in salt tolerance of plants.

The progressive salinization of cultivated land affects over 800 million hectares (Rengasamy, 2010, Funct Plant Biol 37) and is one of the major concerns in agriculture. The processes that produce soil salinization may be complex based on climatic, soil, and groundwater conditions (Rengasamy, 2006 J Ex Bot 57) but the effects of salinity in crop plants are the same in all cases: water deficit and salt toxicity. Despite many efforts of research activity trying to characterize the Na⁺ transport and distribution along the plant, little is known about the molecular mechanisms involved in those processes. Besides, it is well accepted that a high cytoplasmic K⁺/Na⁺ ratio is a key determinant of plant salt tolerance meaning that plants with high Na⁺ accumulation in leaves are more salt sensitive. Our group has been interested in clarifying these aspects with two plant models: Arabidopsis and the bryophyte Physcomitrella patens.
RESEARCH ACTIVITY

Phenotypic approach on Arabidopsis to characterize the determinants involved in Na$^+$ tolerance

We studied the natural variability of different accessions of Arabidopsis on Na$^+$ tolerance considering the Na$^+$ toxicity evidenced by “leaf wilting” or “short root length” as quantitative traits. The difference of this approach compared to others performed earlier is that the NaCl concentrations to which the plants were exposed were not too high to avoid superimposed osmotic effects. Our results indicate that Arabidopsis plants growing in NaCl can suffer root or shoot Na$^+$ toxicities and an ionic growth inhibition, not due specifically to Na$^+$ but also induced similarly by K$^+$. These responses (root toxicity, shoot toxicity and ionic growth inhibition), were independent and showed different intensities across accessions that will be analyzed in future RIL and GWA (genome-wide association) studies addressed to identify the genes involved in those processes. (See Fig. 1)

Na$^+$ uptake by plant roots and osmotic adjustment in saline conditions

We have identified the Na$^+$ transport system that operates in roots of Arabidopsis exposed to saline conditions and that constitutes the major pathway for the accumulation of Na$^+$ in Arabidopsis shoots. This Na$^+$ transport is mediated or controlled by one or more nitrate-dependent transporters. It implies an interesting result since it is shown that the Na$^+$ uptake, considered in many cases as detrimental for plant, is coupled to nitrate uptake, an essential mineral plant nutrient. It suggests that Na$^+$, at least at millimolar concentrations studied (up to 20-60 mM NaCl), has not a toxic but an essential role. Our work also indicates that other anions such as chloride may also function in the Na$^+$ loading into the xylem and thus in its transfer to the shoots. In addition, we also have seen in Arabidopsis that, under high osmolality conditions (400 mOsm), Na$^+$ acts as an osmotic stabilizer in such a way that its uptake prevent plant water loss and shoot wilting. These results encourage us to rethink that Na$^+$ entry by roots, besides being unavoidable, can be beneficial to the plant because it determines osmotic adjustments in plants growing in saline conditions. (See Fig. 2)
Contribution of chloroplast to salt tolerance in plants

It is assumed that the main strategy to maintain a low Na\(^+\) concentration in the cytosol is its compartmentation in the vacuole. However, using Corona Green, a Na\(^+\) binding indicator, we have observed that Na\(^+\) accumulates in chloroplasts of *Physcomitrella* and that this accumulation apparently does not affect the chloroplast function. These results suggest that chloroplast might have a relevant role to control cytosolic Na\(^+\) in saline conditions. We have carried out an ultrastructural analysis of *Physcomitrella* plants exposed to saline conditions and we have observed that in these conditions, protonema cells showed a retraction of plasma membrane from the cell wall, a compaction of the cytosol, an increase in vacuolization and specially, a striking increase in starch granules numbers accumulated in chloroplasts. These results would suggest that a higher starch accumulation in the NaCl-treated plants may be important for salt tolerance. (See Fig. 3). Similar results has also been obtained in Thellungiella (Xuchu Wang et al., 2013 Mol Cell Proteom 12).

In order to know whether the NHAD chloroplast transporters have any role in salt tolerance, we have carried out the cloning, expression and functional characterization of nhad knockout mutants in *Physcomitrella*. The results indicate that NHAD transporters have not any relevant role by modifying Na\(^+\) accumulation in the chloroplast, but probably in the regulation of osmotic or pH changes occurring under saline conditions.

Fig. 3. Ultrastructure of protonema cells of *Physcomitrella patens* grown in the absence or in presence of 200 mM NaCl. Evident starch accumulation is shown in saline conditions.

**Publications and awards**


**Funding**

AGL2012-36174. Entrada de Na\(^+\) en la raíz y tolerancia al NaCl en las plantas: aplicación para su estudio de un análisis funcional y genético. MICINN.
BIO2014-56153-REDT. Red de Excelencia “Sistemas de transporte de sodio y potasio en plantas” (KNaTs). MICINN.
AGL2016-80593-R. Entrada de sodio en arroz en condiciones de salinidad. Efecto de la simbiosis con Piriformospora en la respuesta de la planta. MICINN.
BIO2016-81957-REDT. Red de Excelencia “Sistemas de transporte de sodio y potasio en plantas (KNaTs)”. MICINN.
Research Group: Ecological and molecular factors involved in fungal endophytism and pathogenesis

Group Leader: PhD Students

María Sacristán Soledad Sandra Díaz González
Benayas

Technical staff: Master Students

Palmira del Prado Diana Ramírez Zapata
Polonio

GOALS

- To find model systems for the study of the endophytic lifestyle of fungi
- To determine de ecological factors that affect fungal endophytes incidence, diversity and function in wild populations of Arabidopsis thaliana
- To find molecular determinants of the endophytic, pathogenic and mutualistic interactions between fungi and hosts
- To find mutualistic endophytes that can be used to improve the productivity of crops

A better knowledge of the principles that regulate the endophytic lifestyle will provide clues to identify common points and switches between mutualism and pathogenesis that can be used for a better disease management and to optimize the benefits and minimize the risks of using plant associated microorganisms in agriculture

GENERAL OVERVIEW

Endophytes are fungi that grow inside the plant without causing apparent symptoms of disease. The vast majority of plants of natural ecosystems are colonized by endophytes, and this situation can be beneficial for the plant in many cases. However, some of the endophytic fungi are related to latent pathogens and saprotrophs that cause severe pre and post-harvest losses. In fact, the endophytic lifestyle of fungi is poorly characterized, particularly concerning the molecular interactions established during penetration, infection and colonization of plant tissues.

The aim of this research line is to study the endophytes of natural populations of Arabidopsis thaliana in order to establish model systems to generate and test hypothesis about the general principles underlying the endophytic lifestyle. A. thaliana is not just a lab model plant: the knowledge generated in the lab is being applied to the study of its wild populations, so it has become a model, as well, for the study of plant ecology, adaptation and evolution. Thus, the availability of endophytic isolates naturally infecting A. thaliana offers a great opportunity for an integral approach to better understand the principles of the endophytic lifestyle, taking advantage of the molecular tools and the abundant knowledge accessible from the host plant.
RESEARCH ACTIVITY

Real-time monitoring of circadian rhythms

We investigate the presence, diversity and function of cultivable fungal endophytes in natural populations of Arabidopsis thaliana situated at different ecological environments at the Central Plateau of Spain. We have studied the effect of biotic and abiotic factors in the frequency of fungal endophytes in plant specimens, and in the species composition of the endophytic community. Our results indicate that the frequency of Arabidopsis plants hosting endophytes depends on the time of the year and the phenological stage of the plant, and that the probability of endophyte colonization increases as the life cycle of the plant progresses. The diversity of the endophytic assemblages of natural A. thaliana populations is high (Figure 1), and precipitation and temperature are the two main factors determining the diversity and species composition of the communities. In summary, our results indicate that the diversity, structure and composition of the fungal endophytic assemblages of natural occurring A. thaliana populations is similar to those found in surveys of other hosts in temperate regions. This was the first study of the endophytic mycobiota of A. thaliana at different ecological environments, and showed that this community can be used as a model system to improve our knowledge about the ecological function of endophytes and their contribution to plant adaptation (García et al., 2013). The isolation and establishment of culture collections of microbiota components is fundamental to conduct studies to fully understand their function and permits the application of their potential benefits. During four years of surveys, we have obtained nearly 1000 fungal isolates from surface disinfected organs of asymptomatic A. thaliana plants whose phenological and fitness related parameters have been recorded.

In collaboration with the company Plant Response Biotech S.L., we are currently testing these isolates under controlled conditions in order to determine the type of interaction established with A. thaliana plants. The most interesting isolates will be further analysed for a better knowledge of the mechanisms involved in plant colonization and the conditions that maximize their beneficial effect. In a final stage, this effect will be validated on plants of interest, such as vegetables or crops.

An important milestone of these analyses has been the discovering of the mutualistic interaction between Colletotrichum tofieldiae (Ct) and A. thaliana. We have shown that Ct increases seed production when applied to leaves of A. thaliana plants (Patent ES-2439393). We have further characterized the interaction between Ct and A. thaliana at the cellular and molecular level in collaboration with the research group of Paul Schulze-Lefert (Max Planck Institute of Plant Breeding Research, Germany), showing that root infection by Ct increases the growth of A. thaliana roots and shoots under phosphate (P) limiting conditions, (Hiruma et al., 2016, Figure 2).

Comparative genomics of Ct with related pathogens of the same genus indicates that the transition of Ct from parasitism to mutualism is relatively recent (Hacquard et al., 2016). Mutualistic behaviour is revealed by a narrowed repertoire of secreted effector proteins. We have shown that hybridization of complementary DNA (cDNA) libraries from A. thaliana plants infected with Ct and A. thaliana plants infected with the pathogenic C. incanum (Ci) identified several Arabidopsis genes with Ct-specific expression, some of which may play a role in the mutualistic interaction. We have further characterized the interaction between Ct and A. thaliana at the cellular and molecular level in collaboration with the research group of Paul Schulze-Lefert (Max Planck Institute of Plant Breeding Research, Germany), showing that root infection by Ct increases the growth of A. thaliana roots and shoots under phosphate (P) limiting conditions, (Hiruma et al., 2016, Figure 2).

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proteins and a limited activation of pathogenicity-related genes in planta. Transcriptomic analyses indicate that beneficial responses are prioritized in Ct colonized A. thaliana roots under phosphate-deficient conditions, whereas defence responses are activated under phosphate sufficient conditions. The host phosphate starvation response system (PSR) controls Ct root colonization and is required for plant growth promotion (Figure 2).

Plant growth promotion also requires PEN2-dependent indole glucosinolate metabolism, a component of innate immune responses, indicating a functional link between innate immunity and the PSR system during beneficial interactions with Ct (Figure 2). The nutrient status of the plant might have facilitated the transition from pathogenic to beneficial lifestyles of this fungus, which is in fact replacing the function of mycorrhizas in Arabidopsis. Ct has been found in different parts of the world, but its association with A. thaliana has only been found in the phosphorus-poor soils of the wild populations of A. thaliana in Central Spain. Other interesting endophytes isolated in our surveys are closely related to the species Plectosphaerella cucumerina. P. cucumerina is well known as a plant pathogenic fungus that naturally infects A. thaliana and has been deeply studied as model for necrotrophic interactions by the “Plant Innate Immunity and Resistance against Necrotrophic Fungi” group at the CBGP. Within this group, we are carrying out the phenotypic and genomic comparison of endophytic, pathogenic and non-pathogenic isolates of P. cucumerina (Figure 3). This study will bring more information about the genomic and functional signatures that determine the endophytic or pathogenic lifestyles.

Publications and awards


Patents

ES2439363 B2. Método para incrementar la producción de flores, semillas y/o frutos en una planta. Authors: Mª Soledad Sacristán Benayas, Evelin Eliseth Cueva González, Ángela Alonso González. Titularity: Universidad Politécnica de Madrid. Extended to EPO countries; Argentina; Australia; Brasil; Canada; Mexico; USA. Licensed by Response Biotech S.L.

Funding

The challenge of feeding a global population cannot be achieved without major improvements in both water and nutrient efficiency. Irrigated agriculture is a major user of freshwater resources and contributes significantly to food production. Simultaneous application of water and nutrients requires careful management.

GENERAL OVERVIEW

In nature, plants during development usually have to deal simultaneously with multiple nutrient stress conditions. Currently research has been limited to responses to individual stresses, and understanding of adaptation to combinatorial stress is reduced, but indicative of non-additive interactions. A number of transcriptomic and metabolomic analysis and functional characterization of individual genes has discovered a convergence of signaling pathways for water stress and nutrient stress adaptation. Our research line is focus on the study of regulatory networks and signaling pathways involved in plant responses to adverse conditions such as limitation of nitrate and sulfur and to identify new target genes that can be used in a new generation of improved biomass-breeding programs in crops. The tolerance to this kind of stresses is a very complex phenomenon, involving a metabolic adjustment that implies changes in nutrient use efficiency, partitioning of assimilates and changes in plant organs such as shoot and root architecture and cell structures as the plant cell wall and endoplasmic reticulum. Using forward genetic screenings and systems biology approaches we have identified a set of regulatory factors that might be involved in metabolic adjustment in response to those nutrition limitations.

GOALS

- Determination of plant molecular mechanisms and strategies involved in the response dehydration and Nitrogen and Sulphur deficiencies
- Identification and molecular characterization of Factors that control plant responses and crosstalk between dehydration stress and Nitrogen and Sulphur deficiencies
- Establish new methods to construct regulatory networks that improve the analysis of abiotic stress responses in model plants and crops.
- Understanding plant drought and nutrient responses in extremophiles plant species
RESEARCH ACTIVITY

Plant Responses to water and nutrient stress conditions

Nutrient limitation has become a major environmental stress that restricts plant growth and productivity. Nitrogen and Sulphur use efficiency and biomass are multigenic traits that involve several metabolic processes in different plants organs that are essentially based on C and N/S compounds. Although we have an accurate picture of the primary metabolism of these compounds, little is known about their regulation and coordination at the plant level. Using forward genetic screenings and systems biology approaches we have identified a set of Arabidopsis transcription factors that might be involved in metabolic adjustment in response to Nitrogen and Sulphur limitations. Wide Genomic and syntenic analyses allow us identifying putative orthologous genes in dicot and monocot species such as tomato and maize, respectively. Remarkably, overexpression of putative orthologous transcription factors in the model plant Arabidopsis, promotes a significant increase in biomass production and tolerance to several nutrient deficiencies.

Identification of regulatory factors involved in nutrient remobilization and ER/autophagy stress responses.

In nature, plants usually have to deal with multiple stress conditions such as drought and nutrient starvation that may cause disturbances in protein folding in the endoplasmic reticulum (ER) thus promoting ER stress in plants. As a consequence, Unfolded Protein Response (UPR) signaling pathways are triggered, which may ultimately lead to programmed cell death and autophagy. Interestingly, early cellular responses include transcriptional changes to increase the folding and processing capacity of the ER, and eventually autophagy. We systematically screened a collection of inducible transgenic Arabidopsis plants expressing a library of transcription factors for resistance toward UPR-inducing chemicals. We identified 23 candidate genes that may function as novel regulators of the UPR.

The putative role of identified candidate genes in the UPR signaling is supported by favorable expression patterns in both developmental and stress transcriptional analyses. We demonstrated that WRKY75 is a genuine regulator of the ER-stress cellular responses.

CDFs factors integrate flowering time and abiotic stress responses

Plants have developed sophisticated molecular, biochemical and physiological mechanisms to adjust growth according to the availability of resources and to environmental conditions. Detailed transcriptomic analyses using data of Arabidopsis plants exposed to different nutrient deficiencies allow us to identify a group of factors known as CDFs that might play a central role in abiotic stress responses. In particular, we identified the roles of CDF3, in abiotic stress responses and developmental processes like flowering time. The CDF3 T-DNA insertion mutant cdf3-1 is much more sensitive to drought and low temperature stress, whereas CDF3 overexpression enhances the tolerance of transgenic plants to drought, cold and osmotic stress and promotes late flowering.

Transcriptome analysis revealed that CDF3 regulates a set of genes involved in cellular osmoprotection and oxidative stress, including the stress tolerance transcription factors CBFs, DREB2A, and ZAT12, which involve both GIGANTEA dependent and-independent pathways.
Novel way of representing network systems to identify master regulatory genes.

In order to find new methods to identify new regulatory factor involved abiotic stress responses we investigate new ways to represent networks. Here, we introduce a novel way of representing as networked systems such collections of isolated, possibly heterogeneous, scalars. The final result is the creation of a network for each subject, where nodes represent features, and links are weighted according to the deviation between the values of two features and their corresponding typical relationship within a studied population. The result is what we term a parenclitic network representation, the Greek term for “deviation”. Such a representation allows defining a system the identity of which parts and relationships are continuously “deviated” in a context dependent manner. We apply our method to investigate gene expression in the response to abiotic stress condition such as drought responses of the model Arabidopsis. Network reconstruction method and in vivo experiments allowed identifying 15 previously unknown key regulatory genes, and provided models of their mutual relationships.

**Publications and awards**


7. "Patents


**Awards**

Salvador de Madariga Fellowship as a Research visitor to VIB Center Belgium (2013-14).
Salvador de Madariga Fellowship as a Research visitor to the Catholic University of Chile (2016).

**Funding**

RTA2012-0008-C02 Transcription factors as a tool to increase production, biomass and tolerance to abiotic stresses in solanaceae species. INIA.
OPTISOL.Optimización de la Producción de lignocelulosa solanaceae canopy. PROGRAM “Inspire” REPSOL.
PUC1566-2016-2017, Internationalization Program PUC. (Pontificia Universidad Católica de Chile).
New strategies for the improvement of crops under conditions of climate change: Identification of new genes involved in the assimilation of micro-nutrients
4.3
Young Investigator
Research lines
Our research aims to establish a solid foundation for a mechanistic understanding of how epigenetic phenomena regulate key developmental processes in plants. This will allow enhancement of crop performance by modulating agronomic traits at the molecular level and will be to address the problem of food security.

GOALS

- Unveil the epigenetic regulation of plant development by histone demethylases.
- Profile the epigenomic landscape of histone marks in plants.
- Identify novel crop traits controlled by epigenetic mechanisms.
- Transfer knowledge from model plants to Brassica crops systems.

GENERAL OVERVIEW

Epigenetics, the study of heritable changes in gene function that take place without alterations in the DNA sequence, is a relevant topic nowadays. Epigenetic mechanisms, such as DNA methylation or histones post-translational modifications, are crucial for the proper development of eukaryotic organisms like plants. One of the most fascinating developmental responses induced by the environment is vernalization, the acceleration of flowering time in response to winter cold. This and other epigenetic responses are important crop traits and relevant targets for molecular breeding programs. In the lab we work with the model plant Arabidopsis thaliana and related crop species. Arabidopsis belongs to the Brassicaceae family, which includes several important vegetable cultivars like cauliflower, broccoli, mustard, and rapeseed, which is the third source of vegetable oil worldwide. The long-term objective of our research is to translate all the current knowledge on epigenetic regulation from Arabidopsis to its close Brassica crops relatives. We are currently studying the epigenetic regulation of important agronomic traits, like flowering time, in Arabidopsis and oleseed *Brassica rapa* cultivars. We are performing genetic analyses of mutant lines together with genomics and epigenomics approaches to characterize the role of key epigenetic factors that govern plant development. The obtained results will improve our knowledge about the epigenetic mechanisms regulating key developmental traits, and will help us to increase crop yield by engineering new Brassica varieties.
RESEARCH ACTIVITY

Epigenetic regulation of flowering time: role of the histone demethylases ELF6 and REF6

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. Our main research aim is to deeply characterize the role of the histone demethylases ELF6 and REF6 in Arabidopsis and B. rapa R-o-18. We showed that the histone lysine demethylase ELF6 is key epigenetic factor controlling flowering time in Arabidopsis. Mutant elf6 plants are early flowering and are impaired in the epigenetic reprogramming of flowering time after vernalization. Arabidopsis plants deficient in REF6, the ELF6 paralog, show late flowering time and other developmental alterations. Jumonji proteins ELF6 and REF6 counteract the H3 lysine 27 trimetylation (H3K27me3) activities of repressive Polycomb complexes during plant development. We also characterized an allelic series of B. rapa R-o-18 tilling mutant lines for ELF6 and REF6 homologs. Our results showed that Bra.elf6 and Bra.ref6 mutant lines showed altered flowering time response and plant architecture (Figure 1). To characterize the molecular mechanism responsible for the observed phenotypes, state-of-the-art next generation sequencing approaches are being performed to determine the genes regulated by these epigenetic factors. In addition, we are characterizing the dynamics of a key epigenetic regulatory mark, H3K27me3, across B. rapa development and in response to environmental stimuli.

Figure 1. Histone H3K27me3 demethylase mutant tilling plants show delayed flowering time initiation when compared to wild-type Brassica rapa R-o-18 plants.

Interplay between Arabidopsis SWR1 and NuA4 chromatin remodeling complexes: role of YAF9 and SWC4 proteins.

We characterized the role of the Arabidopsis YAF9 and SWC4 proteins within the “Molecular Bases of Plant Developmental Phase Transitions” group. SWR1 complex regulate histone H2A.Z deposition whereas NuA4 complex is a histone acetyltransferase complex. Yeast Yaf9 and Swc4 are shared subunits between SWR1 and Nu4 complexes. Genes encoding for these two proteins are conserved in most eukaryotes, and Arabidopsis genome contains one gene encoding for SWC4 and the two YAF9 homologs, YAF9A and YAF9B. Therefore, to understand the interplay between SWR1 and Nu4 complexes in plant development, we studied in detail Arabidopsis SWC4 and YAF9 subunits using a combination of molecular genetics, chromatin biology and biochemical approaches. By proteomic studies we found that SWC4 and YAF9 proteins are bona fide components of the Arabidopsis SWR1 complex and that they interact with Nu4 complex subunits, suggesting the existence of this complex in plants. Phenotypic characterization and genetic analyses of loss-of-function mutant lines revealed that SWC4 and YAF9 regulate multiple aspects of plant development including flowering time. Transcriptomic analyses and ChIP immunoprecipitation followed by sequencing showed that these chromatin factors regulate H2A.Z function at key developmental genes. Our data define a novel regulatory mechanism of plant gene expression by H2A.Z deposition (Figure 2).

Figure 2. Working model for the role of SWC4 in the SWR1-C mediated deposition of H2A.Z at target loci. SWR1-C catalyzes ATP-dependent deposition of H2A.Z, but the recruitment mechanism of SWR1-C to promoter regions remains unclear. In Arabidopsis H2A.Z is preferentially located in the transcription start site but lacks upstream H2A.Z nucleosomes and H2A.Z occupancy at nucleosome +1 is inversely correlated with gene expression levels. Our results demonstrate that Arabidopsis SWC4 is able to bind DNA, recognizing preferentially AT-rich DNA elements that are overrepresented in the promoters of a subset of genes where H2A.Z eviction enhances transcription.
Publications and awards


Awards

Marie Curie Intra-European Fellowships (SP3-PEOPLE 298790 Flowering Chromatin: Control of flowering time by chromatin remodelling)

Ramón y Cajal Fellowship (RYC-2103-14689)

Funding

SP3-PEOPLE 298790 Flowering Chromatin: Control of flowering time by chromatin remodelling.
RYC-2103-14689 Ayuda Contrato Ramón y Cajal.
BIO2015-68031-R REGULACION EPIGENETICA DEL TIEMPO DE FLORACION EN CULTIVOS OLEAGINOSOS DE BRASSICA.
Stable and high expression of transgenes is essential for the development of any genetically engineered crop. We work in the design of synthetic constructs that maximize transgene expression while minimizing variability amongst lines due to gene silencing.

GENERAL OVERVIEW

RNAi pathways are responsible for silencing transgenes as a result of the activation of plant defense mechanisms evolved to confront invasive nucleic acids such as transposons and viruses. The key questions we want to address in our lab are how do plants distinguish “self” from “non-self” nucleic acids and what are the signals in the transgenic transcriptional units that license the silencing response. Our approach consists on designing synthetic constructs with different features regarding insulator sequences, promoters, transgenes and terminators and analyzing the effect of each of these elements in expression level and stability. The identification of the signals responsible for silencing and the development of methods to avoid them will have a big impact in plant biotechnological applications, making it possible to obtain higher yields of recombinant protein production in molecular farming and to simplify the complexity of gene expression regulation in crop improvement exploits.
**RESEARCH ACTIVITY**

**Efficient ways to avoid positional effects on transgene expression**

It has been known for many years that the structure of a transgenic locus and the state of the chromatin in the site of its integration can have a major influence on the level and stability of transgene expression. Genetic insulators are sequences that function flanking transgenes to shield them from outside signals preventing inappropriate activation or repression of expression by nearby regulatory elements (Figure 1). Many of these sequences have been described and tested for different species and in different conditions. We have performed the first systematic study in Arabidopsis of 5 insulator sequences and are working on analyzing the characteristics and effectiveness of each of them in providing a higher and more stable transgene expression.

**Role of promoters and terminators in the initiation of gene silencing**

Even though the events that occur after the establishment of silencing and that help maintain it are very well known, the steps that initiate this pathway in response to transgenes remain unknown. We have evidence that shows that different transgenes license different silencing responses, even when they are located in the same region of the host genome (Figure 2). This suggests that the composition of the transgenic transcriptional unit might be determinant in triggering or evading silencing.

**Figure 1:** Scheme showing how genetic insulators can shield transgenes from outside signals preventing positional effects caused by heterochromatin spreading from the integration site in the genome.


In particular, promoters seem to have a key role in the recognition of exogenous DNA by the host plant and the initiation of transcriptional gene silencing. We have generated a tool that allows us to measure and compare the level of transgene silencing induced by different constructs by measuring luciferase expression and activity. This assay provides a sensitive method for the accurate quantification of small changes in transcription and translation. By using this tool, we are analyzing a set of almost 50 promoters with different features in Arabidopsis thaliana, in the search for signals responsible for the plant recognition of transgenes and the licensing of their silencing. In the case of terminators, it seems that an inefficient termination of transcription can lead to the generation of aberrant RNAs, preferred substrates for RDR proteins to generate double-stranded RNA and develop post-transcriptional gene silencing. A plausible scenario is that cap-, poly(A)- and other RNA-binding proteins normally prevent RDRs and RNA-silencing proteins from interacting with mRNAs, but that in misprocessed RNAs with aberrant characteristics siRNAs can be produced by the RNA-silencing cofactors. We have generated lines with different terminator sequences or no terminator at all and plan to test this hypothesis in them.

**Figure 2:** Left panel, scoring of Hygromycin resistance in 3 lines of plants transformed with p35S::Fluc::FUS3short. Right panel, LUC activity scoring of a mature leaf of plants of the same lines. Note that all seedlings show resistance to Hygromycin while the levels of LUC expression are very variable, suggesting different levels of silencing for the two different transgenic transcriptional units.
Suitability of plastids to host oxygen-sensitive nitrogenase proteins

We are part of a consortium led by Professor Luis Rubio to investigate the transfer of nitrogen fixation genes to plants with the aim of obtaining cereals with minimum requirements of nitrogen fertilizers that will produce higher and more consistent returns on their crops. Our part within this initiative consists on exploring the transfer of bacterial nif genes to plastids through nuclear transformation. In complex approaches like this where coordinated expression of multiple genes is required for stoichiometric synthesis and assembly of proteins in a pathway, gene silencing is an especially worrisome problem since its effects do not only negatively impact the final yield of protein production, but can also impair the whole functioning of the pathway. Therefore, it is of vital importance to develop effective strategies to achieve uniform and predictable expression of nitrogen fixation genes.

The nitrogen fixation genes contain a very high GC content (due to their bacterial origin) and no introns, a feature shared with transposons, which are prime targets for gene silencing. We have generated different synthetic designed and intron-containing versions of nifH, nifM, nifU and nifS and are searching for the ones that allow for higher and more stable transgene expression (Figure 3).

Figure 3: Pipeline for the creation and selection of the more stable nif transcriptional units. The performance of different variants assembled with the MoClo modular cloning system will be tested and compared for expression in BY2 cells.

Publications and awards


2. Ana Pérez-González was awarded with a UPM pre-doctoral fellowship “Ayuda para la realización del Doctorado” (Programa Propio UPM).

Funding

GOALS

- How do plants locate new roots at specific positions?
- How do plants root de novo?
- How do plants form and pattern new roots from founder cells?
- How do plants regenerate root organs after chemical or physical wound?

GENERAL OVERVIEW

Multicellular organisms are made up of organs that are critical for survival and perform specific functions: reproduction, water and nutrient uptake, photosynthesis, etc. Plant embryos, unlike animal embryos, only contain primary organs. However, most organs at a plant’s adult or reproductive state are newly made after embryogenesis. Thus, in order to complete their life cycle, plants need to develop postembryonic organs for nutrition, anchorage, reproduction, etc. We use as a model the root of the plant Arabidopsis thaliana. This is an excellent model because we can observe morphogenetic processes in vivo. Positioning of organs determines plants’ morphology. We have shown that organ positioning requires a developmental clock: the Lateral Root Clock (Moreno-Risueno et al., 2010), which uses auxin hormone and periodic oscillations of gene expression (~6 hours) to reprogram cells that will first become prebranch sites and subsequently organ founder cells. The genetic and molecular bases of cell selection and reprogramming remain largely unexplored. We have identified gene mutants with altered positioning of postembryonic roots (Figure 1). We are currently studying the interaction of these genes with the Lateral Root Clock and auxin signaling. Root founder cells divide following certain rules and organizing principles to form new organs. An unexplored key aspect of this process is how new tissues and cell types are formed and arranged following specific patterns, namely the first divisions of root founder cells. Part of our research involves marking certain cells in vivo with fluorescent markers, isolating those cells and studying their developmental programs. We are also studying how roots form new tissues after chemo-toxic treatment as part of their regenerative mechanism. We investigate the role of certain factors in establishing cell lineages and tissues from stem cells. Finally, green parts of plants, such as leaves, are mechanisms shared with other morphogenic developmental processes. able to root. We are investigating the role of auxin signaling and cell-type specific factors in forming these new roots, aiming to discover if there are common mechanisms shared with other morphogenic developmental processes.
RESEARCH ACTIVITY

POTENT sets the pace of oscillating gene expression to position postembryonic (lateral) root founder cells

Organogenesis starts with the formation of organ founder cells. These cells have the developmental potential of giving rise to all distinct tissues which make up an organ. Plants form organs continuously and in Arabidopsis thaliana, we have found that (lateral) postembryonic root positioning is dependent on oscillating gene expression, which is part of a developmental clock, the Lateral Root Clock. Some gene expression oscillations (so called in phase) associate with periodic auxin biosynthesis at the root tip to precede static sites of DR5 expression (prebranch sites), from where new roots will be formed through the specification of founder cells. To gain further insight into morphogenetic regulators of oscillating gene expression and founder cell specification we have generated plants carrying specific cell identity markers and have performed a mutagenesis screen. In one of these mutants, which we named potent, many pericycle cells change their identity becoming organ founder cells, which results in overproduction of lateral roots. Our results indicate that POTENT is required to integrate auxin signaling into oscillating gene expression. Specifically POTENT, which is degraded by auxin, interacts with an AUXIN RESPONSE FACTOR oscillating in antiphase that is required for correct positioning of founder cells. POTENT functions as a transcriptional repressor of in-phase gene expression as shown by RNA sequencing data and therefore we propose that a double repression mechanism is required to set the pace of organ founder cell positioning. Future modeling of this pathway could shed light into how specific genetic modifiers translate auxin information into organ positioning.

The pericycle tissue gives rise to lateral root founder cells (LRFC) through a reprogramming process, and subsequently, distinctive cell fates are specified through asymmetrical divisions. Morphogenesis of lateral roots initiates with the asymmetric division of LRFC to generate small and large cells. These divisions require external inputs (auxin hormone) and are driven by intrinsic cues (such as polarity and nuclear migration). The mechanism(s) regulating these developmental transitions and specifying different cell fates is not well understood. We hypothesize that self-organizing properties of founder cells are controlled by a regulatory network which incorporates external cues such as auxin. Trough Fluorescent Activated Cell Sorting (FACS) we will be able to know the expression levels of genes in pericycle, lateral root founder cells and its daughters. To these end, we have already generate a range of plants carrying cells markers and perform RNA sequencing after FACS of the cell types of interest: a) pericycle cells, b) founder cells, c) daughter cells, d) daughter cells with auxin signaling, e) small daughter cells and f) organizing stem cells. This approach will define the regulatory program between crucial developmental stages (pericycle, lateral root founder cells and its daughters) associated to root organ morphogenesis addressing how two distinct fates are specified from a single cell. We expect that our approach provides novel relationships between pluripotency and cell identity.

Profiling the gene expression programs of lateral root morphogenesis and patterning from founder cells.

FIGURE 1. Rooting of Arabidopsis roots. A) Maxima in auxin and gene expression commit cells (prebranch sites) to be specified as founder cells. Scheme of potent and arf mutant phenotypes. B) Prebranch site (*) number is altered in potent mutant as marker by imaging of DR5::Luciferase. C) Overproduction of lateral roots (*) in potent mutant as marked by fluorescent reporters

FIGURE 2: Cell type specific transcriptional programs during postembryonic root organogenesis. Cell types during initiation and patterning of lateral root formation have been marked using promoter fusions to the mCherry and Green Fluorescent Proteins. Cell types of interest have been isolated using Fluorescent Activated Cell Sorting followed of mRNA profiling using Hi-Seq RNA sequencing. Gene specific expression to developmental stages will be identified using bioinformatics and statistical analysis.
A developmental framework for organogenesis and cell differentiation during rooting Arabidopsis thaliana leaves

Rooting of aerial cuttings is a morphogenetic process that occurs naturally without exogenous hormone supply in many plant species. Although some species of economic importance are propagated in vitro from leaf cuttings, the underlying morphogenetic mechanisms are not well understood. We have investigated the developmental changes occurring during rooting of whole leaves in the Arabidopsis thaliana leaf model. Our results showed that rooting requires proliferation of some vascular-associated tissues, primarily those expressing the J0121 marker, as well as cytokinin and auxin biosynthesis, transport and signaling which leads to the formation of a wound-induced callus with rooting competence. We have identified specific auxin and cytokinin signaling factors required for rooting such as IAA28, SOLITARY-ROOT-IAA14, CRANEIAA18, WOODEN LEG, and RESPONSE REGULATORS (ARR) 1, 10 and 12. Our data showed that wound-induced callus formation precedes the specification of postembryonic root founder cells, and must require a reprogramming process shared with lateral root development, although with obvious morphological differences. We found that root initiation requires SHORT-ROOT and PLETHORA pathways, as rooting is blocked in shrplt1plt2 mutant. Interestingly, shrplt1plt2 form functional primary roots. Finally, we identified additional stem cell regulators required for normal patterning of adventitious roots, such as SCHIZORIZA, JACKDAW, BLUEJAY and SCARECROW, likely through the regulation of lineage-specific stem cell specification. Our work highlighted key stages and regulators, providing, therefore, a developmental framework to further understanding de novo organ formation in plants.

FIGURE 3. Organ positioning in Arabidopsis roots requires stem cell regulators. A) Scheme of Arabidopsis rooting. Leaves were excised and grown on hormone-free medium. New roots are visible after 7-8 days. B) shr1 plt1-4 plt2-2 leaves do not show visible roots after 10 days of culture. C) Confocal imaging show that shr2 plt1-4 plt2-2 leaves do not initiate root primordia at 6 or even 19 days of culture.

Publications and awards


Funding

A MOLECULAR, GENOMICS AND GENETIC ANALYSIS OF NEW STEM CELL FORMATION DURING ROOT MORPHOGENESIS. FP7-PEOPLE-2012-CIG- 322082- Curie Integration Grant. UE. 2012-2016.
SPECIFICATION OF NEW STEM CELLS IN THE ARABIDOPSIS THALIANA ROOT. Ministry of Science and Innovation –MICINN- Programa Ramon y Cajal. RYC-2011-09049.
Research Group: Environmental control of plant development

GOALS

- Understanding the signal transduction pathways in response to photoperiod and temperature in poplar.
- Identification of new seasonal regulators of axillary and apical shoot growth in poplar

We study plant growth by a developmental and environmental perspective aiming to identify new strategies to improve aerial biomass yield and fruit production in crops.

GENERAL OVERVIEW

Plant development must be coordinated with the environment to optimize growth and survival. We investigate how the photoperiod and the temperature control plant axillary and apical shoot growth. We are interested to identify new signal transduction pathways and transcription regulatory networks potential targets for biotechnological application in crops.
RESEARCH ACTIVITY

Signal transduction pathways in response to the environmental cues

Plants continued sense the environment modulating growth and development. Photoperiod provides reliable information for seasonal growth regulation. When day length fall below a certain threshold, poplars cease growth. Levels of Flowering Locus T (FT) has been shown to be necessary for the photoperiodic control of shoot apical growth. We found that FT mRNA levels quantitatively response to night length (Fig. 1a and b). Through molecular genetics and genomics, we are investigating the signal transduction pathway that transmits night length information to FT, which is critical for poplar trees survival.

Identification of new seasonal regulators of axillary and apical shoot growth in poplar

Seasonal control of shoot axillary and apical bud outgrowth in woody species require specific genetic programs integrated to the environmental signals. This trait has direct influence in plant shoot architecture and it is positively correlated with high biomass yields in poplar plantations. Earlier work has shown that overexpression of the circadian RAV1 transcription factor in poplar exhibits dormant axillary bud outgrowth or syllepsis without any visible impairment of wood anatomy. Our goal is to decipher the regulatory principles of dormancy eluding and the seasonal control of axillary and apical shoot development through the understanding of the gene network of the circadian gene RAV1 in poplar.
Publications and awards


Funding

AGL2014-53352-R Desarrollo de herramientas genéticas para modular los ciclos de dormanciacrecimiento en especies leñosas MECYT 2015-2018

PCIG13-GA-2013-631630 Underpinning the significance of circadian clock winter disruption in Poplar FFP7-PEOPLE-2013-CIG-European Commission

RYC-2012-10194 Ramón y Cajal programme of MINECO
Research Group: Determinants of plant virus transmission and speciation

Group Leader
Jesús Israel Pagán Muñoz

PhD Students
Cristina Rodríguez Nevado
Viji Vijayan

Master Students
Alberto Cobos Piñuela
Sara Rodríguez Domínguez

GOALS
- Understanding how plant virus speciation processes and virus transmission mode affect virus evolution and disease emergence.

GENERAL OVERVIEW

The FAO estimates that, in the next 35 years, global agricultural production will need to increase by 60% to achieve acceptable levels of food safety for a human population that would reach nine billion. One of the most serious challenges to achieve this goal is to reduce the impact of emerging infectious diseases in crop production. Emerging diseases in plants are mainly caused by RNA viruses, which affect negatively sustainable food production by reducing crop quality and yield. The main focus of our research is to understand the processes of plant virus emergence, with the ultimate goal of contributing to control emerging diseases. Specifically, we are interested in exploring the effect virus speciation processes and of virus transmission mode in virus emergence. Plant viruses have a high capacity to generate genetic diversity, which favors the appearance of viral genetic variants with new properties that may eventually lead to virus speciation. The appearance of these new genetic variants is often associated with virus emergence, such that analysing speciation processes may contribute to understand virus emergence. We explore how the diversity of host species in an ecosystem and their population genetic diversity influences virus speciation, and whether this affects disease emergence. For an emerging virus to be successfully established in a host population, it is necessary not only acquire characteristics that increase the fitness of the new variant as compared with its competitors, it is also mandatory to be able to maintain transmission cycles in the host population. Thus, virus transmission is a central aspect of emergence. We are interested in understanding how the mode of transmission: horizontal (from plant-to-plant) or vertical (from parent-to-offspring) affect virus prevalence and genetic diversification.
RESEARCH ACTIVITY

A key feature of RNA viruses, often associated with disease emergence, is their high potential to generate genetic diversity. This characteristic provides RNA viruses with a high capacity for adaptation to new environments, including new hosts. Adaptation to new hosts may lead to diversification of the virus population, which may result in the emergence of new diseases caused by the appearance of new viral lineages or species: that is, by 'speciation' events. Although virus speciation is central to understand emergence, its determinants are still poorly understood. One of the major research goals of our group is characterizing the ecological and genetic factors that drive speciation processes of RNA virus populations. We are especially interested in understanding how ecosystem composition influences virus speciation and emergence. To address this subject, we analyze in evergreen oak forests and riparian forests of the Iberian Peninsula how changes of species identity and richness, plant density, host spatial aggregation and other environmental traits affect the population genetic diversity and prevalence of plant RNA viruses of the genus Potyvirus. These two ecosystems account for 75% of the wild landscape in Spain, being the most frequently adjacent to agroecosystems. Understanding the role of ecosystem ecological traits in the evolution of viruses in these natural ecosystems may contribute to clarify the determinants of virus emergence in both wild and domesticated plants, as these ecosystems may act as source of inoculum for the adjacent agricultural areas. Indeed, potyviruses, which are important crop pathogens, are commonly found in evergreen oak and riparian forests.

Evergreen oak and riparian forest in the Iberian Peninsula

Biodiversity is a two-sided coin. On one side it encompasses the diversity of species within an environment, while on the other it reflects the diversity of genotypes within species. Accumulating evidence indicates that host diversity has an important impact on parasite evolution and emergence. However, the vast majority of studies in this area have only utilized species diversity as an overall measure of biodiversity, overlooking the role of host genetic diversity. Although theoretical models propose that host genetic diversity shapes that of the infecting parasite population, and hence modulates the risk of disease emergence, this has seldom been tested empirically. We are interested in understanding the role of host genetic diversity in virus evolution and emergence. For that, we work with the experimental model formed by the plant virus Turnip mosaic virus (TuMV) and its natural host Arabidopsis thaliana. In controlled greenhouse conditions we analyze how TuMV virulence, multiplication and genetic diversity evolves in host populations with different levels of genetic diversity. Our ultimate goal is determining the level of host population genetic diversity that minimizes virus emergence such that this knowledge can be translated into more efficient control strategies.

Another characteristic of plant viruses that facilitates disease emergence is their great potential to spread rapidly. About 25% of all known plant viruses are vertically transmitted from parent to offspring through the seeds, and this is surely an underestimate as more viruses are reported to be seed transmitted every year. Given that approximately 90% of the food crops grown worldwide are propagated from seeds, seed transmitted viruses are an important threat for crop production. Seed infection provides the virus with a mean to persist for long periods of time when hosts or vectors are not available, allows for long distance dissemination of the virus via infected seeds, and represents an important source of primary inoculum for many viruses, which are disseminated afterwards via insect vectors. Current strategies to reduce the impact of seed transmitted viruses mostly involve routine seed health testing for seed certification and plant quarantine. However, the efficacy of these methods is limited. We are interested in the characterization of the plant and virus genes that control seed transmission. To attain this goal, we use the model system formed by the plant Arabidopsis thaliana and two viruses that infect natural populations of this host and are seed-transmitted: Cucumber mosaic virus and Turnip mosaic virus. Our aim is using this knowledge with biotechnological purposes to obtain plant varieties immune to seed transmission. Thus, we work with seed and biotechnology companies to transfer our findings into economically important crops.

Schematic representation of the direct and indirect routes of virus seed invasion
Publications and awards


Funding

Titulo: Analysis of speciation modes in plant RNA viruses
Referencia del proyecto: PCIG11-GA-2012-322100
Entidad financiadora:European Union (Marie Curie Career Integration Grants)
Duración: 01/10/2012 a 30/09/2016
Titulo: Effects of plant domestication in plant RNA virus evolution
Referencia del proyecto: RYC-2011-08574
Entidad financiadora: Ministerio de Ciencia e Innovación
Research Group: Gene regulation by TOR pathway in Rice blast fungus

Group Leader
Julio L. Rodríguez-Romero

Master Students
Francisco Borja Cuevas
Sara Rodríguez Domínguez

GOALS
- Rbp35 protein acts as a modulator of the TOR pathway, plant virulence and fungal growth, and it carries out this function by regulating alternative pre-mRNA 3'end processing.
- Identification of at least eighteen genes of TOR pathway with altered 3' UTRs in Rbp35 delta mutant.
- Identification of more than 12 Δrbp35 revertants strains.
- Identification of 13 novel genes involved in overcoming rapamycin lethal effects.

“Results derived from our research are relevant for food security and crop protection. This project also has practical implications to develop durable and sustainable strategies for disease control, which directly depend on a better understanding of the disease process.”

GENERAL OVERVIEW
Rice is the most widely distributed dietary staple in the world. It also represents a significant percentage of global farmland reaching up to 160 million hectares each year. One of the most devastating rice pathogen is the blast fungus, Magnaporthe oryzae. Yield losses caused by blast disease oscillate between 10-30 % per annum, which, even at the most conservative estimate, are sufficient to feed 60 billion people (Skamnioti&Gurr, 2009). M. oryzae is considered a hemibiotrophic fungus. Fungal metabolism plays an essential role during M. oryzae plant invasion. We are interested in identifying genes involved in the nutritional adaptation of the fungus by dissecting M. oryzae TOR signaling pathway. Given the significant role that the TOR pathway plays in the cell, the new genes identified as a result of this project could be used as targets for developing small molecule inhibitors. The knowledge of how plant pathogens interact with important crops will ultimately provide Spanish agriculture with new sustainable strategies to maintain and improve crop production and will strengthen the Spanish research effort in plant pathology. Spain and Italy are the two main producers of rice in Europe and they are affected by blast disease. The development of effective and durable strategies for disease control depends on a better understanding of the disease process. This, in turn, will enhance the European ‘knowledge-based bio economy’ (a concept originally conceived in the Lisbon agenda), which is of strategic importance. Additionally, RNA metabolism and TOR pathway in the fungal kingdom has been poorly studied in filamentous fungi (more complex organisms compared to model yeasts). Thus results derived from this research will therefore not only be relevant for the scientific community working on rice blast, but for the mRNA processing community in general. This research area has a broad relevance for post-transcriptional mechanisms of gene regulation and TOR pathway in all eukaryotic cells.
RESEARCH ACTIVITY

TOR is a conserved serine/threonine kinase present in all eukaryotes from fungi to humans. It is also a key component of the most central nutrient-sensing signal transduction pathways in eukaryotic cells (Figure 2). While TOR kinases are broadly conserved, distinct strategies in utilizing the TOR signaling cascade have been developed by fungal organisms. Rbp35 is a polyadenylation factor present only in filamentous fungi. The nutrient-dependent behaviour of the rbp35 mutant, together with its accelerated autophagy and higher tolerance to rapamycin, strongly suggests that the target of rapamycin (TOR) pathway is significantly altered (Figure 3). In fact, TOR is the most severely affected signalling pathway in this mutant. A comparative genomewide poly(A) site mapping analysis has identified the involvement of Rbp35 in alternative polyadenylation (APA) in M. oryzae. In carbon-depleted cells, eighteen out of the thirty components of the TOR signalling pathway are alternatively polyadenylated (APA). Based on these results, Rbp35 acts as a key modulator of the TOR pathway. Therefore, we will identify new proteins that are relevant for the adaptation of the fungus to external environment. These experiments will also enable us to understand how the expression of genes involved in the TOR signalling pathway is being affected at post-transcriptional level. This will be achieved initially by identifying genetic interactions between the TOR pathway and pre-mRNA 3’end processing.

Figure 1. Up-left a Rice field, and down-left Rice leaves infected with M.oryzae. Right, microscopy picture from a spore with an appressorium.

What type of genetic interactions link Rbp35 with the TOR pathway?

In this study, we used two complementary approaches to find new genes and decipher the mechanism by which the Rbp35 protein can control the TOR pathway.

Identification of Δrbp35 revertants:
After subculturing several consecutive rounds of the Δrbp35 mutant in CM, we already isolated about 50 reverted sectors. We confirmed stability and homogeneity of these revertants by growing them on a new plate of CM and performing single-spore isolations before their long-term storage. Out of the 50 revertants, we selected 12 that have overcome the rapamycin-dependent defects of Δrbp35. Genome of 12 selected revertants will be sequenced. We expect to find mutations in the same gene, in different genes and/or related pathways. This will provide us with a set of genes involved in the recovery of the TOR-associated defects in Δrbp35. Finally, the two most interesting gene candidates identified from the isolated revertants will be selected for further characterisation.

Identification of novel genes involved in TOR pathway:
Moreover, we used random T-DNA insertional mutagenesis to identify novel genes involved in overcoming rapamycin resistance. We randomly mutagenized the wild-type strain and isolated 57 T-DNA transformants that were insensitive to rapamycin and identified the locus of 13 T-DNA transformants.

Figure 2. TOR is a conserved serine/threonine kinase present in all eukaryotes from fungi to humans. It is also a key component of the most central nutrient-sensing signal transduction pathways in eukaryotic cells. While the TOR kinases are broadly conserved, distinct strategies in utilizing the TOR signaling cascade have been developed by fungal organisms.
After several screening stages, we proceeded to characterize selected strains by looking at their growth rates in different media and subsequently their pathogenic behavior. We also conclude that the use of random T-DNA insertion mutagenesis and selection of transformants in MM-T supplemented with rapamycin is a good method to find new genes related with the TOR signaling cascade. Finally, the two most interesting gene candidates identified from T-DNA mutagenesis will be selected for further characterization.

In summary, we expect that sequencing of the genomes of the revertant strains and further characterization of the some T-DNA mutants will provide us more insights into the interconnections found between 3'end processing and TOR signalling.

Main topics:
TOR, mRNA, APA, rice, rice blast, alternative polyadenylation, phytopathogen, fungi, Magnaporthe oryzae

Figure 3. Model diagram of the M. oryzae TOR signalling network based on S. cerevisiae. TORC1 is found at the upper-left corner. TORC1 promotes cell growth by stimulating anabolic processes such as translation initiation and ribosome synthesis. TORC1 also blocks transcriptional stress responses mediated by RIM15, MSN2/4, GIS1 and 14.3.3. Arrows and bars denote positive and negative interactions, respectively. Solid arrows and bars refer to direct interactions, dashed arrows and bars refer to indirect and/or potential interactions.

Publications and awards

5. Artículos de divulgación:
- Magnaporthe oryzae, un hongo de difícil control y de efectos devastadores en nuestros cultivos de arroz (https://www.interempresas.net/Grandes-cultivos/Articulos/158284-Magnaporthe-oryzaehongo-dificil-control-efectos-devastadores-nuestros-cultivos-arroz.html)
- Magnaporthe oryzae, la gran amenaza de los cultivos de arroz y trigo (http://www.qcom.es/v_portal/informacion/informacionver.asp?idcode=30303&te=2&idade=33794&vap=0)

Funding

Genetic interaction between the TOR pathway and mRNA processing in the rice blast fungus (MINECO, 2015–2018. BIO20014-54233-JIN) PI: Julio L. Rodríguez-Romero
**Research Group: Microscopy Facility**

**Group Leader**
Pablo González-Melendi de León

**Technical staff**
Beatriz Mora Rojas

**GOALS**
- Training and assistance to users
- Management of the equipment
- Sample preparation: fixation, paraffin-wax embedding, sectioning and staining

**GENERAL OVERVIEW**

The following imaging equipment is available in the facility:
- Fluorescence inverted microscope Leica DM IRB with CCD camera Leica DC 200
- Fluorescence upright microscope Zeiss Axiohot with colour CCD camera Leica DFC 300FX
- Fluorescence upright microscope Leica DM RB
- Fluorescence upright microscope Leica DM2000 with colour CCD camera Leica DFC 300FX
- Fluorescence stereomicroscope Leica MZ9 with CCD camera Leica DC 280
- Fluorescence stereomicroscope Leica MZ10 F with colour CCD camera Leica DFC 420C
- Fluorescence stereomicroscope Olympus SZX9
- CCD camera Berthold NightOwl LB 983 NC100, for bioluminescence and fluorescence
- Confocal microscope Leica TCS SP8 on an inverted microscope Leica DMI6000CS
  - six laser lines (450, 458, 488, 514, 561 and 633 nm)
  - four detectors (2 PMTs and 2 HyDs)
  - galvanometric Z-stage & motorized XY scanning stage
  - LAS X software with wizard for FRET & FRAP analysis and 3D viewer

*Unveiling plant cells structure and function*
In 2015 a new equipment (FLumaZone) for high sensibility fluorescence and bioluminescence imaging was acquired. It comprises:
- motorized stereomicroscope Leica M205FA with:
  - three types of illumination: bright field, Rotermann contrast and dark field
  - apochromat objectives: 1X (NA 0.17; WD 61.5 mm) and 2X (NA 0.35; WD 20.1 mm)
- fluorescence filters for DAPI, GFP and mCherry
- high sensitivity Electron Multiplying Hamamatsu ImagEM X2 EM-CCD camera with:
  - Electron Multiplying Back Thinned Transfer CCD technique
  - high frame rate format with 512 x 512 pixels of 16x16 μm
  - readout speed of the full image 70 fps
  - EM (electron multiplying) gain between 1 - 1200x
  - QY > 90%
  - analog gain 0.5x to 1x for EM mode and 1x to 5x for non-EM mode
  - air (-65ºC) and water (-80ºC) cooling
- Metamorph for image acquisition, integration and analysis

The equipment of the Facility is used for 20 groups of the CBGP. In most cases bookings can be done by intranet.
In the period 2014-2016, on average, the microtomes and microscopes of the Facility have been used nearly 4000 hours/year, of which 1000 correspond to the confocal microscope.
The Facility collaborates with the “Science week” activities organized by the CBGP.
5. Science and Society

One strategic objective of CBGP is the transmission of information on plant biotechnology and genomics and, thus, CBGP is concerned about the perception of Plant Biotechnology, and Plant Science at large, by society. Accordingly, CBGP researchers actively participate in events to introduce and explain Science. These activities include imparting seminars and conferences specifically aimed at improving the social perception of biotechnology, participating in workshops and fora with private companies of the Agro-Food and Forestry Productive Sectors or collaborations in radio, TV and written media.

Of these activities we would like to single out those aimed at introducing science to primary school and high school students, such as Madrid "Semana de la Ciencia" (2014-2016).

National meetings:
- I-VII Jornada Científica CBGP
- IV Meeting of the Spanish Network Plant - Pathogen Interaction

International meetings:
- I and II Workshop "New Frontiers in Plant Biology"
- 3rd European Workshop on Plant Chromatin
- Annual ENPER Meeting 2012
- International Symposium: Understanding plant disease emergence: evolutionary ecology meets genomics (Madrid 8-9 February 2012)
  - MERIT 1st Annual Meeting

CBGP Activities
- Fascination of Plants Day
- Backing up science
- Do you want to see what we do in the EpiTRAITS Project?
- Biotech Week
- UPM Poster Competition in Biotechnology Dissemination
- Workshops for kids
- Summer Science Camp
- Science Week
6. PhD Theses

2014

Azotobacter vinelandii nitrogenase: kinetics of nif gene expression and insights into the roles of fdxn and nifq in femo-co biosynthesis

Qualification: Sobresaliente cum laude
Date of defense: 22/09/2014
Author: Emilio Jimenez Vicente
Director: Luis Manuel Rubio Herrero

Mechanisms of sense and response to plant and environmental signals in Dickeya dadantii 3937 and Pseudomonas syringae pv tomato DC3000

Qualification: Sobresaliente cum laude
Date of defense: 06/06/2014
Author: Isabel del Rio Alvarez
Director: Emilia Antonia Lopez Solanilla

Análisis molecular de sistemas génicos implicados en la homeostasis de niquel y eficiencia simbiótica en rhizobium leguminosarum

Qualification: Sobresaliente
Date of defense: 26/03/2014
Author: Laura Rubio Sanz
Director: Jose Manuel Palacios Alberti

Caracterización funcional de los genes SGB (Suppressors of agb1-2 susceptibility to pathogens): Nuevos reguladores de la respuesta de inmunidad innata de Arabidopsis

Qualification: Sobresaliente
Date of defense: 30/04/2014
Author: Viviana Pamela Escudero Welsch
Director: Antonio Molina Fernandez
Lucia Jorda Miro

Caracterización Molecular del Banco de Germoplasma de vid del Rancho de la Merced

Qualification: Apto cum laude
Date of defense: 12/12/2014
Author: Ana Jiménez Cantizano
Director: Rosa Adela Arroyo Garcia
Alberto García de Luján

Chaperonas moleculares y tolerancia a estrés abiótico en especies arbóreas

Qualification: Sobresaliente cum laude
Date of defense: 21/03/2014
Author: Irene María Merino Sierra
Director: Luis Gomez Fernandez
<table>
<thead>
<tr>
<th>Title</th>
<th>Qualification</th>
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<tr>
<td>Factores transcripcionales de la clase DOF en Brachypodium distachyon: caracterización molecular de BdDOF24 durante la germinación de las semillas</td>
<td>Sobresaliente cum laude</td>
<td>20/03/2014</td>
<td>Virginia González de la Calle</td>
<td>Pilar Carbonero Zalduegui Cristina Barrero Sicilia</td>
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<td>Papel de EARLY BOLTING IN SHORT DAYS (EBS) en la regulación de la dormición de semillas en Arabidopsis thaliana.</td>
<td>Sobresaliente cum laude</td>
<td>24/04/2014</td>
<td>Laura Narro Diego</td>
<td>Manuel Angel Piñeiro Galvin Jose Antonio Jarillo Quiroga</td>
</tr>
<tr>
<td>Strukturelle Aspekte der Katalyse der Allen Oxid Zyklase 2 aus Arabidopsis thaliana und der Rubisco aus Thermosynechococcus elongates</td>
<td>Sobresaliente</td>
<td>08/09/2014</td>
<td>Barbara Terlecka Ruhr-Universität Bochum</td>
<td>Eckhard Hofmann (Ruhr-Universität Bochum) Stephan Pollmann (UPM)</td>
</tr>
<tr>
<td>2015 Bioinformatics tools for the analysis of plant-associated bacterial genomes</td>
<td>Sobresaliente cum laude</td>
<td>26/06/2015</td>
<td>Pedro Manuel Martínez García (Universidad de Málaga)</td>
<td>Cayo Juan Ramos Rodríguez (Universidad de Málaga) Pablo Rodríguez Palenzuela</td>
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<td>Caracterización fenotípica molecular de vides silvestres (V Viniera ssp sylvestris) en la Península Ibérica</td>
<td>Apto cum laude</td>
<td>19/12/2015</td>
<td>Alejandro Benito</td>
<td>Rosa Adela Arroyo Garcia F. Cabello</td>
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<td>Compromisos en la adaptación a distintos huéspedes en virus de plantas</td>
<td>Sobresaliente cum laude</td>
<td>06/05/2015</td>
<td>Manuel Guillermo Moreno Pérez</td>
<td>Fernando Garcia-Arenal Rodríguez</td>
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<td>Cycling DOF Factors: Molecular and functional characterization of Arabidopsis thaliana AtCDF3 and tomato (Solanum versicolorio L.) SlCDF3 in response to abiotic stress.</td>
<td>Sobresaliente cum laude</td>
<td>06/03/2015</td>
<td>Alba Rocío Corrales Duciara</td>
<td>Joaquin Medina Alcazar, Jesus Vicente Carbajosa</td>
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<td>Functional characterization of YODA, a mitogen-activated protein kinase kinase kinase (MAP3K) that regulates a novel innate immunity pathway in Arabidopsis thaliana</td>
<td>Sobresaliente cum laude</td>
<td>23/07/2015</td>
<td>Sara Sopeña Torres</td>
<td>Antonio Molina Fernandez</td>
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<tr>
<td>Identificación de nuevos genes implicados en la iniciación y desarrollo de las raíces laterales en Arabidopsis thaliana</td>
<td>Sobresaliente cum laude</td>
<td></td>
<td>Ricardo Duran Wendt</td>
<td>Luis Rey Navarro, Tomas-Andres Ruiz Argueso</td>
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<td>New insights into the regulation of NADPH oxidase dependent reactive oxygen species signaling during the plant immune response</td>
<td>Sobresaliente cum laude</td>
<td>19/06/2015</td>
<td>Jorge Morales Bello</td>
<td>Miguel Angel Torres Lacruz</td>
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<td>Role of the NuA4 complex in the regulation of flowering time in Arabidopsis thaliana</td>
<td>Sobresaliente cum laude</td>
<td></td>
<td>Alfonso Mouriz Villar</td>
<td>Jose Antonio Jarillo Quiroga, Manuel Angel Piñeiro Galvin</td>
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<td>Nuevos mecanismos moleculares de tolerancia a sequía y otros tipos de estrés abiótico en especies arbóreas de interés económico.</td>
<td>Sobresaliente cum laude</td>
<td>21/01/2016</td>
<td>Ángela Bibiana Contreras Mogollón</td>
<td>Luis Gómez Fernández</td>
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<tr>
<td>Arabidopsis thaliana como modelo de estudio de la coevolución planta-virus.</td>
<td>sobresaliente</td>
<td>27/01/2016</td>
<td>Nuria Montes Casado</td>
<td>Fernando García-Arenal Rodríguez</td>
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<td>Biotecnología forestal aplicada a la producción de madera de nogal</td>
<td>sobresaliente cum laude</td>
<td>05/02/2016</td>
<td>Ricardo Julián Licea Moreno</td>
<td>Luis Gómez Fernández</td>
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</tbody>
</table>
Estudio de la alergenicidad en Alt a 1, una proteína única de hongos.

Qualification: sobresaliente cum laude
Date of defense: 09/05/2016
Author: María Garrido Arandia
Director: Araceli Díaz Perales
Luis Fernández Pacios

Genomics of Specificity in the Symbiotic Interaction between Rhizobium leguminosarum and Legumes

Qualification: sobresaliente cum laude
Date of defense: 01/06/2016
Author: Beatriz Jorrín Rubio
Director: Juan Imperial Ródenas
Manuel González Guerrero

FAIR approaches applied to unraveling plant-pathogen interactions data and RNA processing evolution

Qualification: sobresaliente cum laude
Date of defense: 15/11/2016
Author: Rodríguez Iglesias, Alejandro
Director: Ane Sesma Galarraga
Mark Denis Wilkinson

2016

Nuevos mecanismos moleculares de tolerancia a sequía y otros tipos de estrés abiótico en especies arbóreas de interés económico.

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**Director:** Ane Sesma Galarraga
Mark Denis Wilkinson

Efecto del aluminio, del ayuno de fosfato y de la luz en el crecimiento y arquitectura radicular.

**Qualification:**
**Date of defense:** 2016
**Author:** Javier Silva Navas
**Director:** F Javier Gallego
(UCM) y J. Carlos del Pozo
7. Seminars at CBGP

2014

January 4
miRNA networks and their central roles in plant development.
Ignacio Rubio-Somoza
Max Planck Institute for Developmental Biology, Tübingen, Germany

January 24
Auxin homeostasis in plants: functions and evolution
Jutta Ludwig-Müller
Technical University of Dresden, Germany

February 7
Chromatin dynamics during transcription elongation: histone eviction versus RNA polymerase backtracking
Sebastián Chávez - Universidad de Sevilla, España

February 14
Structural and functional diversity of bacterial chemoreceptors and chemosensory pathways
Tino Krell - Estación Experimental del Zaidín, Granada, España

February 21
Adaptation to osmostress
Francesc Posas - Universidad Pompeu Fabra, Barcelona, España

February 28
Herramientas de ingeniería multigénica para Biología Sintética y Plantas Biofactoría
Diego Orzáez - IBMCP, CSIC-UPV, Valencia, España

March 7
Mechanisms of induction of immune tolerance
Domingo Barber - IMMA-CEU, Madrid, España

March 14
Threshold dependent transcriptional discrimination underlies stem cell maintenance in Arabidopsis shoot apical meristem
Mariano Perales - CBGP, Madrid, España

March 21
Plant signal transduction in symbiosis with nitrogen fixing bacteria and phosphate-acquiring fungi
Martin Parniske - LMU, Munich, Germany

March 28
Small noncoding RNA production pathways in fungi
Yi Liu - Univ. of Texas Southwestern Medical Center, USA

April 4
Selective uptake: new players and roles of clathrin mediated endocytosis in plants
Clara Sánchez - MPI of Molecular Plant Physiology, Potsdam, Germany

April 25
Technology drives insight – multiscale analysis of signalling networks in Arabidopsis thaliana
Klaus Palme - University of Freiburg, Germany

May 9
Roots and microbes: Selection, signalling and domination
Phil Poole - Oxford University, UK

May 23
GEOMETAGENOMICS: A second metagenomics generation to better understand plant virus ecology and evolution
Philippe Roumagnac - CIRAD, Montpellier, France

May 30
Environmental control of molecular machines that activate bacterial transcription
Ray Dixon - John Innes Centre, UK
September 19
Evolution of dominant and recessive virus resistance genes in wild plant populations
Nil Poulicard - CBGP, Madrid, España

October 3
Sequence specific heterochromatin formation in response to environmental and developmental signals in Arabidopsis
Elena Caro - CBGP, Madrid, España

October 10
Multidimensional genomics for dissection of complex traits in crop plants
Rod Snowdon - Justus Liebig University, Giessen, Germany

October 24
How diverse is the prokaryotic biosphere?
Ramón Rosselló-Mora - IMEDEA (CSIC-UIB), Palma de Mallorca, España

October 17
Association genetics for reproductive traits in grapevine.
Javier Ibañez - Instituto de las Ciencias de la Vid y el Vino, Logroño, España

November 14
Integrating photoreceptor signals to adjust shade avoidance responses to neighbors
Ronald Pierik - Utrecht University, Netherlands

November 21
Multiple resistance to insecticides in the Mediterranean fruit fly, Ceratitis capitata
Felix Ortega - CIB-CSIC, Madrid, España

November 28
Traffic control for pattern recognition receptors
Silke Robatzek - The Sainsbury Laboratory, Norwich, UK

December 12
Small signalling peptides, receptor kinases and phosphatases shape the plant
Ive de Smet - VIB Department of Plant Systems Biology University of Ghent, Belgium

December 19
Plantas en el espacio: la ausencia de gravedad altera la competencia meristemática en células proliferantes
Javier Medina - CIB-CSIC, Madrid, España

2015

January 16
Regulation of nitrogen fixation and molybdate transport genes in Rhodobacter capsulatus
Bernd Masepohl - Ruhr-Universität, Bochum, Germany

January 23
Cellular and regulatory basis of floral organ growth
Robert Sablowski - John Innes Centre, UK

January 30
Diversity, Evolution and Adaptation to Symbiosis in legume nodulating Burkholderia
Lionel Moulin - IRD Montpellier, France

February 6
Traducción de RNAs de virus de plantas independiente de cap: análisis del modelo experimental MNSV/melón y aplicaciones biotecnológicas
Miguel Aranda - CEBAS, Murcia, España

February 13
Hormone signaling and rhythmic organogenesis in plants
Teva Vernoux - CNRS-INRA, UCBL, Université de Lyon, France

February 20
Learning plant biology from Darwin in the 21st Century
Rafael Rubio de Casas - EEZA-CSIC de Almería, España

February 27
Evolution of genomes by copy and paste: phylogenomics reveal non-vertical modes of evolution in fungi
Toni Gabaldón - Centro de Regulación Genómica, Barcelona, España
<table>
<thead>
<tr>
<th>Date</th>
<th>Title</th>
<th>Speaker</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>March 6</td>
<td>Interspecies and interkingdom signaling in plant-associated bacteria</td>
<td>Vittorio Venturi</td>
<td>Internacional Centre for Genetic Engineering and Biotechnology, Trieste, Italy</td>
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<tr>
<td>March 13</td>
<td>Gibberellin and the control of reproductive development in Arabidopsis</td>
<td>Miguel Ángel Pérez Amador</td>
<td>IBMCP, Valencia, España</td>
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<tr>
<td>March 27</td>
<td>Roles for redox processes in plant responses to drought</td>
<td>Christine Foyer</td>
<td>University of Leeds, UK</td>
</tr>
<tr>
<td>April 10</td>
<td>Plantas en el espacio: la ausencia de gravedad altera la competencia meristemática en células proliferantes</td>
<td>Javier Medina</td>
<td>CIB-CSIC, Madrid, España</td>
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<td>April 17</td>
<td>Exocytosis and apical growth in <em>Aspergillus nidulans</em></td>
<td>Miguel Peñalva</td>
<td>CIB-CSIC, Madrid, España</td>
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<tr>
<td>April 24</td>
<td>Signalling pathways that establish symbiotic associations in plants</td>
<td>Giles Oldroyd</td>
<td>The John Innes Centre, Norwich, UK</td>
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<tr>
<td>May 8</td>
<td>Regulation of phenology in Arabidopsis, sugar beet and aspen trees - same problem - different solutions</td>
<td>Ove Nilson</td>
<td>Umea Plant Science Centre UPSC, Sweden</td>
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<tr>
<td>May 22</td>
<td>Extracting mechanism-based biomarkers from genomic big data</td>
<td>Joaquín Dopazo</td>
<td>Centro de Investigación Príncipe Felipe-CIPF, Valencia, España</td>
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<tr>
<td>May 29</td>
<td>It takes two to tango: resistance gene pairs that recognize and respond to pathogen effectors</td>
<td>Jonathan Jones</td>
<td>The Sainsbury Laboratory, Norwich, UK</td>
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<tr>
<td>September 18</td>
<td>C1A peptidases-cystatins interplay in barley biological events</td>
<td>Mercedes Díaz-Mendoza.</td>
<td>CBGP, Madrid, España</td>
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<tr>
<td>October 2</td>
<td>Virus interactions with agricultural and native species in Poaceae-dominated landscapes</td>
<td>Carolyn Malmstrom</td>
<td>Michigan State University, East Lansing, USA</td>
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<tr>
<td>October 16</td>
<td>Prospects and challenges of plant made pharmaceuticals</td>
<td>Julian K-C. Ma</td>
<td>Institute for Infection and Immunity, St. George’s Hospital Medical School, London, UK</td>
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<tr>
<td>October 23</td>
<td>Enzymatic control of stereoselective biosynthesis of jasmonates and lignans: What structural biology can tell us (and what not)</td>
<td>Eckhard Hofmann</td>
<td>University of Bochum, Germany</td>
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<tr>
<td>November 13</td>
<td>Entry of Pi into Plants: role of PHT1 transporters</td>
<td>Lauren Nussaune</td>
<td>IBEB/SBVME/LBDP, CNRS-Marseille, France</td>
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<td>November 20</td>
<td>Symmetry Matters in Arabidopsis Gynoecium Development</td>
<td>Lars Ostegaard</td>
<td>John Innes Centre, Norwich, UK</td>
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<td>November 27</td>
<td>The benefits of a colourful diet: the science behind 5-a-day</td>
<td>Cathie Martin</td>
<td>John Innes Centre, Norwich, UK</td>
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<tr>
<td>December 4</td>
<td>New paradigms in therapeutic effectiveness against cancer</td>
<td>María del Carmen Ramírez Castillejo</td>
<td>CBGP, Madrid, España</td>
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</table>
Multiple Resistance Pathways Elicited by TMV Interacting with the N Gene Products
Peter Palukaitis - Seoul Women's University, Seoul, Korea

Crowdfunding: una forma alternativa para comenzar una Investigación
Alberto Jiménez - CNIO, Madrid, España

2016

January 15
Novel roles for translation elongation as a regulator of gene expression
Tobias von der Haar - University of Kent, Canterbury, UK

January 22
Diversidad genética e historia evolutiva de la dualidad olivo-acebuche
Pablo Vargas Gómez - CSIC, Real Jardín Botánico de Madrid, España

January 29
Gibberellin and the control of reproductive development in Arabidopsis
Miguel Ángel Pérez Amador - IBMCP, Valencia, España

February 5
Andrea Chini - CSIC-CNB, Madrid, España
Pim-kinase inhibitors modulate JA and auxin responses

February 12
Marc Valls - Universitat de Barcelona, España
Bacterial virulence and plant resistance using R. solanacearum as a model system

February 19
Alex Webb - Department of Plant Sciences University of Cambridge, UK
Signaling to and from the circadian clock of Arabidopsis

February 26

Sebastien Thomine - Institute for Integrative Biology of the Cell, Gif-sur-Yvette, France
Managing essential and toxic metals in plant cells with NRAMP transporters

March 4
Jaime Martínez-García – CRAG, Barcelona, España
Shades of greens: illuminating plant responses to vegetation proximity

March 11
Gabrielle Berg - Graz University of Technology, Austria
Deciphering the plant microbiome for health and biocontrol

March 18
Jane Parker - Max Planck Institute for Plant Breeding Research, Köln, Germany
Connecting intracellular pathogen recognition to the stress response network in plant immunity

April 1
José Tomás Matus - CRAG, Barcelona, España
Towards the characterization of the grapevine R2R3-MYB family: phenylpropanoid regulators as a case of study

April 8
Concha Gómez-Mena CSIC-IBMCP, Valencia, España
Using parthenocarpic varieties to decipher the molecular basis of fruit set and fruit development in tomato

April 15
Alejandro Ferrando - CSIC/Universidad Politécnica de Valencia, España
Biological relevance of the axis Spermidine/eIF5A and the translation of proline repeat-rich proteins

April 22
Judith Armitage - University of Oxford, UK
Protein localisation and dynamics in bacterial cells

6 May
David Posé - Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga, España
Key regulators of floral transition and fruit ripening

May 13
Julio Rodríguez-Romero - CBGP, Madrid, España
Interconnections between mRNA processing, TOR pathway and plant pathogenesis in the rice blast fungus

May 20
Susana Moreno - Centro de Investigaciones Biológicas, Madrid, España
Nuclear lamina in plants: distinct proteins for a conserved structure and function

May 27
Pablo Vera - IBMCP (UPV-CSIC), Valencia, España
Domesticación de *Euphorbia lathyris* como cultivo energético

October 7
F. Javier Cejudo - Universidad de Sevilla, España
A novel model of chloroplast redox regulation

October 14
Víctor Parro - Centro de Astrobiología INTA-CSIC Madrid, España
Subsurface microbiology in extreme environments and the search for life in planetary exploration

October 21
Luis Vidali - Worcester Polytechnic Institute, Massachusetts, USA
The moss *Physcomitrella patens* as a model system for Plant Cell Biology

November 4
Antonio Di Pietro - Universidad de Córdoba, España
Host plant directional sensing and chemical reprogramming by the fungal pathogen *Fusarium*

November 11
M. Ángel Blázquez - Instituto de Biología Molecular y Celular de Plantas IBMCP, Valencia, España
A role for auxin methylation during differential growth

November 25
Pilar Cubas - Centro Nacional de Biotecnología, CSIC, Madrid, España
To branch or not to branch: a bud’s question

December 16
Angélique Déleris - Institut de Biologie de l'École Normale Supérieure, Paris, France

Reactivation of repeated sequences during plant innate immunity