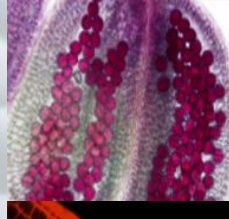
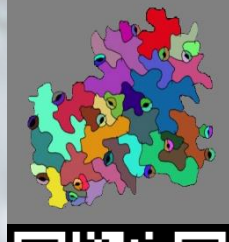
# Plant Organ Growth Symposium 2017

## Elche (Spain)
## March 15-17th, 2017

**Covering the latest advances in the biology, modeling and automated phenotyping of root, leaf, shoot and reproductive growth and development**





**http://pogs2017.edu.umh.es**

**@POGS2017Elche**

UNIVERSITAS Miguel Hernández

Instituto de Bioingeniería
Universidad Miguel Hernández

GENERALITAT VALENCIANA

visitelche.com

VIB science meets life

# Plant Organ Growth Symposium 2017

**15-17 March 2017**

**Centro de Congresos "Ciutat d'Elx"**
**Elche, Spain**

# Local Organising Committee

**Prof. José Luis Micol**
**Prof. María Rosa Ponce**
**Dr. David Wilson-Sánchez**
**Dr. Raquel Sarmiento-Mañús**

# Scientific Committee

**Prof. José Luis Micol (UMH, Elche, Spain)**
**Prof. Dirk Inzé (VIB, Ghent, Belgium)**
**Dr. Nathalie Gonzalez (VIB, Ghent, Belgium)**

# Sponsors

**Ayuntamiento de Elche**
**Generalitat Valenciana**
**Instituto de Bioingeniería**
**Universidad Miguel Hernández de Elche (UMH)**
**VIB-UGent Department of Plant Systems Biology**

# Table of contents

# Program

## Wednesday, March 15th

8:00-9:00 Registration

9:00-9:05 Welcome and introduction to the meeting by José Luis Micol

**Session 1: Root growth and development**

*Chair: Malcolm Bennett*

9:05-9:45 Malcolm Bennett, University of Nottingham, UK

9:45-10:25 Alexis Maizel, University of Heidelberg, Germany

10:25-11:05 Masaaki Umeda, Nara Institute of Science and Technology, Japan

11:05-11:35 Coffee break

11:35-13:30 Short presentations

Gregory Vert, Institute for Integrative Biology of the Cell, Paris, France
Idan Efroni, The Hebrew University, Rehovot, Israel
Thomas Blein, Institute of Plant Sciences, Orsay, France
Naoki Takahashi, Nara Institute of Science and Technology, Japan
Jos Schippers, Rheinish-Westphalian Technical University Aachen, Germany
Benjamin Péret, CNRS Biochimie et Physiologie Moléculaire des Plantes, Montpellier, France
Kris Vissenberg, University of Antwerp, Belgium
Kiflemarian Y. Belachew, University of Helsinki, Finland
J. Carlos del Pozo, Centro de Biotecnología y Genómica de Plantas, Madrid, Spain

13:30-14:30 Lunch

**Session 2: Reproductive growth and development**

*Chair: Esther van der Knaap*

14:30-15:10 Esther van der Knaap, University of Georgia, Athens, USA

15:10-15:50 Loïc Lepiniec, Institute Jean-Pierre Bourgin, Versailles, France

15:50-16:30 Jose Feijó, University of Maryland, USA

16:30-17:00 Coffee break

17:00-18:15 Short presentations

Antonio Vera, Universidad Miguel Hernández, Sant Joan d'Alacant, Spain
Sam van Es, Wageningen University and Research, The Netherlands
Constance Musseau, University of Bordeaux, France
Fernando Pérez-Martín, Universidad de Almería, Spain
Martine Lemaire-Chamley, University of Bordeaux, France
Marcos Egea-Cortines, Universidad Politécnica de Cartagena, Spain

18:15-20:00 Refreshments and poster viewing

## Thursday, March 16th

**Session 3: Leaf and shoot growth and development I**

*Chair: Neelima Sinha*

9:00-9:40 José Luis Micol, Universidad Miguel Hernández, Elche, Spain

9:40-10:20 Neelima Sinha, University of California, Davis, USA

10:20-11:00 Hirokazu Tsukaya, University of Tokyo, Japan

11:00-11:30 Coffee break

11:30-13:00 Short presentations

Katharina Bürstenbinder, Leibniz Institute of Plant Biochemistry, Halle, Germany

Sander Corneille,VIB-UGent Center for Plant Systems Biology, Belgium

Marieke Dubois, Institut de Biologie Moléculaire des Plantes, Strasbourg, France

Sander Hulsmans, University of Leuven, Belgium

Bulelani Sizani, University of Antwerp, Belgium

Alicja Kunkowska, Rheinish-Westphalian Technical University Aachen, Germany

Enrique Rojo, Centro Nacional de Biotecnología, Madrid, Spain

13:00-14:30 Lunch

**Session 4: Leaf and shoot growth and development II**

*Chair: Marja Timmermans*

14:30-15:10 Marja Timmermans, Cold Spring Harbor Laboratory, USA, and University of Tübingen, Germany

15:10-15:50 Miltos Tsiantis, Max Plank Institute for Plant Breeding Research, Köln, Germany

15:50-16:20 Coffee break

16:20-17:10 Short presentations

Miguel A. Blázquez, Instituto de Biología Molecular y Celular de Plantas, Valencia, Spain

Sara Farahi-Bilooei, Royal Holloway University of London, UK

Binish Mohammed, John Innes Centre, Norwich, UK

Reidunn Aalen, University of Oslo, Norway

**Keynote lecture**

*Chair: José Luis Micol*

17:10-18:05 Dirk Inzé, VIB-UGent Center for Plant Systems Biology, Belgium

18:05-20:00 Refreshments and poster viewing

## Friday, March 17th

**Session 5: Modeling and phenotyping**

*Chair: Thomas Altmann*

9:00-9:40 Thomas Altmann, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany

9:40-10:20 Nathalie Wuyts, VIB-UGent Center for Plant Systems Biology, Gent, Belgium

10:20-11:00 Olivier Loudet, Institute Jean-Pierre Bourgin, Versailles, France

11:00-11:30 Coffee break

11:30-12:45 Short presentations
Beata Orman, University of Liège, Belgium
Urs Fischer, Umeå Plant Science Centre, Sweden
Radka Slovak, Gregor Mendel Institute, Vienna, Austria
Chvan Youssef, Université de Lorraine, Champenoux, France
Jim Murray, Cardiff University, Wales,UK
Nuria De Diego, Palacký University, Olomouc, Czech Republic

12:45-13:00 Symposium adjournment and announcement of next meeting by Nathalie Gonzalez

13:00-14:30 Lunch

# Index of talks, oral communications, and posters

**SESSION 1: Root growth and development**



**SESSION 2: Reproductive growth and development**

**SESSION 5: Modeling and phenotyping**



**POSTERS**

# Abstracts

# SESSION 1:
# Root growth and development

# New angles on root growth and development using systems & phenotyping based approaches

Bennett, M.J.

Centre for Plant Integrative Biology (CPIB) & Hounsfield Facility, University of Nottingham, UK (www.cpib.ac.uk).

Our understanding of root biology has accelerated over the last decade in large part to genetic and genomic approaches in model plants such as *Arabidopsis* and rice (reviewed in [1]). Researchers have started to study the mechanisms controlling root growth and development using systems approaches (reviewed in [2]). Modeling is set to become much more important as our knowledge of root regulatory pathways becomes increasingly complex and their outputs less intuitive. To relate root genotype to phenotype we must move beyond the network scales and employ multi-scale modeling approaches to predict emergent properties at the tissue, organ, organism and rhizosphere levels. The interplay between scales is complex and a multi-disciplinary approach is essential to understand the underlying biological mechanisms. To illustrate this point, I will describe examples relating to regulation of root angle in *Arabidopsis* and rice.

Despite these advances in understanding, roots have remained the '*hidden half*' of plant biology due to challenges of non-invasively visualize roots in their natural soil environment. At Nottingham, we have employed an interdisciplinary research approach to image roots directly in soil using X-ray CT based techniques. This has enabled us to discover or characterise novel root adaptive mechanisms [3,4]. I will conclude by describing the Hounsfield Facility, a root phenotyping platform, designed to study root developmental and rhizosphere processes.

[1] Atkinson, J.A., *et al.* (2014). Branching out in roots: uncovering form, function and regulation. *Plant Physiol.* 166, 538-550.

[2] Benfey, P.N., *et al.* (2010). Getting to the root of plant biology: impact of the Arabidopsis genome sequence on root research. *Plant J.* 61, 992-1000.

[3] Bao, Y., *et al.* (2014). Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proc. Natl Acad. Sci. USA* 111, 9319-9324.

[4] Hill, K., *et al.* (2013). Root systems biology: integrative modeling across scales, from gene regulatory networks to the rhizosphere. *Plant Physiol.* 163, 1487-1503.

# Auxin dependent cell remodelling during lateral root morphogenesis in Arabidopsis

Maizel, A.[1], Vilches-Barro, A.[1], Bald, L.[1], Schubert, M.[1], Ruiz-Duarte, P.[1], Vodermaier, V.[1], Vermeer, J.[2], Smith, J.[1], and Doe, J.[1,2]

[1]Center for Organismal Studies (COS), University of Heidelberg, Germany.
[2]Department of Plant and Microbial Biology, University of Zürich, Switzerland.

Plants form new organs with patterned tissue organization throughout their lifespan. As plants cells are encaged in a rigid cell wall cell migration is impossible. In consequence, plants rely on oriented cell divisions and anisotropic growth to shape their organs and precisely organise their tissues.

Lateral roots are formed postembryonically and determine the final shape of the root system, a determinant of the plants ability to uptake nutrients and water. Lateral root formation commences when founders cells located in the pericycle divide and create a dome-shaped lateral root primordium (LRP), which has to cross three overlying tissues to emerge at the surface of the parent root: the adjacent endodermis, the cortex and the outermost layer, the epidermis. In a previous work [1], we combined modelling with empirical observations using light sheet microscopy of whole organ development to identify the principles governing lateral root formation in Arabidopsis. Lateral roots derive from a small pool of founder cells, in which some take a dominant role as seen by lineage tracing. The first division of the founders is asymmetric, tightly regulated, and determines the formation of a layered structure. Our recent results indicate that auxin plays a crucial role in the control of this first essential formative division. To preserve the structural and functional integrity of the primary root, it is necessary to coordinate growth and proliferation within the LRP and the responses of the overlying tissues. Auxin plays a pivotal role in coordinating these responses. Our results show in particular that auxin controls the dynamics of cortical microtubule rearrangements which has a critical impact on the control of plane of division and the ability of cells to alter their geometry.

[1] von Wangenheim, D., *et al*. (2016). *Curr. Biol.* 26, 439-449.

## Hormonal control of genome integrity in roots

Umeda, M.[1,2], and Takahashi, N.[1]

[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara 630-0192, Japan.
[2]JST, CREST, Nara 630-0192, Japan.

Cell division is usually suppressed in response to environmental stress to minimize energy consumption in organ growth. Under such circumstances, plants control the cell cycle duration and the transition from the mitotic cell cycle to the endocycle, in which DNA polyploidization occurs without mitosis. DNA is constantly damaged during DNA replication, and various internal and external stimuli also cause DNA damage; for example, reactive oxygen species produced by photosynthesis, high aluminum or boron levels in soil, and pathogen attack are known to induce single- or double-strand DNA breaks. In animals, severe DNA damage causes cell death, but we previously found that plants promote the endocycle onset, but not cell death, upon genotoxic treatments. This means that cell death is not the common strategy to cope with DNA stress in plants. However, stem cells and their daughters are exceptional in that they undergo cell death in response to DNA damage. In *Arabidopsis* roots, dead stem cells are replaced by newly generated cells provided by QC cell division. This represents an elaborate mechanism preserving stem cells during continuous root growth. We shall present how these responses to DNA damage are mediated by hormonal signaling, and how plants control cell division and cell death to maintain genome integrity in roots.

## Brassinosteroid signaling-dependent root growth responses to prolonged elevated ambient temperature in Arabidopsis

Martins, M., Montiel-Jorda, A., and Vert, G.

Institute for Integrative Biology of the Cell (I2BC), CNRS/CEA/Univ. Paris Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette, France.

As sessile organisms, plants have to cope with and adjust to their fluctuating environment. Temperature elevation stimulates the growth of Arabidopsis aerial parts. This process is mediated by increased biosynthesis of the growth-promoting hormone auxin. How plant roots respond to elevated ambient temperature is however still elusive. Here we present strong evidence that prolonged temperature elevation impinges on brassinosteroid hormone signaling to alter root growth. We show that elevated temperature leads to increased root elongation, independently of auxin or factors known to drive temperature-mediated shoot growth. We further demonstrate that brassinosteroid signaling negatively regulate root responses to elevated ambient temperature. Increased growth temperature specifically impacts on the level of the BR receptor BRI1 n the root by ubiquitin-mediated endocytosis and degradation and thus downregulates BR signaling to mediate root elongation. Our results establish that BRI1 integrates temperature and BR signaling to regulate root growth upon long-term changes in environmental conditions and allow us to anticipate on the consequences of global warming.

## Roots regeneration activates an embryonic developmental sequence guided by antagonistic hormonal interactions

Efroni, I.[1,3], Mello, A.[2], Nawy, T.[2], Ip, P.L.[2], DelRose, N.[2], Rahni, R.[2], Powers, A.[3], Satija, R.[2,3], and Birnbaum, K.[2]

[1]The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University, Rehovot, Israel.
[2]Center for Genomics and Systems Biology, New York University, New York, USA.
[3]New York Genome Center, New York, USA.

Plant roots can regenerate after excision of their tip, including the stem cell niche, but it is not clear what developmental program mediates such repair. We used a combination of lineage tracing, single-cell RNA-Seq, and marker analysis to test different models of tissue reassembly. We showed that the root tip regenerates by recruitment of cells from multiple remnant tissues to form a new stem cell niche which then orchestrates growth. The transcriptomic dynamics of regenerating cells prior to stem cell activation reveals rapid identity transitions in a developmental sequence which resembled the embryonic root initiation. Consistent with a re-activation of an embryonic program, regeneration defects were more severe in mutants defective in embryonic, rather than adult root formation. Furthermore, the signaling domains of the hormones auxin and cytokinin mirrored their embryonic dynamics, and manipulation of both hormones could alter the position of new tissues and the stem cell niche within the regenerating root. Our findings suggest that plant organ regeneration resembles the developmental stages of embryonic patterning and is guided by spatial information laid down by complementary hormone domains. I will address these conclusions in the context of tomato stem-borne root formation as a new model system to study root initiation.

# Ecotype-related long non-coding RNAs in environmental control of root growth

Blein, T.[1,2], Balzergue, C.[3,4,5], Gabriel, M.[6], Scalisi, L.[1,2], Sorin, C.[1,2], Christ, A.[1,2], Nussaume, L.[3,4,5], Hartmann, C.[1,2], Gautheret, D.[6], Desnos, T.[3,4,5], and Crespi, M.[1]

[1]Institute of Plant Sciences Paris Saclay IPS2, CNRS, INRA, Université Paris-Sud, Université Evry, Université Paris-Saclay, Batiment 630, 91405 Orsay, France.
[2]Institute of Plant Sciences Paris-Saclay IPS2, Paris Diderot, Sorbonne Paris-Cité, Bâtiment 630, 91405, Orsay, France.
[3]CEA, Institut de Biologie Environnementale et de Biotechnologie, Laboratoire de Biologie du Développement des Plantes, Saint-Paul-lez-Durance, F-13108, France.
[4]CNRS, Unité Mixte de Recherche 7265 Biologie Végétale & Microbiologie Environnementale, Saint-Paul-lez-Durance, F-13108, France.
[5]Aix-Marseille Université, Saint-Paul-lez-Durance, F-13108, France.
[6]I2BC, Institute for Integrative Biology of the Cell, CEA, CNRS, Université Paris Sud , 1 avenue de la terrasse, 91198 Gif sur Yvette, France.

The plant root system is characterised by its high developmental plasticity. This adaptability allows the plant to face constantly variable environments. In case of phosphate starvation, the adaptation of root architecture varies widely between species but even between ecotypes of the same species despite the conservation of the protein-coding portion of the genome. Long non-coding RNAs (lncRNAs) have been shown to quantitatively regulate the expression of specific genes and hence may play roles in the quantitative modulation of the root architecture between ecotypes. The Columbia (Col-0) and Landsberg *erecta* (L*er*) ecotypes respond differently to phosphate starvation. Indeed, after transfer to a –P medium, primary root growth in L*er* remains unchanged whereas it stops in the Col-0 ecotype. Using RNA sequencing, we compared complete transcriptional response (mRNAs, lncRNAs and small RNAs) of root tips from these two ecotypes during early phosphate starvation response. We identified thousands of new lncRNAs largely conserved at the DNA level. However, in contrast to coding genes, many lncRNAs were specifically transcribed in one ecotype. These ecotype-specific lncRNAs were further characterized by analysing their variability among sequenced Arabidopsis

ecotypes and their ability to generate siRNA. In contrast to coding genes, a majority of lncRNAs were differentially regulated according to genotype than to phosphate starvation, suggesting a role in ecotype adaptation. Our analysis identified 746 lncRNAs whose expression is different between the two ecotypes and mis-regulation of two ecotype-specific lncRNAs affects primary root growth. The non-coding genome may reveal novel mechanisms for the adaptation of roots to the soil environment.

# Brassinosteroids are involved in stem cell replenishment in Arabidopsis roots under DNA damage

Takahashi, N.[1], and Umeda, M.[1,2]

[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Takayama 8916-5, Ikoma, Nara 630-0192, Japan.
[2]JST, CREST, Takayama 8916-5, Ikoma, Nara 630-0192, Japan.

Maintenance of stem cells is crucial for guaranteeing continuous organ growth in plants. In roots, quiescent center (QC) cells are important for maintenance of stem cells. QC cells divide at low frequency under normal growth conditions. However, when stem cells surrounding the QC are died in response to DNA damage, QC cell division is activated, and newly generated cells replace dead stem cells, restoring the stem cell niche (SCN). However, it has remained unknown how QC cell division is activated under DNA stress conditions. Here we show that brassinosteroids (BRs) are required for QC cell division triggered by DNA damage. By a comprehensive expression analysis, we found that expression of one of the BR receptors was induced by treatment with DNA damaging agents.

## The N-end rule controls root meristem cell proliferation through a Myb-like transcription factor

Krings, J., Schmidt, R., and Schippers, J.H.M.

Institute of Biology I, RWTH Aachen University, Worringerweg 1, 52074, Aachen, Germany.

Organ growth relies on the coordination of cell proliferation and differentiation. Roots display indeterminate growth, which requires maintenance of the root meristem and allows plants to forage for nutrients. How the size of the root meristem is maintained and balanced with the rate of differentiation is still only partially understood.

Here we show that the arginylation-branch of the N-end rule pathway regulates cell proliferation in the root meristem of Arabidopsis. The arginylation-branch controls the lifetime of peptides or proteins that contain N-terminal Asp, Glu or oxidized Cys. To reveal how the N-end rule modulates root meristem cell proliferation, we aimed at identifying the molecular targets it controls during root growth. Interestingly, we found a Myb-like transcription factor that contains a destabilizing N-terminal Cys residue and represents a novel target of the N-end rule pathway. Furthermore, the transcription factor acts as a negative regulator of cell proliferation as loss of function mutants display an increased root meristem size, whereas overexpression results in an opposite effect.

As there is no single way to cause oxidation of Cys residues, we set out to understand the gradients of reactive oxygen and nitrogen species within the root meristem. Based on our analysis and follow-up experiments we propose a novel role for one of these species in the regulation of root meristem cell proliferation, which potentially is linked to the regulation of the stability of the here identified Myb-like transcription factor through the N-end rule.

## Unravelling cluster root development in white lupin

Péret, B.

Centre National de la Recherche Scientifique, Biochimie et Physiologie Moléculaire des Plantes, Montpellier SupAgro, 2 Place Pierre Viala, 34060 Montpellier, France.

Plants show a strong level of developmental plasticity that is controlled by a complex combination of perception, integration and response. Root systems are a fantastic tool to study this plasticity since the number and position of lateral roots is deeply altered by the environment. We are trying to understand the fundamental mechanisms governing lateral root development and its control by the environment. Our research focuses on two main biological systems: the model plant *Arabidopsis thaliana* and white lupin (*Lupinus albus*). Our new project (ERC Starting grant LUPIN ROOTS) aims at understanding the formation of cluster roots in white lupin. These roots are specific lateral roots that are dedicated towards efficient phosphate acquisition and are produced as a response to its deficiency. From a developmental point of view, they consist in the induction of numerous rootlet primordia that will emerge to produce a "bottlebrush"-like structure. We believe that studying these extraordinary structures will help us understand plant organ formation as a response to their environment.

We generated and started to screen a mutagenized population. We have identified constitutive cluster root mutants that are now being amplified for confirmation of their phenotype. In parallel, we will sequence the genome of white lupin, a diploid species (2n=50) and will perform detailed timecourse transcriptomics analysis by RNAseq. We also perform histology approaches to study cluster root development and we are developing genetic transformation to generate markers and tools for functional analysis.

More information at www.plasticity.fr

# The auxin-regulated kinase ERULUS controls root hair growth through cell wall pectin modifications in *Arabidopsis thaliana*

Schoenaers, S.[1], Balcerowicz, D.[1], Breen, G.[2], Hill, K.[3], Zdanio, M.[1], Markakis, M.N.[1], Mouille, G.[4], Holman, T.J.[3], Oh, J.[3], Wilson, M.H.[3], Swarup, R.[3], Grierson, C.S.[2], Bennett, M.J.[3], and Vissenberg, K.[1,5]

[1]Integrated Molecular Plant Physiology Research, Biology Department, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium.
[2]School of Biological Sciences, University of Bristol, BS8 1UG, United Kingdom.
[3]Centre for Plant Integrative Biology, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom.
[4]INRA Centre de Versailles-Grignon, Route de St-Cyr (RD10), 78026 Versailles, France.
[5]UASC-TEI, Plant Biochemistry & Biotechnology Lab, Dept. Agriculture, School of Agriculture, Food & Nutrition, Stavromenos, Heraklion, Crete, Greece.

Root hair morphogenesis is an auxin-regulated process ultimately dependent on localized synthesis, secretion and modification of the apical cell wall. However, (1) direct RH-specific targets for auxin-mediated transcriptional regulation are still lacking, (2) the RH cell wall is poorly understood and (3) the link between auxin and cell wall dynamics remains elusive.

Two microarray datasets on root hair elongation mutants identified 151 genes that are positively correlated with root hair growth in *Arabidopsis thaliana*. A reverse genetics approach identified *erulus*, a mutant in a putative cell wall sensing receptor-like kinase with a striking root hair (RH) phenotype. *Eru* root hairs are often swollen at the tip, exhibit a 69% length reduction and showed irregular and slower growth. ERU transcription is confined to trichoblast cell files only and occurs just before the visible outgrowth of bulges. *In vivo* spinning disc confocal microscopy of *ERUp*::*ERU-GFP* showed that ERU is delivered by vesicles to the apical plasma membrane throughout RH development.

*In silico* analysis revealed several ARF transcription factor-binding sites in the ERU promoter, suggesting auxin-dependent transcription. Micro-array data of control and *arf7/arf19* mutant roots treated with auxin

confirmed this hypothesis. Furthermore, ChIP-qPCR confirmed that the promoter of *ERU* is a direct target of ARF7 and ARF19. In addition, several well-studied core RH genes upstream of ERU were found to be auxin-inducible in an ARF7/ARF19-dependent manner, suggesting that *ERU* is both directly and indirectly regulated by auxin.

*ERU* belongs to the family of putative cell wall sensing CrRLK1L proteins. Micro-Fourier Transform-Infrared (FT-IR) microscopy revealed compositional cell wall changes in *eru* mutant RH. Whole mount immunolocalization of cell wall components*, in vivo* visualization of pectin $Ca^{2+}$ egg-box oscillations and enzymatic determination of pectin methylesterase activity lead to the conclusion that ERU exerts its tip-growth control through modulation of cell wall pectin dynamics by negatively regulating pectin methylesterase activity. In addition, ERU expression was specifically reduced in pectin-perturbed mutants, suggesting a close relationship between ERU and pectin.

We conclude that *ERU,* as a first*,* provides a direct link between ARF7/ARF19-mediated auxin signaling and pectin cell wall dynamics during RH morphogenesis. Moreover, our results highlight the underestimated complexity by which auxin regulates RH development.

# Root and shoot traits for drought tolerance in faba bean (*Vicia faba L.*)

Belachew, K.Y.[1], Nagel, K.A.[2], and Stoddard, F.L.[3]

[1]Department of Agricultural Sciences, Viikki Plant Science Centre, University of Helsinki, Helsinki, Finland.
[2]IBG-2: Plant Sciences, Forschungszentrum Jülich GmbH, 52425 Jülich, Germany.
[3]Department of Food and Environmental Sciences, Viikki Plant Science Centre, University of Helsinki, Helsinki, Finland.
kiflemariam.belachew@helsinki.fi

Faba bean (*Vicia faba* L.) is an important legume crop throughout the world. It is used for both food and feed, and its use in crop rotations has environmental benefits. It is considered relatively sensitive to water deficit, and in the era of climate change and increased temperature, drought poses a great challenge to the sustainable production of the crop. Many accessions show leaf wilting symptoms even at moderate soil water potential, and yield gap and instability are the main problems of the crop in drought-affected areas.

In earlier work, 200 accessions from drought-affected regions and 200 from seldom droughted regions were screened in glasshouse conditions. For this work, these numbers were reduced to 50 + 50 on the basis of recorded differences in canopy temperature depression, country of origin and availability of seeds. These 100 were grown again in the glasshouse in spring 2016 and 8 were selected for further investigation on the basis of contrasts in stomatal conductance, canopy temperature, root and shoot dry weight, and root to shoot dry weight ratio. In order to identify root traits associated with drought tolerance in faba bean, these 8 accessions were grown in peat-based potting medium in GROWSCREEN-Rhizo boxes under well watered and water-limited conditions. The experiment was arranged in a split-plot design with treatment as the main plot and accession as the subplot, in 4 replications, and ran from 24 January to 20 February 2017 at the Forschungszentrum Jülich GmbH, Germany in collaboration with Jülich Plant Phenotyping Center (JPPC). Key measurements were the lengths of tap, lateral and tertiary roots, root system depth and width, stomatal conductance, chlorophyll concentration and leaf number.

The genotype by treatment interaction was significant in primary and tertiary root lengths, but not in other root measurements. Accession DS70622 showed the maximum value of all root measurements in all conditions, and was the best at maintaining its root length when exposed to water deficit. It combined these traits with maximum chlorophyll concentration and leaf count, so it is a valuable new source of potential drought avoidance by water uptake. The lowest values of root length, depth and width were found in either Mélodie/2 or WS99501. Total root length of DS70622 was almost four times that of Mélodie. The maximum setback of root length due to drought was in DS74573, although it is from a drought-affected zone.

Use of the GROWSCREEN-Rhizo boxes allowed detection of useful differences in root response to water deficit in faba bean. Drought-tolerant accessions maintained their primary and tertiary root lengths, reduced their stomatal conductance and increased leaf chlorophyll content. These phenotypic markers will be associated with the genotyping data being generated in parallel work.

## Light and flavonols limits root growth and responses

del Pozo, J.C.[1], Silva-Navas, J.[1], Moreno-Risueno, M.A.[3], Manzano, C.[1], Téllez-Robledo, B.[1], Navarro-Neila, S.[1], Carrasco, V.[3], Pollmann, S.[3], and Gallego, F.J.[2]

[1]Centro de Biotecnología y Genómica de Plantas (CBGP). Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria. Campus de Montegancedo, Pozuelo de Alarcón, 28223 Madrid, Spain.
[2]Dpto. de Genética, Facultad de Biología, Universidad Complutense de Madrid, Madrid 28040, Spain.
[3]Centro de Biotecnología y Genómica de Plantas (CBGP). Universidad Politécnica de Madrid. Campus de Montegancedo, Pozuelo de Alarcón, 28223 Madrid, Spain.

Roots normally grow in darkness, but they may be exposed to light. However, in the lab we normally cultivate roots in presence of light. We have demonstrated that root illumination is a stress that affects root growth and responses. Light increases the level of several metabolites in roots. Among them, flavonols accumulated to high levels along the root. Dark-grown roots, after perceiving light, bend to escape from illumination (root light avoidance). In this response, flavonols rapidly accumulate at the side closer to light in the transition zone. This accumulation promotes asymmetrical cell elongation and causes a differential growth between both sides, leading to root bending. If the illumination persists, flavonol content increased to high levels and root growth is retarded. We found that high level of flavonols diminishes root growth and cell division by reducing auxin signaling, PLETHORA gradient and superoxide radical content in the root meristem. In other hand, cytokinin and hydrogen peroxide, which promote root differentiation, induce flavonol accumulation in the root transition zone, decreasing root meristem size. We propose that flavonols function as positional signals, integrating hormonal and ROS pathways to regulate root growth direction and rate in response to light.

# SESSION 2:
# Reproductive growth and development

# The role of OVATE Family Proteins in tomato fruit patterning

Van der Knaap, E.[1,2], Wu, S.[1], Keyhaninejad, N.[1,2], and Meulia, T.[1]

[1]The Ohio State University, Wooster OH 44691, USA.
[2]University of Georgia, Athens GA 30602, USA.

The final shape and size of fruits result from coordinated cell proliferation and expansion along different axes of growth. The shape of many elongated and pear-shaped tomato varieties is controlled by a naturally occurring premature stop mutation in *OVATE*, a member of the Ovate Family Proteins (OFPs) class. Histological analyses demonstrated that the mutation results in elongated shape associated with an altered cell division pattern. Mapping of the suppressors of the *ovate* (*sov*) led to the identification of another member of the family, *SlOFP20*, as the candidate gene underlying *sov1*. A synergistic interaction was found between *ovate* and *sov1* in controlling fruit elongation, which suggests that *OVATE* and *SlOFP20* are involved in the same pathway. Yeast 2 Hybrid experiments showed that OVATE and SlOFP20 interact with members of a protein complex that regulates the formation of preprophase band and organization of cortical microtubule array. Transient co-expression in *N. benthamiana* resulted in relocalization of OVATE and SlOFP20. Our findings are starting to shed light on the role of OFPs in proximal-distal patterning of fruit and provide insights into fundamental aspects of plant organ growth.

## Dissecting the conserved *LAFL* gene regulatory network that controls seed development

Dubreucq, B., Baud, S., Boulard, C., Fatihi, A., Fiume, E., Kelemen, Z., Miquel, M., Thévenin, J., To, A., and Lepiniec, L.

IJPB, INRA-AgroParisTech-CNRS, Université Paris-Saclay, France.

Genetic and molecular analyses have demonstrated the key role of Arabidopsis "LAFL"transcription factors in controlling seed development and maturation. *LEAFY COTYLEDON 1 (LEC1), ABSCISIC INSENSITIVE 3 (ABI3), FUSCA3 (FUS3), and LEC2* encode a homolog of the *NF-YB* protein and three transcriptional regulators of the "B3-domain"family, respectively.

The LAFL factors have pleiotropic and partially overlapping function in seed maturation. For instance, LEC2 induces the genes coding for seed storage proteins and proteins of the oil bodies (*e.g.* OLEOSIN1), or regulatory genes such as *WRINKLED1* (*WRI1*) and *MYB118*. WRI1 is a member of the AP2 family that controls glycolytic and fatty acid biosynthetic genes and oil accumulation. MYB118 represses the maturation program during early endosperm development.

We will illustrate recent progress made in the characterization of these LAFL proteins, their regulation, partners, target genes and evolutionary conservation among angiosperms. Last, we will show the interest of investigating further the environmental and epigenetic regulation of this network for the coming years

# Glutamate Receptor-Like (GLR) channels in plants: evolution and function on $Ca^{2+}$ homeostasis in sperm and male reproduction

Feijó, J.A.

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, Maryland, USA.

I will report on advances on the biology of Glutamate- Receptor Like (GLR) Ca2+-channels. A growing body of literature published in recent years has demonstrated that GLRs act as Ca2+ channels in plant cells, and are involved in various fundamental physiological functions, from reproduction to stress and pathogen resistance. In pollen, where we described their function for the first time, they were hypothesized to participate on Ca2+ homeostasis. I will present data suggesting an evolutionary conservation of these channels related to a male reproductive function in *Physcomittrela patens* (*Pp*). Double GLR KO plants of Pp show no visible defects on somatic development, but are almost sterile. We have genetically determined that sterility is carried by the male gamete lineage. We thus designed a sperm navigation assay and determined that chemotaxis is affected in the KO sperm. In accordance to a role in CA2+ homeostasis, KO sperm has lower levels of cytoplasmic Ca2+. We fully characterized the patch-clamp electrophysiology of the GLR on protoplasts and heterologous mammalian expression, and show that, despite chloride and strong cation non-selective conductances, PpGLRs do have a defined Ca2+ conductivity associated. Surprisingly, on the rare events that fertilization occurs on the KO plants, the sporophyte does not develop normally or at all, on what seems like an extra checkpoint for fertilization dependent on GLR function. We have queried our transcriptomic databases, and found that the BELL2 transcription factor, which is fundamental for the haploid to diploid transition in Chlamydomonas, is almost totally repressed on the double KO. We hypothesized that the sporophyte development is dependent on BELL2, and generated complementation lines of the double GLR KO with BELL2 under a the GLR2 promoter, which is specifically expressed during gamete formation. The complemented line rescued the phenotype thus confirming the dependency of BELL2 for transition to diploid status. Our work revealed an unexpected dependency of GLR for male reproduction, leading us to suggest this role as the major selective pressure to conserve this family of genes throughout terrestrial plant evolution.

# The HUA-PEP ribonucleoproteins regulate ovule development in Arabidopsis via post-transcriptional control of D-function identity genes

Rodríguez-Cazorla, E.[1], Ortuño-Miquel, S.[1], Ripoll, J.J.[2], Candela, H.[3], Bailey, L.J.[2], Yanofsky, M.F.[2], Martínez-Laborda, A.[1], and Vera, A.[1]

[1]Área de Genética, Universidad Miguel Hernández, Campus de Sant Joan d'Alacant, Sant Joan d'Alacant, Alicante, Spain.
[2]Division of Biological Sciences, Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA, USA.
[3]Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

In eukaryotes, correct gene expression relies on the production of functional transcripts, which requires a complex interplay between transcription and RNA processing activities. RNA-binding proteins (RBPs) play key roles as they assemble into multifunctional complexes on nascent transcripts [1]. Co-transcriptional RNA modifications are surveilled by the carboxyl-terminal domain (CTD) of the RNA polymerase II (RNAP II) large subunit, securing the fidelity of the operation. In this regard, the CTD phosphorylation state is crucial for the final output of gene expression [2].

In Arabidopsis, *HUA1*, *HUA2*, *HEN4*, *FLK* and *PEP* (collectively named *HUA-PEP* activity) encode a set of interacting RBPs that regulate the MADS-box floral homeotic gene *AGAMOUS* (*AG*). HUA-PEP proteins affect flower organ identity by maintaining the floral C-function via accurate processing of the *AG* large second intron. Otherwise, non-functional *AG* transcripts retaining intron sequences accumulate impairing correct flower patterning [3].

Arabidopsis ovule identity is specified by the D-function genes *SHATTERPROOF1* (*SHP1*), *SHP2*, and *SEEDSTICK* (*STK*), closely related to *AG* [4]. Here, we show that mutations in the *HUA-PEP* activity genes lead to homeotic transformation of ovules into flower organ-like structures. Accordingly, *hua-pep* mutants exhibit reduced expression of D-genes and accumulate aberrant transcripts that retain intron sequences, strongly suggesting post-transcriptional misregulation of ovule identity D-genes. Our transcriptomic data and the ability of PEP and HUA1 proteins to interact with the RNAP II CTD regulator C-TERMINAL DOMAIN PHOSPHATASE-LIKE1 (CPL1) further support our current model.

[1] Bentley, D.L. (2014). *Nat. Rev. Genet.* 15, 163-175.
[2] Hsin, J.P., and Manley, J.L. (2012). *Genes Dev.* 26, 2119-2137.
[3] Rodriguez-Cazorla, E., *et al*. (2015). *PLoS Genet.* 11, e1004983.
[4] Pinyopich, A., *et al*. (2003). *Nature* 424, 85-88

Funded by the Spanish Ministry of Economy and Competitiveness (Grant BIO2014-56321-P) to AV and AML.

# How TCP5 keeps the Arabidopsis petal in good shape

van Es, S.W.[1], Rocha, D.I.[2], Silveira, S.R.[3], Bimbo, A.[1], van der Wal, F.[1], Martinelli, A.P.[3], Dornelas, M.C.[2], Angenent, G.C.[1], and Immink, R.G.H.[1]

[1]Plant Developmental Systems (PDS), Wageningen University and Research, Radix building 107, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

[2]Departamento de Biologia Vegetal, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Sao Paulo CEP 13083–862, Brazil.

[3]Laboratório de Biotecnologia Vegetal, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Piracicaba, SP CEP: 13416-000, Brazil.

We studied the functioning of the CIN-type *TCP5*-like genes during floral organ development and performed mutant analyses, detailed spatial and temporal expression studies, and protein complex isolations.

We found that TCP5 regulates petal size; overexpression of *TCP5* in epidermal cells results in smaller petals, whereas the knock-out grows wider petals with an increased surface area. Comprehensive expression studies of loss of function and gain of function mutants by RNA-seq suggests, besides the observation of differential expression in cell cycle, growth regulation, developmental processes and organ growth related genes, a role for ethylene biosynthesis and signalling in petal differentiation. Ethylene is known to influence cell elongation in petals and other tissue and the ethylene biosynthesis genes 1-*amino-cyclopropane-1-carboxylate synthase 2* (*ACS2*) and *ACC oxidase 2* (*ACO2*) are found to be differentially regulated in our mutants in addition to several *ETHYLENE RESPONSE FACTOR* (*ERFs*).

Detailed investigation of the cell shape, size, and structure revealed interesting alterations in the cells at the distal end of the adaxial side of the petal in *tcp5*-like mutant backgrounds. The distal end of a petal is home to conical cells that reflect UV light to attract pollinators. In our mutants however, in both overexpressor and knock-out lines conical cell development is disturbed, apparent by loss of a dome-shaped structure and an increase in cell size due to increased cell elongation, and disorganization of the cuticular ridges.

Ongoing research is focussing on direct transcriptional targets of TCP5 and the function of its physical interaction partners, as well as confirming the role of TCP5 in ethylene biosynthesis and signalling during petal growth.

# Tomato *MEDIATOR COMPLEX SUBUNIT 18* (*MED18*) plays a key role in pollen development: evidences from the functional analysis of *pollen deficient 1* (*pod1*) mutant

Pérez-Martín, F.[1], Yuste-Lisbona, F.J.[1], Giménez, E.[1], Pineda, B.[2], Capel, C.[1], García-Sogo, B.[2], Sánchez, S.[2], Angosto, T.[1], Capel, J.[1], Moreno, V.[2], and Lozano, R.[1]

[1]Centro de Investigación en Biotecnología Agroalimentaria (BITAL). Universidad de Almería, 04120 Almería, Spain.
[2]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia. 46022 Valencia, Spain.

Pollen development is a key step in the life cycle of angiosperms and it depends on a coordinated spatio-temporal regulation of gene expression at early stages of reproductive development. An appropriate pollen formation is essential for the maintenance of biological diversity as well as the production of fruits and seed in agronomical important crop species. Furthermore, in fleshy fruit plants like tomato (*Solanum lycopersicum* L.), defects in pollen ontogeny produces parthenocarpic (seedless) fruits, which are considered to be of great importance since they guaranteed fruit yield under unfavorable conditions and also have a high commercial value.

In this study, we described the tomato enhancer trap T-DNA mutant *pollen deficient 1* (*pod1*) that displayed abnormalities in pollen development, which leads to production of parthenocarpic fruits. Analysis of the genomic T-DNA flanking sequences displayed that a single T-DNA copy was inserted in an intergenic region of chromosome 6 between *ZINC FINGER* HIT-type (*ZF*-HIT) and *MEDIATOR COMPLEX SUBUNIT 18* (*MED18*) genes. Expression analysis and characterization of silencing lines demonstrated that the *pod1* mutant phenotype relies on the tomato *MED18* gene (*POD1/SlMED18*), which encodes a member of the Mediator multi-protein complex involved in the regulation of RNA polymerase II transcription. A detailed histological characterization of anther development indicated that microspores were degenerated at the tetrad stage, although tapetum tissue developed normally. Expression of pollen marker genes confirmed that *POD1/SlMED18* is required for the proper pollen formation and fruit development.

Additionally, we demonstrated that *MED18* homologs share functional homology in Arabidopsis and tomato species as *POD1/SlMED18*

is able to rescue the flowering time and floral organ identity abnormalities of the Arabidopsis *med18* mutant (Zheng et al., 2013). Nevertheless, our results indicated that *SlMED18* has evolved to acquire a novel function in tomato, which is the genetic control of male gametogenesis.

# A direct genetic strategy for identifying novel regulators of fruit tissue morphology in tomato

Musseau, C.[1], Jorly, J.[2], Just, D.[2], Lemaire-Chamley, M.[2], Chevalier, C.[2], Rothan, C.[2], Gévaudant, F.[1], and Fernandez, L.[2]

[1]University of Bordeaux, UMR1332 Biologie du Fruit et Pathologie, INRA Bordeaux Aquitaine, CS20032, F-33882 Villenave d'Ornon cedex, France.
[2]INRA, UMR1332 Biologie du Fruit et Pathologie, INRA Bordeaux Aquitaine, CS20032, F-33882, Villenave d'Ornon cedex, France.

Tissue morphogenesis and overall fruit growth depend on complex cellular and molecular interactions that affect the balance between cell division and cell expansion. The molecular control of fruit tissue morphogenesis and growth remain largely underexplored and only few regulators have been already identified. Forward genetics appears as the most powerful approach to decipher these processes and their regulation, because natural and artificially-induced genetic variability offers invaluable resources for discovering new phenotypes and new allelic variants. Thanks to the availability of tomato genomic sequence and deep sequencing technologies, linking genotypic variations to associated phenotypic changes is now more accessible for fleshy fruit.

The whole strategy, based on tools currently available will be described, from the phenotypic screening of tomato mutants, from our EMS collection, to the identification of the causal mutation by mapping-by-sequencing and its validation by CRISPR technologies. The phenotypic characterization of twelve mutants revealed two additional modules controlling fruit growth that coordinate either the growth of the whole fruit or that of pericarp, through either isotropic or anisotropic cell expansion.

Whole-genome-sequencing and polymorphism analysis of four representative mutants allowed us to demonstrate that those modules were not related to genes and processes previously described. Furthermore, we recently developed the mapping-by-sequencing approach [1] that will give access to the identification of a novel regulator coding for the Guanylate Binding Protein (GBP). Mutant alterations suggest a role of this protein in cytoskeletal organization and cell wall modifications with obvious impact on tissue morphology and fruit growth.

[1] Garcia, V., *et al.* (2015). Rapid identification of causal mutations in tomato EMS populations via mapping-by-sequencing. *Nat. Protoc.* 11, 2401-2418.

# Tomato fruit locular tissue differentiation is regulated by a C2H2 transcription factor

Viron, N.[1,2], Jorly, J.[1,2], Noilhan, B., Brès, C.[1,2], Mauxion, J.P.[1,2], Garcia, V.[1,2], Wong Jun Tai, F.[1,2], Rothan, C.[1,2], and Lemaire-Chamley, M.[1,2]

[1]INRA, UMR 1332 de Biologie du fruit et Pathologie, F-33140 Villenave d'Ornon, France.
[2]Univ. Bordeaux, UMR 1332 de Biologie du fruit et Pathologie, F-33140 Villenave d'Ornon, France.

In tomato, locular tissue or gel differentiates from the fruit central axis a few days after ovule fertilization. Cells from the locular tissue are then undergoing successive processes of cell division and cell expansion which lead to the formation of a gelatinous tissue surrounding the seeds.

As a consequence of tomato transformation using a RNAi construct, we isolated a transgenic line with a fruit gel less phenotype. Fruits from homozygous plants were indeed characterized by a total absence of locular tissue, a change in seed shape and an increase of fruit firmness. According to molecular data, we demonstrated that this phenotype was linked to an insertion in tomato genome. Using a combination of genetic mapping and NGS mapping, we were able to identify the mutation as the insertion of a Ty1/copia retrotransposon in the coding sequence of a C2H2 Zinc Finger gene. The generation of KO lines by CRISPR/Cas9 allowed us to confirm the function of this C2H2 in locular tissue differentiation.

The expression profile of this gene in tomato organs and during fruit development was consistent with the specific phenotypes observed [1]. In-depth molecular and physiological study of the *gel less* mutant lines will be conducted to evaluate the consequences of *gel less* mutation on tomato fruit quality and to elucidate the implication of this C2H2 in locular tissue differentiation.

[1] Weng, L., Zhao, F., Li, R., Xu, C., Chen, K., and Xiao, H. (2015). *Plant Physiol*. 167, 931-949.

# Identification of organ-specific clock structure and outputs of growth using artificial vision systems and Machine Learning processing

Weiss, J.[1], Perez-Sanz, F.[1], Navarro, P.J.[2], Terry, M.-I.[1], and Egea-Cortines, M.[1]

[1]Genética, Instituto de Biotecnología Vegetal Universidad Politécnica de Cartagena 30202 Spain.
[2]Tecnología Electrónica, Escuela Técnica Superior de Ingeniería de Telecomunicaciones, Universidad Politécnica de Cartagena 30202 Spain.

The plant circadian clock is involved in the integration of external cues such as light and temperature, and controls growth, primary and secondary metabolism or plant organ movement among other traits. The current paradigm, based on Arabidopsis leaves and seedlings is that due to the transcriptional repression and activation of the genes comprising the clock, mutations affect via increase or decrease in the clock speed. This results in early or delayed outputs depending on the mutation

We have performed a comprehensive analysis of the Petunia clock in leaves and for the first time we characterized the petal clock. The Petunia circadian clock comprises additional paralogs compared to Arabidopsis with duplicated copies of PRR7, PRR5 and GIGANTEA. The transcriptional structure of the leaf and petal clock differed in the timing of several genes as well as what appears to be an organ specific expression of the paralogs.

Using artificial vision systems and a Machine Learning process to extract image information we found that knockdown of *PaxilZTL* by RNA caused early gating of growth of the stem and early changes in the flower angle. Surprisingly, flower growth was delayed. Our data indicates that the gating of clock outputs maybe organ specific. This may be caused by differences in the local circadian clock transcriptional structure.

# SESSION 3:
# Leaf and shoot growth and development I

# Role of *DESIGUAL1* and auxin in bilateral symmetry of Arabidopsis leaves

Wilson-Sánchez, D., Navarro-Cartagena, S., Lup, S.D., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

Most living organisms exhibit some form of symmetry; however, there is a dearth of mutations affecting bilateral symmetry in all biological systems. This lack of mutations has hampered genetic analysis of bilateral symmetry in multicellular organisms, particularly plants. To examine the regulation of symmetry and other aspects of leaf development, we screened 19,850 Arabidopsis lines from the Salk homozygous T-DNA collection and found 706 leaf mutants. Only one of these mutants exhibited defects in bilateral symmetry; we named this mutant *desigual1-1* (*deal1-1*).

Arabidopsis has bilaterally symmetric leaves with interspersed marginal lobes and indentations along the margin. Several overlapping regulatory pathways establish these marginal features; these pathways involve feedback loops of auxin, the PIN-FORMED1 (PIN1) auxin efflux carrier, and the CUP-SHAPED COTYLEDON2 (CUC2) transcriptional regulator.

The *deal1* mutants have randomly asymmetric leaves that fail to acquire symmetry in the early stages of leaf primordium development, but instead form ectopic lobes and sinuses. In the leaves of *deal1* mutants, improper regulation of cell division (simultaneous over- and under-proliferation) along the organ margins alters bilateral symmetry in the leaf primordium stage. Auxin maxima are mislocalized at the margins of expanding *deal1* leaves and this asymmetry can be enhanced by treatment with the polar auxin transport inhibitor 1-N-naphthylphthalamic acid or alleviated by treatment with the synthetic auxin 1-naphthaleneacetic acid. Among other defects, *deal1* mutants show aberrant recruitment of marginal cells expressing properly polarized PIN1, resulting in misplaced auxin maxima. Normal PIN1 polarization requires *CUC2* expression and *CUC2* genetically interacts with *DEAL1*; *DEAL1* also affects *CUC2* expression in the leaf primordium margin. DEAL1, a protein of unknown molecular function, localizes to the endoplasmic reticulum membrane and functions in the leaf, acting partially redundantly with its two closest paralogs.

## Using gene networks to elucidate developmental processes

Ichihashi, Y.[1,2], Zumstein, K.[1], Nakayama, H.[1], Farhi, M.[1], and Sinha, N.[1]

[1]Department of Plant Biology, University of California, Davis, USA.
[2]Riken, Yokohama, Japan.

How morphological diversity has arisen is a key question in biology. Angiosperms exhibit a great diversity in leaf shape and leaf development has been characterized in several species, making leaves ideal targets to understand the mechanism behind morphological natural variation. Leaves are also functionally significant for generating biomass and leading to agricultural yield. We performed comparative transcriptomics utilizing three *Solanum* species showing different leaf development characteristics. We utilized gene network construction to identify key network modules that play a role in leaf development. Super self-organizing map clustering, which can account for multiple factors by using a separate weighted layer for every factor, identifies major interspecific changes of gene expression patterns in leaf development. Our analyses suggest that not only massive differential gene expression but also changes in the system-level regulation of gene expression pattern differentiate leaf development between the species. We have used *differential correlation interactions (DiffCorr)* between genes in the Gene Regulatory Network (GRN) *across species* to detect *GRN* rewiring and identify genes that play a role in generating developmental differences between these species.

## Cell proliferation and cell expansion in leaves

Tsukaya, H.[1,2]

[1]Grad. Sch. Sci., Univ. Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
[2]Okazaki Institute for Integrative Bioscience, Yamate Build. #3, East-9F, 5-1, Higashiyama, Okazaki, Aichi 444-8787, Japan.

The relationship between cell size and ploidy level is not as simple as has been believed. While epidermal cell size shows a good correlation with ploidy level that is under the control of endoreduplication, as has been well known, parenchymatous cell size does not. Interestingly, the correlation changes if *ATML1,* a transcription factor for epidermal identity, is ectopically expressed in the parenchymatous tissue, indicating that the correlation is governed by some genetic pathways. On the other hand, our meta-data analysis revealed some interesting tendency between the effect of excess endoreduplication on average cell size. More careful re-examination on the linkage between endoreduplication and cell size appears to be needed to understand the mechanisms of leaf size control.

On the other hand, spatial determination of meristematic activity in leaf primordia is also key to regulate leaf size. As we have already reported, AN3/AtGIF1, a transcriptional co-activator, can move between cell layers in leaf primordia, enabling synchronized cell proliferation in leaf primordia. Thus it is very plausible that AN3 can move also along the longitudinal axis, considering that the spatial distribution of the meristematic activity in leaf primordia is much wider than the *AN3*-mRNA expression domain and shows a longitudinal gradient. Unexpectedly, we found that the speed of intercellular movement of soluble protein in leaf primordia is biased: it is more rapid in the distal areas than in the proximal areas. Detailed analyses indicated that the bias is caused by differed cell sizes along the longitudinal axis of the primordia. A mathematical model beautifully reproduced the spatial distribution of meristematic cells by considering the biased intercellular movements of AN3 protein, strongly indicating that the AN3 is the key factor in regulating the spatial patterning of leaf meristems.

[1] Katagiri, Y., *et al*. (2016). *Development* 143, 1120-1125.
[2] Kawade, K., *et al.* (2013). *Curr. Biol.* 23, 788-792.
[3] Kawade, K., *et al.*, submitted.

# Calmodulin-binding IQD proteins are essential for cell plane selection and cell shape formation in *Arabidopsis thaliana*

Bürstenbinder, K.[1], Mitra, D.[1], Abel, S.[1], and Möller, B.[2]

[1]Department of Molecular Signal Processing, Leibniz Institute of Plant Biochemistry, 06120 Halle (Saale), Germany.
[2]Institute of Computer Science, Martin Luther University Halle-Wittenberg, 06120 Halle (Saale), Germany.

Calcium ions ($Ca^{2+}$) act as universal second messengers in all eukaryotes, and play important roles in the control of plant growth. Changes in intracellular $Ca^{2+}$ levels are perceived by $Ca^{2+}$ binding proteins, such as calmodulins (CaM), which transduce $Ca^{2+}$ signals into cellular responses by regulation of diverse target proteins. We previously identified IQ67-domain proteins (IQDs) as novel plant-specific CaM targets, which are encoded by multigene families (23-67 members) in tomato, maize, soybean, rice and Arabidopsis. Despite their large family sizes, modes of IQD action, however, are still largely elusive.

To study IQD functions, we initiated a comprehensive characterization of the 33-membered Arabidopsis IQD family using reverse genetics approaches. We showed, by analysis of the subcellular localization of translational GFP fusion proteins, that most Arabidopsis IQD members localize to microtubules, where they recruit CaM $Ca^{2+}$ sensors. Important functions at microtubules are further supported by altered microtubule organization and plant growth in *IQD* gain-of-function lines. To identify IQDs with potentially overlapping functions and to gain insight into spatio-temporal expression patterns of *IQD* genes, we generated a cellular expression map of the Arabidopsis IQD family. Promoter activity was largely restricted to meristematic and actively elongating tissues, e.g. in the root and shoot meristem, or during embryo development. Molecular and functional analysis of loss-of-function lines revealed defects in leaf epidermal cell shape, and in positioning of the cell plate during cytokinesis. Together, we hypothesize that IQDs function during cell division and elongation to control cell shape and organ growth, possibly by linking CaM $Ca^{2+}$ signaling to the regulation of the microtubule cytoskeleton.

# Effect of higher ploidy levels on plant growth and biomass composition

Corneille, S.[1,2], Vanholme, B.[1,2], and Boerjan, W.[1,2]

[1]Bio-energy and bio-aromatics, VIB-UGent Center for Plant Systems Biology, VIB, Ghent, Belgium.
[2]Bio-energy and bio-aromatics, Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent, Belgium.

Improving plant production is a necessity to guarantee food security in the face of a rapidly growing world population. In addition, plant biomass is currently the only available renewable feedstock to replace petro-based chemicals. In order to increase the use of lignocellulosic biomass as a sustainable source for the bio-economy, both biomass production and composition need to be optimized.

Polyploidization or whole genome duplication can play a role in achieving these goals. The increase in biomass yield upon polyploidization is well known, but the effect on biomass composition is less well studied. To get additional insights into the effects of polyploidization on plant growth and biomass composition, we decided to study the growth, yield, and biomass composition of a series of autopolyploid *Arabidopsis thaliana* plants, including tetraploids, hexaploids and octaploids. Besides an elaborate phenotypic analysis whereby different growth parameters were determined, we performed a thorough cell wall characterization and performed saccharification experiments on the dry biomass.

It is to our knowledge the first time that trends in growth and biomass composition were quantified in a set of plants which differ only in their degree of ploidy. Besides the fundamental insights, our research shows that polyploidy could potentially play a role in breeding programs to obtain economically valuable plants that could be used as a feedstock for the bio-economy.

# The plant-specific CDK-inhibitory proteins SMR1 and SMR5 are regulated post-translationally and stabilized under water-limiting conditions to participate in cell cycle arrest and growth repression

Dubois, M.[1], Marrocco, K.[1,2], Bach, L.[2], Lamy, G.[1], Granier, C.[2], and Genschik, P.[2]

[1]Institut de Biologie Moléculaire des Plantes (IBMP), CNRS, Strasbourg, France.
[2]Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), INRA, Montpellier, France.

The cell cycle of plants is tightly regulated, integrating endogenous cues and environmental signals to adapt plant growth to changing conditions. Under drought, cell division in young leaves is blocked as an active mechanism to save energy resources. While the molecular function of CDK-inhibitory proteins (CKI) in regulating the cell cycle has already been extensively studied, very little is known about the mechanisms to arrest the cell cycle under drought. In this study, we show that among the known CKIs, *SMR1* and *SMR5* transcripts are strongly and quickly induced under moderate drought in Arabidopsis leaves. Functional characterization of these genes further reveal that SMR1 and SMR5 inhibit cell division and affect meristem structure, thereby restricting the growth of leaves and roots. Importantly, we demonstrate that SMR1 and SMR5 are short-lived proteins, which are degraded by the 26S proteasome after being ubiquitinylated by a RING-type E3-ligase and putatively phosphorylated by CDKA. Supporting this, stable lines overexpressing non-degradable SMR1-alleles have much stronger phenotypes than SMR1-overexpressing plants. Under moderate drought, the SMR1 protein turn-over is slowed down. Accordingly, *smr1* mutants are slightly more tolerant to drought, suggesting that SMR1, and likely also SMR5, participate in the cell cycle arrest under drought stress. Surprisingly, not the classical drought hormone ABA but rather the growth-repressive hormone ethylene is a good candidate for acting upstream of transcriptional and post-translational accumulation of SMR1. These results fit within the emerging view of mild drought response in young Arabidopsis leaves, placing ethylene in the center of an active decision to arrest the cell cycle and decrease growth.

# Transient reconstitution of the plant cell cycle regulatory machinery in leaf mesophyll protoplasts to identify direct SnRK1 growth targets

Hulsmans, S. , and Rolland, F.

Laboratory of Molecular Plant Biology, Biology Department, University of Leuven-KU Leuven, Kasteelpark Arenberg 31, 3001 Heverlee-Leuven, Belgium.

As sessile and autotrophic organisms, plants are significantly challenged by the changing environment and continuously need to adjust their growth and metabolism to resource supplies. The cellular carbon and energy status functions as an important point of integration of both developmental and environmental signals. The SnRK1 (SNF1-related kinase 1) kinase acts as a cellular fuel gauge, that is activated in response to diverse energy-depleting stress conditions to maintain energy homeostasis for optimal growth and survival. Although alteration of SnRK1 activity can have dramatic effects on plant development, whether and how SnRK1 directly affects growth-controlling processes, such as cell division and cell expansion, is still unclear.

We started exploring Arabidopsis leaf mesophyll protoplasts as a tool to study possible molecular interactions with the cell cycle regulatory machinery. Interestingly, both application of sugars and hormones and reconstitution of the upstream regulatory pathways by transient over-expression effectively induced S- and M-phase specific gene expression in differentiated leaf cells. Co-expression with the SnRK1 catalytic α subunit (KIN10) now enables identification of direct targets. One reported link between energy availability and cell division is the transcriptional regulation of the G1/S phase transition-specific D-type cyclins by sugar availability. The use of *PrCYCD3;1::LUC* reporter constructs and transient expression of tagged proteins in protoplasts reveals both repression of *CYCD3;1* expression and enhanced proteasome-mediated degradation of the CYCD3;1 protein in response to increased SnRK1 activity.

In conclusion, leaf mesophyll protoplasts can be used to identify and dissect molecular mechanisms linking metabolic status to growth and development, although interactions need to be confirmed in meristematic tissue *in planta* and by genetics.

# Increased leaf size of *Arabidopsis* plants missing multiple *KRP* genes is an indirect consequence of early seed abortion

Sizani, B.L.[a,†], Kalve, S.[a,1,†], Nektarios, M.M., Domagalska, M.A., Stelmaszewska, J.[d], Veylder, L.D.[e], Gerth, S.[b], De Vos, D.[a], Schnittger, A.[c], and Beemster, G.T.S.[a,*]

[a]Department of Biology, Molecular Plant Physiology and Biotechnology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium.
[b]Department of Developmental Biology, University of Fürth, Germany.
[c]Department of Developmental Biology, University of Hamburg, Germany.
[d]Department of Reproduction and Gynecological Endocrinology Medical University of Bialystok, Bialystok, Poland.
[e]Department of Plant Systems Biology, VIB, 9052 Ghent, Belgium.
[1]Current address: Department of Plant and Soil Sciences, Institute for Agricultural Biosciences, Oklahoma State University, 3210 Sam Noble Parkway, Ardmore, OK 73401, USA.
[†]These authors contributed equally to this work.
*Corresponding author.

Possibly due to redundancy, loss of function of individual KRP cell cycle inhibitor genes does not have a significant effect on plant development. However, we observed a progressive increase of leaf size when multiple KRP genes are simultaneously down regulated. Down regulation of three KRP genes (*krp4/6/7*) had the most pronounced effect, increasing mature leaf area by up to 10%. Remarkably, kinematic analysis revealed that the increased leaf phenotype of *krp4/6/7* triple mutant was already established prior to the time frame covered by the analysis, suggesting that the increased leaf phenotype was already predetermined in the seed. Consistently, seed size analysis showed a strong correlation between seed size and leaf area in the wild-type and the *krp4/6/7* mutant. Furthermore, seeds of equal size of Col-0 and *krp4/6/7* mutant had nearly equal leaf phenotype. Noticeably, siliques of the *krp4/6/7* mutant were slightly shortened with less seeds than that of the wild-type. We hypothesise that the abortion of approximately 30% of the seeds in the *krp4/6/7* mutant, increases seed size in the *krp4/6/7* mutant due to reduced competition for of resources. Altogether these results suggest that the enlarged leaf area of the *krp4/6/7* triple mutant is an indirect consequence of seed abortion in the previous generation.

# A transcriptional repressor complex controls ROS homeostasis during leaf growth

Kunkowska, A.B., and Schippers, J.H.M.

Institute of Biology I, RWTH Aachen University, Worringerweg 1, 52074, Aachen, Germany.

Leaf growth starts with an undifferentiated populations of cells which through proliferation and expansion determine final organ size. Although both processes underlie strict genetic control, specific transcriptional networks have remained elusive.

Previously, we identified the Myb-like transcription factor KUODA1 (KUA1) as a specific regulator of cell expansion during leaf growth by direct repression of class III peroxidases (POXs). Members of this class are secreted to the cell wall where they modulate reactive oxygen species (ROS) homeostasis. POXs can both produce and breakdown ROS. The formation of hydrogen peroxide ($H_2O_2$) causes stiffening of the cell wall, restricting cell expansion. Conversion of $H_2O_2$ into the hydroxyl radical, which causes cell wall loosening through cleavage of xyloglucan, promotes cell expansion. KUA1-regulated POXs increase apoplastic $H_2O_2$ level, therefore KUA1 positively regulates leaf growth by remaining low levels of $H_2O_2$ in the apoplast. Interestingly, here we show that KUA1 interacts with members of the TOPLESS/TOPLESS-RELATED (TPL/TPR) family of corepressor proteins. We demonstrate that they are highly expressed at the beginning of the expansion phase. In addition, the *tpl* phenotype is complementary to *kua1*, while *tpr* mutants effect cell proliferation. Although TPL/TPR proteins interact with KUA1, they do not all seem to contribute to the regulation of cell expansion during leaf growth.

As KUA1 controls ROS homeostasis, we decided to determine if *tpl, tpr2* and *tpr4* have an impact on ROS levels. All mutants show different ROS levels during leaf growth as the wild type. Moreover, chemical inhibition of POX activity in *tpl, tpr2* and *tpr4* mutants resulted in different effects on leaf growth. This observation suggests that the different TPL/TPR proteins modulate the level of different POXs that either stimulate or restrict growth. Taken together, we reveal novel insight into the transcriptional network regulating cell expansion through the control of ROS homeostasis.

## IYO and RIMA are co-transported into the nucleus to activate cell differentiation

González-García, M.P., Sanmartín, M., Contreras, R., Muñoz, A., Sánchez-Serrano, J.J. and Rojo, E.

[1]Centro Nacional de Biotecnología-CSIC, Cantoblanco, E-28049 Madrid, Spain.

The transcriptional regulator MINIYO (IYO) is essential and rate-limiting for initiating cell differentiation in *Arabidopsis thaliana*. Moreover, IYO moves from the cytosol into the nucleus in cells at the meristem periphery, possibly triggering their differentiation. However, the genetic mechanisms controlling IYO nuclear accumulation were unknown and the evidence that increased nuclear IYO levels trigger differentiation remained correlative. We have identified an IYO-interacting protein named RIMA, which is homologous to proteins linked to nuclear import of selective cargo in yeast and mammals. The developmental phenotypes and transcriptomic changes caused by *IYO* or *RIMA* knockdown are very similar, supporting a close functional relationship between the proteins. Indeed, RIMA knockdown reduces the nuclear levels of IYO and prevents its pro-differentiation activity, supporting that RIMA-dependent nuclear IYO accumulation triggers cell differentiation in Arabidopsis. We are now testing the hypothesis that IYO and RIMA remain in complex after being transferred into the nucleus, where they would directly regulate transcription to activate cell differentiation.

# SESSION 4:
# Leaf and shoot growth and development II

## Small RNAs as mobile, morphogen-like signals in development

Skopelitis, D.[1], Benkovics, A.[1], Husbands, A.[1], and Timmermans, M.[1,2]

[1]Cold Spring Harbor Laboratory, 1 Bungtown Rd, Cold Spring Harbor, NY 11724, USA.
[2]Center for Plant Molecular Biology, University of Tuebingen, Auf der Morgenstelle 32, 72076 Tuebingen, Germany.

Adaxial-abaxial (top-bottom) polarity drives the flattened outgrowth and patterning of leaves, and represents an important innovation in the evolution of land plants. Patterning of this axis is driven by an intricate gene regulatory network. Integral to this network are two sets of conserved transcription factors that promote either adaxial or abaxial fate, and are expressed in complementary domains on the top or bottom side of the leaf, respectively. The positional information needed to delineate these domains is provided in part by the small RNAs miR166 and tasiR-ARF. We have shown that these small RNAs move outside their defined domain of biogenesis and form opposing gradients across the leaf that polarize expression of key adaxial- and abaxial-promoting transcription factors, HD-ZIPIII and ARF3/4, respectively. Our observations, which will be presented, indicate that mobile small RNAs have the inherent capacity to generate sharp gene expression boundaries, and function as morphogen-like signals in development. Their patterning properties present small RNAs and their targets as highly portable regulatory modules through which to create pattern, and provide a compelling basis for the extensive conservation and repeated co-option of developmentally important small RNA-target modules.

## The genetic basis for diversification of leaf form: from understanding to reconstructing

Tsiantis, M.

Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Köln, Germany.

A key challenge in biology is to understand how diversity in organismal form is generated. Genetic analyses in model systems have identified key regulators that sculpt the body plans of metazoa and seed plants. However, less is known about how the action of such regulators produces particular organ shapes, or how the balance of conservation versus divergence of such form regulating pathways generated the tremendous morphological diversity of multicellular eukaryotes. One impediment to answering these questions is the relative paucity of experimental platforms where genetic tools can be utilized to unambiguously study morphogenesis and its evolution in a genome-wide, unbiased fashion. To circumvent this problem we developed the *Arabidopsis thaliana* relative *Cardamine hirsuta* into a versatile system for studying morphological evolution. We aim to understand the molecular mechanisms through which leaf morphology evolved in these species, resulting in simple, undivided leaves in *A. thaliana* and dissected leaves with distinct leaflets in *C. hirsuta.* This presentation will discuss our progress towards understanding the morphogenetic pathways that specify dissected versus entire leaf shapes and that regulate the number, position and timing of leaflet production. It will also detail how studies in *C. hirsuta* have helped understand to what degree pathways underlying morphological variation between and within species overlap.

# Auxin methylation is required for differential growth in Arabidopsis

Abbas, M.[1,2], Hernández-García, J.[1], Pollmann, S.[3], Samodelov, S.L.[4], Kolb, M.[4], Friml, J.[2], Hammes, U.[5], Zurbriggen, M.[4], Alabadí, D.[1], and Blázquez, M.A.[1]

[1]Instituto de Biología Molecular y Celular de Plantas (CSIC-UPV), Spain.
[2]Institute of Science and Technology, Austria.
[3]Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Spain.
[4]Institute of Synthetic Biology, Germany.
[5]Spemann Graduate School of Biology and Medicine, Germany.

Auxin gradients are instrumental for the differential growth that causes organ bending upon tropic stimuli and curvatures during plant development. It has been shown that local differences in auxin concentrations are mainly achieved by polarized cellular distribution of PIN auxin transporters, but it is not clear if other mechanisms involving auxin homeostasis are also relevant for the formation of auxin gradients. We have found that auxin methylation is required for asymmetric auxin distribution across the hypocotyl, in particular during its response to gravity. In particular, loss-of-function mutants in the Arabidopsis *IAA CARBOXYL METHYLTRANSFERASE1* (*IAMT1*) gene prematurely open the apical hook, and their hypocotyls are impaired in gravitropic reorientation. This defect is linked to an increased polar auxin transport and to the lack of asymmetric distribution of PIN3 in the *iamt1* mutant, which presumably causes the accumulation of auxin on either side of the gravistimulated hypocotyl. Partial inhibition of polar auxin transport in the *iamt1* mutant resulted in the restoration of normal gravitropic reorient¬ation. We propose that IAA methylation is necessary to restrict polar auxin transport within the range of auxin levels that allow differential responses.

# Control of leaf cellular proliferation, differentiation and growth by light: establishing and distinguishing the roles of hormonal- and sugar-signalling

Farahi-Bilooei, S.[1], Mohammed, B.[2], Bogre, L.[1], and Lopez-Juez, E.[1]

[1]Royal Holloway University of London, Egham Hill, Egham, Surrey, TW20 0EX.
[2]John Innes Centre, Colney Lane, Norwich, NR4 7UH, U.K.

Light activates the shoot apical meristem to initiate the production of leaf primordia and eventually leaves, but this process is arrested in the dark. Light energy is itself required for leaf growth. We have in the past observed that the arrested meristem and primordia in the dark show a strong response to auxin. We now report that they also show a strong "starvation"gene expression. These signatures are rapidly turned by light into cytokinin-responsive and strong "feast"gene expression. Both coincide with ribosomal protein gene expression and simultaneous cell proliferation, key components of leaf initiation. The leaf primordia transfer to dark leads to disappearance of mitotic reporter activity but this will reappear in the light. Our data suggest that the seedling meristem and young leaf primordia may specifically experience active carbon starvation in the dark, this being quickly repressed when transferred to the light.

Plants' transfer from low light (LL) to high light (HL) also results in extra proliferation and growth. A leaf grown in HL is composed of several layers of larger cells. Transfer from LL to HL leads to the growth of larger lamina. Thus both multiple layers, and a larger lamina composed of more cells in two dimensions, occur in HL. From those observations, we propose that energy signalling processes are also central to leaf growth under natural, varying light conditions.

Our work currently aims to identify the exact location of the observed gene expression responses of Arabidopsis meristems and leaf primordia in dark and light, using cytokinin and auxin reporters, investigate the signalling pathway of the starvation/feast response of meristematic activity, confirm both control mechanisms and understand whether they have different roles, and test their further involvement in the response to different light quantities.

# Using spatial and temporal interferences to study leaf development

Mohammed, B.[1], Lopez-Juez, E.[2], and Grieneisen, V.A.[1]

[1]John Innes Centre, Colney Lane, Norwich, NR4 7UH, U.K.
[2]Royal Holloway University of London, Egham Hill, Egham, Surrey, TW20 0EX.

Spatio-temporal patterns coordinate leaf development. Cells at the basal zone divide and transition to a mature tip-region of expansion and overtime determine a leaf of terminal size and shape. To study this coordination, quantifying cell shape, size and number can help unravel whether there is a trade-off between cell division rounds and the capacity of cell expansion. I have perturbed the system by stalling mitotic divisions in very young leaves for a certain time-window by bringing young seedlings from light to dark and conversely observed that a shift back to light reintroduces mitotic division 12-24 h later, in a synchronized fashion. Does this interference impact the final size and shape of the leaf? If it does not, how is such compensation established at the level of modified cellular behaviour? Currently, I am using pseudo-Schiff-PI staining to capture, at different developmental points, details of the tissue. The results are being analysed using current methods in the JIC lab (in collaboration with Stan Maree for advanced image analysis).

Finally, after their last divisions, pavement cells (PCs) develop multiple fronts of growth, with an interdigitating pattern in relation to their neighbouring cells. Underlying their complex shape are intracellular players, the Rho of Plants (ROPs). I have chosen to work on these to understand more about the polarity and patterning mechanisms within single cells as well as to the coordination of these patterns between adjacent cells. To date, most genetic analysis of ROPs and their downstream targets has focused on entire organism showing that PCs lose their lobedness. However, to be able to assess the contribution of cell-cell communication versus intercellular patterning in the shape acquisition of these cells, I am instead using clonal analysis by heat-shocking plant tissue to induce sectors of mutant cells. How mutant cells shape in contact with one another in comparison to how they shape in contact with non-mutant cells will really help answer to what extent and how cell-to-cell communication is linked in morphing cellular interfaces.

## A CW-MBD protein, binding methylated DNA and chromatin with H3K4 methylation, controls leaf size in Arabidopsis by regulation of key genes involved in the transition from leaf cell proliferation to cell expansion

Iversen, V., Nenseth, H.Z., Nateland, L., and Aalen, R.B.

Department of Biosciences, University of Oslo, Norway.

DNA-methylation and epigenetic marks on histone tails are important for organ growth and development, as illustrated by the severely diminished organ size of the Arabidopsis ashh2 mutant (alias efs/sdg8). ASHH2 catalyze H3K36me3, and has in addition a CW domain that reads H3K4me [1]. The CW domain is present also in a small Methyl-CpG-binding Domain (MBD) protein. cw-mbd mutants show increased leaf size. To elucidate the mechanisms behind this protein's influence on leaf development we have investigated the chromatin binding preferences and gene regulatory properties and of CW-MBD.

MBD proteins, devoid of the CW domain, reside in heterochromatin. In contrast, the subnuclear localization of CW-MBD-GFP fusion protein is in euchromatin. Using Microscale Thermophoresis, we show CW-MBD binding to methylated DNA and nucleosomes with native histones in a CW-dependent manner. Purified CW-MBD protein efficiently pull down chromatin enriched in Histone H3K4 monomethylation, an epigenetic mark preferably found in gene bodies, where it can co-reside with DNA-methylation. Genes with these characteristics were enriched amongst putative target genes identified by CW-MBD Chromatin pull down (ChroP) seq. RNA seq identified a few hundred differentially expressed genes in cw-mbd mutant leaves that mainly belong to gene clusters important for chloroplast differentiation and photosynthesis, and the switch from cell proliferation to cell expansion during leaf development. Genes encoding transcription factors, including bHLH, MYB and key factors regulating the circadian clock, are also amongst CW-MBD regulated genes.

Models for CW-MBD function will be discussed.

[1] Hoppmann, V., Thorstensen, T., Kristiansen, P.E., Veiseth, S.V., Rahman, M.A., Finne, K., Aalen, R.B., Aasland, R. (2011). The CW domain, a new histone recognition module in chromatin proteins. *EMBO J.* 30, 1939-1952.

# KEYNOTE LECTURE

## Plant Growth: The Final Frontier

Inzé, D.

VIB-UGent Center for Plant Systems Biology, Belgium.

Plant and plant organ growth depends on an exceedingly complex interplay of many genes and their interaction with the ever changing environment. The long-term goal of our research is to obtain a holistic understanding of the cellular and molecular engines driving plant organ growth. Numerous genes of which the modified expression enhances plant organ growth have now been identified and a detailed study of their mode of action has unraveled five different processes that govern organ growth. Furthermore, evidence obtained both in the model plant Arabidopsis and in maize, demonstrated that the combination of multiple growth enhancing genes can have very profound effects on organ sizes. Field experiments with transgenic maize also demonstrates that genes enhancing leaf growth have profound effects on biomass productivity and seed yield.

Tremendous progress has also been made in understanding how environmental cues, such as mild drought stress, negatively affect plant growth. In unpredictable environments, growth reduction enables plants to redistribute and save resources, ensuring reproduction. However, when the episode of stress does not threaten plant survival, and from the agricultural point of view, growth reduction can be seen as counter-productive, leading to unnecessary yield loss. Limiting growth reduction may thus provide a strategy to boost plant productivity under stress. Recent insights show that mild drought stress affects both the growth rate, as well as the growth duration, using maize leaves as model. The extent at which these two traits are affected, is genotype dependent.

I will discuss how our insights open up new perspectives for the identification of optimal growth regulatory networks that can be selected by advanced breeding, or for which more robust variants (e.g. reduced susceptibility to drought) can be obtained through genetic engineering. The ability to improve growth of maize and, in analogy other cereals, could have a major impact in providing food security.

# SESSION 5:
# Modeling and phenotyping

# Plant phenotyping reveals genetic and physiological factors of plant performance

Altmann, T.[1], Muraya, M.[1,2], Chu, J.[1], Zhao, Y.[1], Junker, A.[1], Tschiersch, H.[1], Klukas, C.[1,3], Reif, J.C.[1], Riewe, D.[1], Meyer, R.C.[1], Jeon, H.J.[1], Heuermann, M.[1], Schmeichel, J.[1], Seyfarth, M.[1], Lisec, J.[4], and Willmitzer, L.[4]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany.
[2]Chuka University, Kenya.
[3]LemnaTec GmbH, Germany.
[4]Max-Planck-Institute of Molecular Plant Physiology, Germany.

Knowledge of the structural and functional genetic architecture of agricultural traits is a prerequisite for the systematic exploration and utilization of plant genetic resources in crop improvement strategies. To uncover mechanistic links between genetic variation, physiological factors and whole plant performance strategies, Arabidopsis, maize, and rapeseed are investigated by integrating genotyping, transcript and metabolite profiling, and plant phenotyping. The three facilities at IPK support automated whole plant phenotyping for small, medium, and large plants including cultivation, transport (plant-to-sensor) and imaging of plants in climate controlled phytotron/glasshouse cabins. They are equipped with camera and illumination systems for visible, fluorescence, and near infrared imaging using top view and side views, with 3D laser scanners, with LED panels and CCD cameras for functional (kinetic) chlorophyll fluorescence detection, and with a broad range of environmental sensors. The value of repeated non-invasive monitoring of large plant populations is highlighted by results of the analysis of a collection of 261 maize dent lines characterized for growth / biomass accumulation and water consumption, and thus, for water use efficiency. Through genome-wide association testing with 50k SNPs, QTL of these traits and of the growth dynamics were identified. The detected 12 main effect biomass QTL and 6 pairs of epistatic interactions displayed different patterns of expression through time. Some also showed significant effects on relative growth rates in different intervals. Using nonparametric functional mapping and multivariate mapping approaches, 4 additional QTL affecting growth dynamics were detected. This demonstrates the complexity of the plant

biomass accumulation trait being governed by many small effect loci most of which act at certain restricted developmental phases. It highlights the need to detect and investigate stage-specific growth control genes operating at different developmental phases and to link their activity with physiological parameters such as photosystem II (PS II) efficiency and architectural traits, which have been shown to vary strongly in a collection of 366 maize accessions of the IPK Genbank.

Integrated metabolome analysis and whole plant phenotyping performed in Arabidopsis revealed direct links between a promoter InDel polymorphism of the *FUM2* gene, its mRNA expression, fumarase enzyme activity, and fumarate to malate ratio in leaves of Arabidopsis Col-0/C24 RILs and ILs. It was also significantly associated with the fumarate to malate ratio, with malate and fumarate levels, and with dry weight in 174 natural accessions at 15 days after sowing (DAS) and with biomass production in another set of 251 accessions (at 22 DAS). This supports a role of the cytosolic FUM2, which specifically occurs in *Brassicaceae*, in diurnal carbon storage and points to a growth advantage of accessions carrying the *FUM2* Col-0 allele preferentially occurring in colder climates.

# Cell to whole-plant phenotyping in support of the integrative analysis of growth regulatory networks

Wuyts, N.[1,2]

[1]VIB-UGent Center for Plant Systems Biology, VIB, Technologiepark 927, 9052 Gent, Belgium.
[2]Department of Plant Biotechnology and Bioinformatics, Ghent University, Technologiepark 927, 9052 Gent, Belgium.

Measurement of growth across scales, from the whole-plant to the organ, tissue and cellular levels, is essential in the identification of mutant phenotypes, and the elucidation of molecular growth regulatory networks. Moreover, as it becomes clear that cellular level processes of proliferation and expansion are affected by development- and environment-induced signalling, the design of sampling strategies for molecular analyses capturing these effects requires comprehensive phenotyping that fully accounts for the plant's growth conditions and its developmental timing. Plant phenotyping systems in growth chambers and greenhouse conditions remain therefore important as they allow 'to know how plants grow', and the identification of associated growth processes. High-throughput greenhouse systems enable the phenotyping of crops beyond the seedling stage, providing growth traits beyond early leaf and shoot development, and plant functioning-related traits important for the integrative analysis of growth and development.

## Natural variation for growth response to the environment in *Arabidopsis thaliana*

Loudet, O.

The VAST Lab: www.inra.fr/vast
Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, 78000 Versailles, France.

Following a long history of quantitative genetics in crop plants, it is now quite popular as well to use naturally-occuring variation contained in *Arabidopsis thaliana* accessions as the source of quantitative genomics approaches, designed to map QTLs and try and resolve them at the gene level. Apart from being able to exploit –in multiple genetic backgrounds– allelic variation that cannot be easily retrieved from classical mutagenesis, the success of the QTL studies has often been because of the use of quantitative phenotyping, as opposed to the qualitative scales often used in typical mutant screens. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the abiotic environment (especially drought stress). We are using a combination of our unique high-troughput phenotyping robot (the Phenoscope), RNA-seq, fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale thanks to the Phenoscope should allow to resolve enough quantitative loci to start drawing a more general picture as to how and where in the pathways adaptation is shaping natural variation. I will present recent results obtained when trying to decipher the genetic architecture of dynamic growth response to the environment, to illustrate our strategies and research.

# Rapid repression of lateral root formation under transient water deficit reveals a novel mechanism of ABA-mediated morphological plasticity in cereal species and Arabidopsis

Orman, B.[1,2,3,*], Lavigne, T.[1], Parizot, B.[2,3], Babé, A.[1], Séverin, J.P.[1], Xuan, W.[2,3], Ligeza, A.[1], Novak, O.[4,5], Morris, E.[6,7], Sturrock, C.[6,7], Ljung, K.[4], Rodriguez, P.L.[8], Dodd, I.C.[9], De Smet, I.[2,3,6,7], Chaumont, F.[10], Batoko, H.[10], Périlleux, C.[11], Bennett, M.J. [6,7], Beeckman, T.[2,3], and Draye, X.[1]

[1]Earth and Life Institute, Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium.
[2]Department of Plant Biotechnology and Bioinformatics, Ghent University, B-9052, Ghent, Belgium.
[3]Department of Plant Systems Biology, VIB, B-9052, Ghent, Belgium.
[4]Department of Forest Genetics and Plant Physiology, Umeå Plant Science Centre (UPSC), SLU, SE-901 83 Umeå, Sweden.
[5]Centre of the Region Haná for Biotechnological and Agricultural Research, Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany AS CR, Šlechtitelů 11, CZ-78371 Olomouc, Czech Republic.
[6]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Leicestershire LE12 5RD, United Kingdom.
[7]Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, Sutton Bonington, LE12 5RD, United Kingdom.
[8]Instituto de Biología Molecular y Celular de Plantas, Consejo Superior de Investigaciones Cientificas-Universidad Politecnica de Valencia, ES-46022 Valencia, Spain.
[9]The Lancaster Environment Centre, Lancaster University, LA1 4YQ, UK.
[10]Institut des Sciences de la Vie, Université catholique de Louvain, B-1348 Louvain-la-Neuve, Belgium.
[11]InBioS - PhytoSYSTEMS, Laboratory of Plant Physiology, University of Liège, Chemin de la Vallée 4, B-4000, Liège, Belgium.
*Present address: InBios - PhytoSYSTEMS, Laboratory of Plant Physiology, University of Liège, Chemin de la Vallée 4, B-4000 Liège, Belgium.

Soil exploration patterns can be seen as the addition of global and local trends defined, respectively, by the growth and development of seminal and shoot-borne roots, on the one side, and by the formation and

growth of postembryonic lateral roots (LR), on the other side. As roots expand in heterogeneous soil, the ability of the root system to effectively coordinate root branching with local soil conditions provides the means to exploit different spatial and temporal soil niches and has a profound impact on the capture of soil resources. We have previously reported in cereals that transient water deficit in aeroponics irreversibly represses LR initiation, however the mechanisms involved in this response and the evolutionary significance remain to be elucidated.

Here we demonstrate that in cereal crops, the root entering a soil macropore (large air space) rapidly responds to the loss of soil contact by locally repressing LR formation as long as the root remains inside the pore. This response was visualised by using X-ray microscale computed tomography and observed in trench soil profile. Gene expression analysis during transient water deficit in aeroponics revealed the role of Abscissic acid (ABA) and we used a combination of exogenous ABA treatments and auxin profiling in cereals with a genetic analysis in *Arabidopsis* to address the intrinsic signalling pathway triggering LR repression. We found that exogenous ABA treatment can mimic the inhibitory effect of water deficit on LR formation in barley (*Hordeum vulgare)*, maize (*Zea mays*) and *Arabidopsis*. We demonstrated that transient ABA treatment represses LR initiation by blocking the earliest stages of prebranch site formation.

These results indicate that an ABA response pathway allows a quick adjustment of root branching to local variations in soil structure and presumably, soil water content. The conservation of this process in monocots and dicots suggests that this mechanism of root plasticity was an ancestral adaptation to terrestrial plant life.

# Cell divisions in columella initials trigger root cap abscission at a local auxin response minimum

Dubreuil, C.[1], Jin, X.[1], Grönlund, A.[2], and Fischer, U.[1]

[1]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umeå SE-901 83, Sweden.
[2]Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, Umeå SE-901 87, Sweden.

The root cap protects the meristem and directs growth by sensing environmental cues. A tight balance between cell division, differentiation and separation ensures that the root cap can fulfill its protective role over a prolonged period of time. Even though the root cap is a simple and easily accessible model positional and temporal cues coordinating root cap development are only poorly understood.

With the help of live-cell tracking over a period of several days we established a timeline of Arabidopsis root cap development. Prior to the separation of the outermost cell layer a single cell division took place in the columella initials. Induction of columella cell divisions resulted in an increased rate of cell layer separation, whereas inhibition of initial divisions decreased the frequency of abscission. This suggests that initial division activity is co-regulated with the rate of abscission in order to keep a balance between self-renewal and size homeostasis of the root cap. Intriguingly, the auxin response gradient with a maximum in the outermost columella layer before the onset of cell separation was inverted during cell separation to a gradient with a local minimum in the detaching columella layer. Inhibition of polar auxin transport abolished the auxin response gradient, cell wall remodeling in the detaching cell layer and abscission of the root cap. Auxin efflux carriers were not expressed in the two outermost layers of the columella, supposedly diminishing transporter-mediated auxin flux from the main auxin source in the quiescent center to the separating root cap layer. A dynamic mathematical model indicates that a steady-state auxin gradient can be achieved solely and rapidly on the basis of auxin dilution by cell division and expansion and that such a gradient can robustly predict the site of cell separation.

The combination of live-cell tracking, pharmacological manipulation of root cap development and mathematical modeling allowed us to identify cell divisions in the columella initials together with an auxin gradient as regulators of cell separation.

# An adenylate kinase regulates ribosome biogenesis, cell proliferation, cell size and natural variation in root growth

Slovak, R.[1], Setzer, C.[1], Roiuk, M.[2], Göschl, C.[1], Jandrasits, K.[1], and Busch, W.[1]

[1]Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr.-Bohr-Gasse 3, 1030 Vienna, Austria.
[2]Max F. Perutz Laboratories, Vienna Biocenter (VBC), Dr.-Bohr-Gasse 9, 1030 Vienna, Austria.

Cell proliferation and cell size fundamentally impact the growth of organs. Organ growth is particularly highly relevant for roots, as root growth and length determine the ability of roots to explore the soil to acquire nutrients and water, as well as to anchor the plant. While many regulators with major qualitative effects on root growth are known, we set out to identify genes that quantitatively regulate growth. For this, we sampled the variation in root growth of 253 *Arabidopsis thaliana* accessions and conducted genome wide association studies (GWAS). We subsequently identified a genomic region spanning an adenylate kinase (*AK*) gene. Loss-of-function of this gene leads to significantly slower root growth, resulting in shorter roots. Complementation of the loss-of-function mutant with an allele from faster growing haplotype led to significantly faster root growth compared to complementation with an allele from slower haplotype. This demonstrates that natural genetic variation in the *AK* gene is involved in determining root growth in Arabidopsis accessions. Unexpectedly, the loss-of-function mutant displays slightly longer cells in the root meristem and significantly longer mature cells than wild type. This is accompanied by increased levels of endoreplication. The short root phenotype despite increased cellular length strongly suggests that AK regulates cell proliferation in the root meristem. Surprisingly, loss of AK increases number of cells in G2/M phase, therefore we are currently testing the duration of G2/M phase in mutant and wild type seedlings. At the molecular level, AK is involved in ribosome biogenesis and the loss of AK function leads to accumulation of 80S-like ribosomes, suggesting a link between root growth control and ribosome biogenesis. Overall, utilizing genetic variation of natural alleles, we uncovered a novel growth regulator that links ribosome biogenesis, cell cycle, and cell size control in the *Arabidopsis thaliana* root.

# Assessing the variability of root growth components in poplar

Youssef, C.[1], Hummel, I.[1], Bizet, F.[2], Bastien, R.[3], Legland, D.[4], and Bogeat-Triboulot, M.B.[1]

[1]EEF, INRA, Université de Lorraine, 54280, Champenoux, France.
[2]PIAF, INRA, Université Clermont Auvergne, 63178, Aubière, France.
[3]Department of Collective Behaviour, Max Planck Institute for Ornithology, University of Konstanz, Konstanz, Germany.
[4]Biopolymers, Interactions and Assemblies, INRA, 44316, Nantes, France.

Organ growth results from the coupling of cell production and cell expansion. These two processes being spatially mostly separated in the root apex, this organ appears well suited to decipher their contribution to growth rate. Kinematics is a powerful framework to access to growth determinants such as cell stretching capacity, cell proliferation rate, size of the growth zone, etc [1]. Automatic tracking algorithms have enhanced particle image velocimetry, opening the way for the screening of growth variability.

Taking advantage of the high adventitious rooting capacity of the *Populus* genus, we investigated the variability of growth and its underlying components under optimal conditions. Root growth rate was quantified together with elemental elongation rate (EER), cell production rate, growth zone length and meristem length. We tested whether root growth rate was associated with growth zone length or cell stretching capacity. Poplar cuttings of 8 genotypes were grown in hydroponics. Roots were imaged under infrared light, growth was monitored by time lapse photography and kinematic analyses were conducted with KymoRod [2].

Fifty-seven roots were phenotyped revealing a large range of growth rate (0.1 to 1.3 mm $h^{-1}$). While a special attention was given to the homogeneity of material and growth conditions, this range reflects inter-individual root variability. No correlation was found between root growth rate and root diameter. One genotype, Flevo, differed from the others, showing a significantly higher growth rate. Root growth rate was highly correlated with the elongation zone length ($r^2$=0.93) and, to a lesser extent, to the maximal EER ($r^2$=0.59). EERmax in the root apex co-varied with growth rate but nether exceeding 40 % $h^{-1}$, suggesting this was an intrinsic limit of cell stretching.

[1] Bizet, F., *et al*. (2015) *J. Exp. Bot.* 66, 1387-1395.
[2] Bastien, R., *et al.* (2016). *Plant J.* 88, 468-475

# Cell-size dependent progression of the cell cycle creates both homeostasis and flexibility of plant cell size

Jones, A.R.[1], Forero-Vargas, M.[1], Withers, S.P.[1], Smith, R.S.[2], Traas, J.[3], Dewitte, W.[1], and Murray, J.[1]

[1]Cardiff School of Biosciences, Cardiff University, Cardiff, Wales CF10 3AX, UK.

[2]Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany.

[3]Laboratoire de Reproduction de développement des plantes, INRA, CNRS, ENS Lyon, UCB Lyon 1, Université de Lyon, Lyon, France.

Mean cell size at the point of división in plant cells is generally constant for specific conditions and cell types, but the mechanisms coupling cell growth and cell cycle control with cell size regulation are poorly understood in intact tissues. Here we show that the continuously dividing fields of cells within the shoot apical meristem of Arabidopsis show dynamic regulation of mean cell size dependent on developmental stage, genotype and environmental signals. We show cell size at division and cell cycle length can be effectively predicted using an iterative two-stage cell cycle model linking cell growth and two sequential cyclin dependent kinase (CDK) activities. A single phase model cannot predict the observed effects of alterations in G1/S and G2/M kinsae activities as determined by using mutant and overexpression genotypes. Experimental results concur in showing that progression through both G1/S and G2/M is flexible in response to both genotype and environmental conditions and that both transitions are size dependent. We conclude that the cell-autonomous co-ordination of cell growth and cell division previously observed in unicellular organisms also exists in intact plant tissues, and that observed cell size in growing tissues may be an emergent rather than directly determined property of cells.

# High-throughput screening of Arabidopsis shoot growth in multi-well plates

De Diego, N.[1], Fürst, T.[1], Humplík, J.F.[1,2], Ugena-Consuegra, L.[1], Podlešáková, K.[1], and Spíchal, L.[1]

[1]Department of Chemical Biology and Genetics, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, Šlechtitelů 27, Olomouc, CZ-78371, Czech Republic.
[2]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Faculty of Science, Palacký University, and Institute of Experimental Botany AS CR, Šlechtitelů 27, Olomouc CZ-78371, Czech Republic.

High-throughput plant phenotyping platforms provide new possibilities for automated, fast scoring of several traits of plant growth and development followed over a time-course using non-invasive sensors. In this study we report development of high-throughput *Arabidopsis in vitro* bioassay established at our OloPhen platform suitable for analysis of shoot growth in multi-well plates. This method allows combinatorial testing of high number of compounds and/or genotypes, in various growth conditions limiting plant growth in large scale counting with wide range of concentrations of tested compounds and conditions, respectively. Several traits such as changes in the shoot area, relative growth rate, survival rate and homogeneity of the population are scored using automated RGB imaging and subsequent image analysis. The assay can be applied for fast screening of biological activity of chemical libraries, phenotypes of transgenic or recombinant inbred lines, or to search for potential quantitative trait loci. It is especially advantageous for a selection of stress-tolerant genotypes or compounds that improve stress tolerance.

# POSTERS

## The role of KNOX in shaping leaf forms

Kierzkowski, D., Runions, A., Vuolo, F., Dello Ioio, R., and Tsiantis, M.

Department of Comparative Development and Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linne-Weg 10, 50829 Cologne, Germany.

Altered gene expression of key developmental regulators contributes to organ shape diversity. It is, however, unclear how these genetic changes translate into divergent morphologies. Here we investigate this problem by comparing leaf development in two closely related species, *A. thaliana,* which has simple leaves with serrations, and *C. hirsuta* with compound leaves subdivided into individual leaflets. We show that cellular differentiation is delayed in leaflets compare to serrations coinciding with prolonged growth. This effect depends on *class I KNOTTED-like homeobox (KNOX)* gene called *SHOOTMERISTEMLESS (STM)*. Specifically, ectopic expression of *STM* in the *A. thaliana* leaf shifts the developmental patterns of serrations toward those observed in leaflets. We further explore how STM influences expression of the PINFORMED1 auxin efflux carrier which is required for formation of leaflet primordia along the leaf margin. Our work indicates that KNOX proteins contribute to morphological diversity by delaying differentiation, prolonging growth and influencing organogenic activity of the leaf margin.

## Modeling secondary growth in the Arabidopsis root cambium

Adibi, M.[1], Smetana, O.[2], Broholm, S.[2], Smith, R.S.[1], and Mähönen, A.P.[2]

[1]Max Planck institute for plant breeding research, Cologne, Germany.
[2]Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

Secondary root growth in plants is marked with continuous production of vascular tissues and radial thickening of plant organs. This process is essential for various aspects of plant growth and physiology, such as water transport and response to mechanical stress. Analysis of clonal sectors of vascular cells, induced at the onset of secondary growth, has revealed that the bulk of the tissue produced during secondary growth is comprised of the descendants of procambial cells in contact with primary xylem. Intriguingly, the size of these sectors correlates with the number of secondary xylem vessels formed in each sector. Additionally data reveals that rapid cell expansion associated with vessel formation appears to result in compression and dislocation of neighboring cells. The ability of vascular tissue to accommodate the fast growing xylem vessels while maintaining overall tissue form, requires detailed regulation of growth and cell mechanics.

In order to investigate the patterns of tissue growth and mechanics during secondary growth, we developed a physically-based growing model of a transverse section of root vasculature. Our simulation results suggest that regulation of cellular pressure plays a central role in regulating secondary growth; model results are consistent with xylem expansion driven by elevation of cellular pressure. Results also suggest that pressure regulation is a mean by which the cells adjacent to an expanding xylem resists major deformations, otherwise caused by increasing pressure in the expanding xylem vessels. Overall, model simulations show that regulation of cellular pressure can account for xylem formation and how xylem expansion subsequently impacts overall tissue arrangement.

## A developmental framework for adventitious root development in Arabidopsis thaliana

Ibáñez, S., Sánchez-García, A.B., Fernández-López, M., Micol, J.L., and Pérez-Pérez, J.M.

Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, Avda. de la Universidad s/n, 03202 Elche, Spain.

As Adventitious roots (ARs) are ectopic roots that arise either naturally or in response to stress from various plant tissues, such as stems and leaves; they may also be induced by mechanical damage or following in vitro tissue culture regeneration. The formation of ARs is a complex genetic process regulated by both environmental and endogenous factors, among which the plant hormone auxin plays a central role.

Using *Arabidopsis thaliana* excised leaves as a model for *de novo* root organogenesis, we characterized both at the histological and molecular level the different stages during AR formation. Our results indicate that, shortly after excision, a localized auxin maximum is established on a subset of vascular cells near the wound. Then, cytokinin-dependent cell proliferation leads to callus formation in this region which will later acquire root identity markers.

To identify additional gene functions required for AR development we previously screened the *Arabidopsis thaliana* unimutant collection with a visible leaf phenotype. Here, we present new data on a subset of these mutants selected on the basis of their defective AR formation from excised leaves.

Work funded by MINECO/FEDER (AGL2012-33610 and BIO2015-64255).

# Hormonal signaling of adventitious root formation in tomato hypocotyls after wounding

Alaguero, A.[1], Villanova, J.[1], Cano, A.[2], Acosta, M.[2], and Pérez-Pérez, J.M.[1]

[1]Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, Avda. de la Universidad s/n, 03202 Elche, Spain.
[2]Departamento de Biología Vegetal (Fisiología Vegetal), Universidad de Murcia, 30100 Murcia, Spain.

Adventitious roots (ARs) are formed from non-root tissues, such as stems or leaves, in response to some stresses (i.e. flooding) or after wounding. Tomato is an attractive model to study the genetic basis of *de novo* adventitious organ formation, since there is a considerable natural genetic variation for this trait among wild relatives.

Our results indicate that active polar auxin transport through the hypocotyl leads to a localized auxin gradient required for AR formation in the hypocotyl base. Quantitative histology allowed us to define the cellular dynamics during the early stages of AR initiation. Gene expression profiling at different stages of AR formation have been analyzed. AR formation has been analyzed on a number of tomato mutants affected in hormonal signaling and a model for wound-induced organ regeneration from hypocotyl explants in this species will be presented.

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

Work funded by MINECO/FEDER (AGL2012-33610 and BIO2015-64255).

## Communication of loss: A novel peptide ligand-receptor pair is involved in root cap sloughing in Arabidopsis

Shi, C., Herrmann, U., Wildhagen, M., and Aalen, R.B.

Department of Biosciences, University of Oslo, Norway.

Peptide ligands are playing important roles in plant growth and developmental processes. *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*) encodes a peptide ligand required for floral organ abscission and lateral root emergence in *Arabidopsis*, signaling through its receptor-like kinases (RLK), HAESA (HAE) and HAESA-LIKE 2 (HSL2).

*IDA-LIKE1 (IDL1)*, closely related to *IDA*, is expressed in the the oldest columella cells and neighbouring lateral root cap (LRC) cells in the outermost layer of the root cap at the tip of the primary root ,while *HSL2* is most strongly expressed in the youngest LRC layers. Enhanced expression of *IDL1* (*EnhIDL1*) from its own promoter, using an inducible two-component system, results in an increased number of detached root caps; however, without a reduction in the number of attached root cap layers. The increased frequency of root cap sloughing cannot be induced in *hsl2* mutant background. By using different root reporters, we are exploring the role of IDL1 and HSL2 in the sloughing process and in maintenance of the homeostatic balance between loss and generation of new root cap layers.

## A genome-wide association study identifies new loci for root formation after wounding

Justamante, M.S., Ibáñez, S., and Pérez-Pérez, J.M.

Instituto de Bioingeniería, Universidad Miguel Hernández de Elche, Avda. de la Universidad s/n, 03202 Elche, Spain.

We are interested to contribute to the identification of some of the genetic determinants involved in *de novo* organ formation after wounding using *Arabidopsis thaliana* as a model organism. We have analyzed lateral and adventitious root formation in response to wounding on a collection of 120 natural accessions of Arabidopsis. Genome-wide association studies have been performed to identify some of the genomic regions that may be involved in the phenotypic variation for some of the studied traits.

We found statistically significant associations between the number of lateral roots after wounding and several polymorphic markers at the coding region of some genes on chromosomes 1, 3 and 5. Likewise we have found some markers associated with lateral root density and with the timing of lateral root primordial initiation. However, we found no significant association between the number of adventitious roots and the studied molecular markers. Our results suggest some specificity in the genetic mechanisms that regulate the formation of lateral and adventitious roots after wounding. Subsequent studies will allow us to confirm whether the polymorphisms identified contribute to the phenotypic differences observed in the studied accessions.

Work funded by MINECO/FEDER (AGL2012-33610 and BIO2015-64255).

## Characterization and diversity of rhizobia nodulating *Lablab purpureus*

Benselama, A.[1,2], Ounane, S.M.[2], and Bekki, A.[3]

[1]Laboratory of Biology of Soil. University of Science and Technology Houari Boumedienne USTHB Algiers.
[2]Laboratory of Amelioration Integrative of Production Vegetal (AIPV).
[3]Laboratory of Biotechnology of Rhizobia and Amelioration of Plant. University d'Oran-Es-Senia Algeria.

The objective of this study is to investigate the genetic diversity of 20 isolates from our collection nodulating *Lablab purpureus* in three soil of Algeria, These isolates have been authenticated by seedling inoculation grown in jars containing sand. The results obtained after two months of culture have revealed that the 20 isolates (100% of the isolates) are able to nodulate their host plants.

The determination of the taxonomic position of these isolates and evaluation of the level of approximation or divergence between these strains and the reference strains belonging to different genera of rhizobia. Amplification of the ribosomal 16S rDNA gene (PCR / RFLP of 16S rDNA) was digested with four different restriction enzymes: Msp I, HinfI, Hha I and Taq I.

The results of different electrophoretic profiles of fragments obtained shown the selection of the most discriminating enzymes Msp I and Hinf I. In addition, length polymorphism of the restriction fragments (RFLP) analysis of PCR amplified 16S rDNA were compared with those of reference strains. Numerical analysis of molecular characteristics showed that 20 strains studied fall into three distinct groups, we noted that three isolates only *Lablab purpureus* have a high level of similarity with the reference strain "*Bradyrhizobium*", while 17 isolates did not exhibit precise taxonomic status and therefore their exact phylogenetic classification to be determined.

# Meiotic abnormalities during gamete formation in triploid *Limonium algarvense* (*Plumbaginaceae*)

Conceição, S. , and Caperta, A.D.

Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

The induction of polyploidy (i.e. genome doubling) in flowering plants has been considered one of the main drivers of plant speciation [1,2]. A major route of polyploidization rely on alterations of the meiotic cell cycle involving gametic nonreduction or meiotic nuclear restitution during micro- and megasporogenesis, originating unreduced gametes [3,4]. Triploid taxa can be used as a natural model to investigate these cellular phenomena to infer the polyploid status of the species under study (auto- or allopolyploidy) [5].

*L. algarvense* Erben is a poor known triploid species (2n=3x=25 chromosomes) [6] belonging to the *Limonium* Mill. genus, which is well distributed in coastal areas and saline steppes with a great diversity in the Mediterranean region [7]. Few information regarding cytogenetic variability and reproductive biology of this species is available. The present study aimed to characterize male sporogenesis and gametogenesis of this species. Micro-sporogenesis was analysed through cytology using squashes stained with DAPI and with aceto-carmine. Callose detection and gamete viability tests were also performed. The results showed several abnormalities during male gametes formation such as chromosomes bridges and laggard chromosomes, First and Second division restitution nuclei, as well as cytomixis. These anomalies led to the formation of nonreduced pollen grains with different morphology with low viability and germination rates.

The results indicate that *L. algarvense* is an allopolyploid since there appears to be a lack of homology in some meiosis stages and also a possible intergenomic recombination. Besides this, the results on pollen grain with low fertility and seed with high capacity of germination indicate that this species might reproduce through apomixis.

[1] Ramsey, J., and Schemske, D.W. (1998). Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annu. Rev. Ecol. Syst.* 29, 467-501.

[2] Adams, K.L., and Wendel, J.F. (2005). Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8, 135-141.
[3] Bretagnolle, F., and Thompson, J.D. (1995). Gametes with the Stomatic Chromosome Number: Mechanisms of Their Formation and Role in the Evolution of Autopolypoid Plants. *New Phytol.* 129, 1-22.
[4] De Storme, N., and Mason, A. (2014). Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. *Curr. Plant Biol.* 1, 10-33.
[5] Lavia, G.I., Ortiz, A.M., Robledo, G., Fernández, A. & Seijo, G. (2011). Origin of triploid Arachis pintoi (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behaviour. *Ann. Bot.* 108, 103-111.
[6] Erben, M., (1993). Limonium Mill. In: Castroviejo S, Aedo C, Cirujano S, Lainz M, Montserrat P, Morales R, Garmendia FM, Navarro C, Paiva J, Soriano C [eds.] Flora Iberica 3, 2-143, Real Jardín Botánico, CSIC, Madrid.
[7] Kubitzki, K. in *Flowering Plants Dicotyledons SE - 62* (eds. Kubitzki, K., Rohwer, J. & Bittrich, V.) 2, 523-530 (Springer Berlin Heidelberg, 1993).

# Patterns of Ser 10 phosphorylation of histone H3 and of tubulin during male sporogenesis in diploid and polyploid Limonium species (Plumbaginaceae)

Conceição, S., Silva, N., and Caperta, A.D.[1]

[1]Linking Landscape, Environment, Agriculture and Food (LEAF), Instituto Superior de Agronomia (ISA), Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Unreduced gamete formation is considered to be the main source of polyploidy (genome doubling) in nature [1,2]. The main cytological mechanisms responsible for meiotic non-reduction in plants are First division restitution (FDR) or Second division restitution (SDR) [3]. Limonium Mill. (Plumbaginaceae) is a genus of halophytes with ploidy levels spanning diploids to octoploids and aneuploidy [4]. In the present study, male sporogenesis was studied by cytology using immunostaining of phosphorylated histone H3 at serine-10 (H3S10ph) and α-tubulin in diploid and polyploid species. Our results showed differences in distribution patterns of both antibodies associated to the production of unreduced male gametes.

[1] Tayalé, A., and Parisod, C. (2013). *Cytogenet. Genome Res.* 140, 79-96.
[2] Ramsey, J., and Schemske, D.W. (1998). *Annu. Rev. Ecol. Syst.* 29, 467-501.
[3] De Storme, N., and Geelen, D. (2013). *New Phytol.* 198, 670-684.
[4] Róis, A.S., *et al.,* (2016). *Ann. Bot.* 117, 37-50.

## Antagonistic interaction between A and C floral homeotic activities is critical for ovule development in Arabidopsis

Rodríguez-Cazorla, E.[1], Ortuño-Miquel, S.[1], Cruz-Valerio, M.[1], Bailey, L.J.[2], Yanofsky, M.F.[2], Ripoll, J.J.[2], Martínez-Laborda, A.[1], and Vera, A.[1]

[1]Área de Genética, Universidad Miguel Hernández, Campus de Sant Joan d'Alacant, Sant Joan d'Alacant, Alicante, Spain.
[2]Division of Biological Sciences, Section of Cell and Developmental Biology, University of California San Diego, La Jolla, CA, USA.

Plant ovules are essential for reproductive success. Ovules develop inside the gynoecium, housing the female gametophyte and giving rise to the seeds after fertilization. In Arabidopsis, ovule identity is redundantly conferred by the D-function genes *SHATTERPROOF1* (*SHP1*), *SHP2*, and *SEEDSTICK* (*STK*), encoding MADS-box transcription factors closely related to the floral homeotic gene *AGAMOUS* (*AG*)[1]. *HUA1*, *HUA2*, *HEN4*, *FLK* and *PEP* (collectively, the *HUA-PEP* gene activity) encode a set of interacting ribonucleoproteins that regulate *AG*, thus affecting flower organ identity and determinacy[2].

A tenet of the classic ABC model for flower patterning is antagonism between A and C activities, represented by *APETALA1* (*AP1*) and *AG* genes, respectively. Here, we report that mutational perturbation of the *HUA-PEP* gene function leads to homeotic transformation of ovules into flower organ-like structures. Accordingly, *hua-pep* mutants exhibit reduced expression of D-class genes. Unlike previous studies in which converted ovules were reported to resemble carpeloid structures[1,3], transformed ovules in *hua-pep* mutant backgrounds display obvious sepaloid features. Transformed ovules also show *AP1* ectopic expression likely due to simultaneous fall of *AG*. Indeed, loss of *AP1* or increase of *AG* gene dosage rescue ovule identity in *hua-pep* mutants and favor the appearance of carpeloid traits. These results suggest that proper ovule development may require the exclusion of factors such as *AP1* from those tissues to prevent the adoption of alternative cell fates. Overall, our findings are in line with the classic A-C antagonism and strongly suggest that the interplay between A and C(+D) functions is critical for ovule development.

# PPD-KIX, a conserved protein repressor complex regulating leaf growth in dicots

Baekelandt, A.[1,2], Gonzalez, N.[1,2], Pauwels, L.[1,2], Swinnen, G.[1,2], Doorsselaere, J.V.[3], Jaeger, G.D. [1,2], Goossens, A.[1,2,4], and Inzé, D.[1,2,4]

[1]Center for Plant Systems Biology, VIB, Technologiepark 927, 9052 Ghent, Belgium.
[2]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium.
[3]VIVES University College, Wilgenstraat 32, 8800 Roeselare, Belgium.
[4]These authors contributed equally to this work.

Final leaf size is regulated by a multitude of pathways, among which cell division plays a pivotal role. In dicots, a large fraction of epidermal cells is derived from the stomatal lineage. In this lineage, stem cell-like precursor cells, called meristemoids, undergo asymmetric divisions generating pavement cells adjacent to two guard cells constituting a stoma. PEAPOD2 (PPD2) is a transcriptional regulator known to negatively regulate meristemoid division in *Arabidopsis thaliana* (Arabidopsis). We found that PPD2 interacts with KIX8 and KIX9, acting as adaptor proteins to recruit the co-repressor TOPLESS (TPL) [1]. Interestingly, the *kix8-kix9* mutant and a transgenic line over-expressing an amiRNA targeting *PPD1* and *PPD2* (*ami-ppd*) both have enlarged dome-shaped leaves resulting from increased meristemoid amplifying divisions. Downstream targets of this PPD2-KIX8/9 repressor complex were identified, including several transcription factors and D3-type cyclins.

Genes encoding the members of the PPD-KIX repressor complex are absent from Poaceae (grasses), but conserved in dicots. To shed light on the functional conservation of this complex across different dicot plant species, CRISPR/Cas9 mediated genome editing was used to simultaneously knock-out the *KIX8* and *KIX9* orthologues in *Solanum lycopersicum* (tomato). Primary transformants were obtained where the *SlKIX8* and *SlKIX9* alleles contain indels leading to a frame shift. These plants and their progeny exhibited enlarged dome-shaped leaves, reminiscent of Arabidopsis. Consistently, SlKIX8 and SlKIX9 could interact with tomato orthologues of the PPD and TPL proteins.

The identified PPD-KIX complex is conserved in dicots only and down regulation of *KIX* expression has similar effects on leaf morphology in

Arabidopsis as in tomato, indicating that this is a key complex to regulate leaf growth in dicots. Most likely, this complex plays a role in determining leaf growth in the second dimension, a developmental program that is absent from monocot grasses

[1] Gonzalez, N., Pauwels, L., Baekelandt, A., De Milde, L., Van Leene, J., Besbrugge, N., Heyndrickx, KS., Pérez, A.C., Durand, A.N., De Clercq, R., *et al.* (2015). A repressor protein complex regulates leaf growth in Arabidopsis. *Plant Cell* 27, 2273-2287.

## TCTP interacts with TIP to control cell proliferation and organ development in *Arabidopsis thaliana*

Betsch, L., Pontier, G., Brioudes, F., Boltz, V., Tissot, N., Bendahmane, M., and Szécsi, J.

Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Laboratoire de Reproduction et Développement des Plantes, Université de Lyon, 46 Allée d'Italie, 69364 Lyon Cedex 07, France.

Organ development and size determination require the tight regulation of cell proliferation and cell growth. Recently TCTP (Translationally Controlled Tumor Protein) was shown to control the G1/S transition, cell cycle progression and morphogenesis in plants and in animals. The molecular pathway by which TCTP controls cell cycle in animals involves the negative regulation of the p53 protein. However in plants, no p53 homologous has been identified and the TCTP pathway controlling cell cycle remain unknown. To dissect how TCTP controls cell cycle progression in plants we identified its interacting proteins. We demonstrate that TCTP interacts with a novel protein named TIP (TCTP Interacting Protein) to accomplish its functions. To address the biological significance of this interaction, we performed molecular and biochemical analyses as well as genetic interaction studies. We demonstrate that TCTP and TIP require each other to control the G1/S transition as well as organ and plant development. The latest data will be discussed.

## Characterization of the regeneration process in plant´s wounds: from the nanostructure to the molecular processes

Díaz, A.A.[1], Floriach-Clark, J.[2], Capellades, M.[1], Fuentes, J.[2], Laromaine, A.[2], and Coll, N.S.[1]

[1]Centre for Research in Agricultural Genomics (CRAG), CSIC-IRTA-UAB-UB, Campus UAB, Bellaterra, Barcelona, 08193, Spain.
[2]Institute of Material Science of Barcelona (ICMAB-CSIC), Campus UAB, Bellaterra, Barcelona, 08193, Spain.

Our work focuses on the combination of techniques from physics and chemistry field to plant biology to elucidate the molecular processes underlying plant tissue regeneration in this case promoted by natural exopolysaccharides. This compound has a high degree of polymerization and crystallinity, maintains humidity and protects against UV light. When applied to plant wounds induce regeneration and anti-microbial capacity. Scanning and transmission electron microscopy along with optical microscopy, were used to evaluate the new tissue formation around the wounded area. Kinetic studies of the wound healing process revealed that regeneration starts around 48 hours after wounding and the wound is completely healed after 7 days. We are currently analyzing the identity of the newly formed cells, proteins and genes involved in this regenerating process.

## A novel role of PREFOLDIN in alternative splicing

Esteve-Bruna, D.[1], Carrasco-López, C.[2], Blanco-Touriñán, N.[1], Iserte, J.[3], Perea-Resa, C.[2], Calleja-Cabrera, J.[1], Yanovsky, M.J.[3], Blázquez, M.A.[1], Salinas, J.[2], and Alabadí, D.[1]

[1]Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV, 46022, Valencia, Spain.
[2]Centro de Investigaciones Biológicas, CSIC, 28040, Madrid, Spain.
[3]Fundación Instituto Leloir, C1405BWE, Buenos Aires, Argentina.

PREFOLDIN (PFD) is an evolutionarily conserved heterohexameric chaperonin that presents unfolded tubulin to the main cytosolic chaperone CCT. This function takes place in the cytosol, but PFD can also accumulate in the nucleus of Arabidopsis cells through the interaction with the DELLA transcriptional regulators. Although this interaction is meant to impair PFD function in the cytosol, the possibility still exists that PFD also performs a role in the nucleus.

Published yeast, fly and human interactomes revealed extensive interaction between all six PFD subunits and nuclear proteins, including members of the LSm2-8 complex involved in mRNA splicing. Interestingly, inspection of public databases compiling hundreds of transcriptomic analyses in Arabidopsis showed that several *PFD* genes are extraordinarily coexpressed with genes coding for LSm proteins, suggesting functional relationship. The LSm2-8 complex binds and stabilizes the U6 snRNA forming one of the five small nuclear ribonucleoproteins of the spliceosome.

Here we show that the interaction between several PFD and LSm subunits is conserved in Arabidopsis. Also, loss-of-function alleles of genes encoding PFDs and LSm8 interact genetically. Importantly, loss of PFD alters the stability of the U6 snRNA. Since both *pfd4* and *lsm8* mutants show an altered response to low temperature, we performed a high-coverage RNA-seq to investigate alternative splicing (AS) alterations in these mutants both in standard conditions and after a cold treatment. Using the R package ASpli, we found hundreds of AS events altered both in *pfd4* and *lsm8* under cold acclimation, whereas only AS alterations were found in *lsm8* under standard conditions, suggesting a role for the PFD complex in the control of AS under cold stress.

## Isolation and characterization of an albino T-DNA mutant shows that 1-deoxy-D-xylulose-5-phosphate synthase (DXS1) is essential during tomato plant growth and development

García-Alcázar, M.[1], Giménez, E.[1], Pineda, B.[2], Capel, C.[1], García-Sogo, B.[2], Sánchez, S.[2], Yuste-Lisbona, F.J.[1], Angosto, T.[1], Capel, J.[1], Moreno, V.[2], and Lozano, R.[1]

[1]Centro de Investigación en Biotecnología Agroalimentaria (BITAL). Universidad de Almería, 04120 Almería, Spain.
[2]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia. 46022 Valencia, Spain.

In tomato (*Solanum lycopersicum* L.), the 1-deoxy-D-xylulose-5-phosphate synthase 1 (DXS1) catalyses the first step of the 2-*C*-methyl-D-erythritol-4-Phosphate (MEP) pathway and it is required for carotenoid biosynthesis during fruit ripening. However, its functional role during plant development remains unknown. This work reports the isolation and molecular characterization of the tomato *white lethal seedling*-2297 (*wls*-2297) T-DNA mutant. After seed germination, albino seedlings of the *wls*-2297 mutant expanded cotyledons, but they were unable to develop true leaves from the shoot apical meristem, which resulted in premature lethality. Cloning of the genomic sequences flanking the T-DNA insertion site followed by a co-segregation analysis revealed that the mutant albino phenotype was caused by a 38.6 kb-deletion, which affected the *DXS1* and three *PEROXIDASE* (*POX*) genes. Phenotypic and expression analyses of *DXS1* and *POX* silencing lines indicated that the *wls-2297* mutant phenotype is due to a loss of function of the *DXS1* gene, a result also supported by both *in vivo* complementation assays with 1-Deoxy-D-xylulose-5-phosphate (DXP) and *DXS1* overexpression on the *wls-2297* mutant. Further characterization of genes involved in the MEP pathway suggested a role for DXS in transcriptional regulation of the first steps of the MEP pathway. Taken together, these results indicate that DXS1 may play other important roles besides to that proposed during fruit carotenoid biosynthesis, being it required for the growth and survival of tomato plants at early developmental stages.

## Arabidopsis *DENTICULATA10* encodes FTSHI4, a chloroplast protein with a role in leaf dorsoventrality

Gutiérrez-Nájera, N., Sarmiento-Mañús, R., Robles, P., Quesada, V., Ponce, M.R., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

In Arabidopsis, loss-of-function alleles of a number of genes encoding ribosomal proteins (RPs) cause weak developmental phenotypes. For example, we previously isolated Arabidopsis *denticulata* (*den*) mutants, which exhibit pointed and dentate leaves; these mutants fall into 19 complementation groups. We previously identified five of these *DEN* genes, all of which encode RPs [1]. Many mutant alleles (*rp*) of genes encoding RPs synergistically interact with alleles of the *AS1* (*ASYMMETRIC LEAVES 1*) and *AS2* genes. AS1 is a MYB-domain protein and AS2 a plant-specific nuclear protein belonging to the LATERAL ORGAN BOUNDARIES family. The *rp as1* and *rp as2* double mutants exhibit strong alterations in leaf dorsoventrality. These observations point out the possible involvement of translational regulation in leaf development.

Here, we describe the *den10* mutant, which exhibits dentate and reticulate leaves, abnormal patterning of leaf venation, and abnormal palisade mesophyll cells that are larger in size but fewer in number, compared with wild type. Using mapping-by-sequencing, we identified the *den10* mutation as the first viable allele of the *FTSHI4* (*FILAMENTING TEMPERATURE-SENSITIVE MUTANT H INACTIVE PROTEASE4*) gene, which encodes a thylakoid membrane-associated protein essential for chloroplast development [2]. The *den10 as2-1* double mutants showed a synergistic phenotype of dwarf rosettes with strongly radialized leaves. The *den10 as1-1* plants also exhibited a synergistic phenotype. Surprisingly, we found that the floral gene *AGAMOUS* is ectopically expressed in *den10* leaves. In conclusion, the *FTSHI4* gene, which does not encode a RP, seems to be required for chloroplast biogenesis and leaf polarity.

[1] Horiguchi, G., *et al.* (2011). *Plant J.* 65, 724-736.
[2] Lu, X., *et al.* (2014). *PLOS ONE* 9, e99741.

## Rice matrix metalloproteinase 1 gene, a key regulator of cell shape and tissue development

Kumar Das, P., and Kumar Maiti, M.

Department of Biotechnology. Indian Institute of Technology Kharagpur, India- 721302.

Matrix metalloproteinases (MMPs) are a group of proteins present normally in the extracellular matrix (ECM) of both animal and plant systems. MMPs, being zinc-dependent endopeptidases, are a major group of enzymes that regulate cell–matrix composition in animal system. These proteins belong to the metzincin super family, and have diverse and critical functional roles in development and defence.

We have identified a MMP homologue gene in rice (*Oryza sativa*) genome through bioinformatics, and designated as *OsMMP1*. One genetic construct was used for stable transgenic expression in tobacco plant to develop '*gain-of-function*' phenotype. To investigate the spatio-temporal regulation of *OsMMP1* gene, transgenic tobacco plants were developed through *OsMMP1* promoter- reporter gene fusion construct.

Transgenic tobacco lines expressing OsMMP1 had developed different morphological and cellular alterations. Delayed anther dehiscence was observed in transgenic tobacco. The cell shape of transgenic tobacco lines were much resistance to cell wall biosynthesis inhibitor, where control plant failed to develop normal growth. The expression of green fluorescence protein in anther, stigma, stem and root strongly evident that the gene plays a pivotal role in development. Results obtained from immunodetection indicates that this protein is highly expressed in plasma membrane region as well as cell wall region.

## CW-MBD proteins may provide a link between methylated DNA, histone methylation and euchromatin

Iversen, V., Nenseth, H.Z., Nateland, L., and Aalen, R.B.

Department of Biosciences, University of Oslo, Norway.

Epigenetic gene regulation particularly important at the transition from one developmental stage to the next in a plant's life, e.g. the switch from embryonic to vegetative growth, and later when switching to the reproductive stage. Leaf development does also go through a transformation, from cell proliferation to cell expansion. We have identified a small reader of epigenetic marks, more specifically DNA-methylation and Histone 3 lysine 4 monomethylation (H3K4me1), operating in this switch. Mutation in the gene, encoding a protein with a CW and a Methyl-CpG-Binding Domain, results in enlarged leaves. We have used RNA seq and Chromatin pull down seq to identify putative direct and indirect targets of the CW-MBP protein. One approach has been to map the chromatin states of all putative targets genes. Nine different chromatin states have been described in Arabidopsis, and State 7, mainly associated with long genes and intronic regions and with enrichment in H3K4me1, H2Bub, and H3K36me3 marks, is strongly overrepresented at locations where CW-MBD seems to bind. In contrast, binding peaks were not found at the transcriptional start site or in intergenic regions. We are presently studying in more detail putative direct target genes of CW-MBD.

## Roles of epigenetic regulation in inducing endoreplication in plants

Takatsuka, H.[1], and Umeda, M.[1,2]

[1]Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan.
[2]JST, CREST, Japan.

One of the unique features in plant development is frequent occurrence of endoreplication. Endoreplication is a distinct mode of the cell cycle in which mitosis and cytokinesis (M phase) are skipped, and DNA synthesis (S phase) is repeated, leading to DNA polyploidization. It is well known that endoreplication increases cell size, thereby enhancing organ growth. Although endoreplication is widely observed in higher plants, about 30 % of angiosperm, including useful crops and trees, does not undergo endoreplication. Therefore, a reasonable strategy to increase plant biomass is to induce endoreplication in non-endocycling plants. However, why different plant species have distinct abilities of endoreplication remains completely unknown.

Previous studies have proposed that inhibition of G2/M progression caused by decreased mitotic CDK activity is sufficient to induce endoreplication, based on studies in plant species with a high level of endoreplication, such as *Arabidopsis*. However, our studies using non-endocycling plants demonstrated that a reduction of mitotic CDK activity does not lead to DNA polyploidization, suggesting that an addtional factor(s) is (are) required to induce endoreplication in non-endocycling plants. Recently, we found that epigenetic modifications controlling chromatin structure, such as DNA methylation, histone methylation and acetylation, are highly associated with the competence of endoreplication. Moreover, we observed specific epigenetic modifications are transiently reduced just before the onset of endoreplication. We shall propose a model how control of chromatin structure is coordinated with cell cycle progression in induction of endoreplication.

## Arabidopsis *CUPULIFORMIS* genes: new players on the epigenetics scene

Mateo-Bonmatí, E., Juan-Vicente, L., Cerdá-Bernad, D., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

The critical developmental and physiological events in the plant life cycle depend on the proper activation and repression of sets of genes; plants accomplish this by several mechanisms, including epigenetic regulation. A number of Arabidopsis mutants with defects in the epigenetic machinery exhibit pleiotropic phenotypes, including two common traits: incurved leaves and early flowering [1,2], caused by the ectopic and heterochronic derepression of key developmental regulators.

Loss-of-function mutations of *INCURVATA11* (*ICU11*) also show these traits; *ICU11* is the founding member of a small gene family that we named the *CUPULIFORMIS* (*CP*) family. ICU11 and its closest paralog CP2 are nucleoplasmic proteins. Double mutant combinations of *icu11* alleles with loss-of-function alleles of genes encoding components of the epigenetic machinery exhibit synergistic, severe phenotypes, some of which are similar to those of *embryonic flower* mutants [3,4].

RNA-seq analysis showed that *icu11* plants mis-express hundreds of genes, including several members of the MADS-box family. We demonstrated that derepression of *SEPALLATA3* (*SEP3*) causes the leaf phenotype of *icu11* mutants. Bisulfite-seq of *icu11-1* showed no alteration in DNA methylation levels. Instead of affecting DNA methylation, ICU11 and CP2 are required for the deposition of H3K27me3 at the *SEP3* locus. Our results thus reveal a novel family of proteins required for deposition of histone epigenetic marks through an unknown mechanism.

[1] Goodrich, J., *et al.* (1997). *Nature* 386, 44-51.
[2] Barrero, J.M., *et al.* (2007). *Plant Cell* 19, 2822-2838.
[3] Sung, Z.R., *et al.* (1992). *Science* 258, 1645-1647.
[4] Chen, L., *et al.* (1997). *Plant Cell* 9, 2011-2024.

## An analysis of the expression of *CUPULIFORMIS* genes

Juan-Vicente, L., Mateo-Bonmatí, E., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

We are studying the Arabidopsis (CUPULIFORMIS; CP) family of five 2-oxoglutarate/Fe(II)-dependent dioxygenases (2OGDs). At least two CP proteins have epigenetic activity: INCURVATA11 (ICU11), the founding member of the family, and CP2. The superfamily of 2OGDs is the second largest family in plant proteomes and is widespread in bacteria and eukaryotes. The *CP* genes seem to be of ancient origin. Only two *CP* genes are present in genomes of monocots, and only one is found in the liverwort *Marchantia polymorpha* and the cryptophyte *Guillardia theta.* In Arabidopsis, these non-heme iron-containing soluble proteins localize to the cytosol, nucleus, and plastids. However, only some tens of plant 2OGDs have been functionally characterized.

In Arabidopsis, lack-of-function alleles of *CP* genes do not cause visible phenotypes, except *icu11* alleles, which cause leaf hyponasty. The only genetic interaction that we found in the double mutant combinations of alleles of these genes is that of *icu11 cp2* double mutants, which are lethal. No *cp4 cp5* double mutant was obtained, since *CP4* and *CP5* are arranged in tandem. We are obtaining artificial microRNAs and synthetic *trans*-acting siRNAs to simultaneously silence three or four *CP* genes.

We also examined the levels and pattern of *CP* expression to shed light on their potential functions. The number of expressed sequence tags found in databases is much higher for *ICU11* and *CP2* than for *CP3*, *CP4* and *CP5.* However, we obtained GUS transcriptional fusions that showed high levels of expression of *ICU11* and *CP2,* but also of *CP3* in growing tissues. Transgenic plants expressing *$35S_{pro}$:CP:GFP* transgenes showed that ICU11, CP2, and CP5 are nuclear proteins, and suggested that CP4 is nuclear and cytoplasmic and that CP3 is not nuclear. The different expression patterns and subcellular localizations indicate that the members of this small gene family likely have different functions in plant development.

# The *INCURVATA11-CUPULIFORMIS2* paralogous gene pair is essential and exhibits unequal functional redundancy

Nadi, R., Mateo-Bonmatí, E., Juan-Vicente, L., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Spain.

Plant genomes contain many functionally redundant paralogous gene pairs that originated by gene duplication, after which the duplicated genes diverged but retained redundant functions. Many of these pairs of paralogs encode components of signal transduction, metabolic, and developmental pathways, as well as ribosomal proteins [1]. Some of these gene pairs have remained evolutionarily stable—in some cases up to 100 million years—suggesting that they contribute positively to the fitness of the organism by reducing the phenotypic cost of mutations [2].

Here, we studied the Arabidopsis *INCURVATA11* (*ICU11*)-*CUPULIFORMIS2* (*CP2*) gene pair. Redundant paralogs tend to show high sequence identity and the ICU11 and CP2 Arabidopsis proteins show more than 50% amino acid sequence identity. Paralogs also tend to show weak phenotypes as single mutants and very strong phenotypes as multiple mutants. Indeed, severe, synergistic phenotypes were observed in the double mutant combinations of loss-of-function alleles of *ICU11* and *CP2*: seeds of the *icu11 cp2* double mutants germinated but seedlings lacked rosettes leaves, skipped vegetative growth and began flowering upon germination, generating small, sterile flowers. By contrast, the *icu11* single mutants exhibit mildly hyponastic leaves and early flowering, and the *cp2* mutants are indistinguishable from wild type, except for some infertility.

The relatively mild phenotype of *icu11* alleles, the completely wild-type phenotype of *cp2* alleles, and the severe, lethal phenotypes of their double mutant combinations, indicate that *ICU11* and *CP2* are a pair of redundant genes with an essential function. However, ICU11 and CP2 do not contribute equally to their redundant function, as the *ICU11/icu11-2*;*cp2-3/cp2-3* plants are phenotypically wild type, but the *icu11-2*/*icu11-2*;*CP2/cp2-3* plants have a lethal phenotype. We also interchanged the promoters of *ICU11* and *CP2,* and found that the promoters of these genes, but not the proteins that they encode, are equivalent.

[1] Kafri, R., *et al.* (2009). *Cell* **136**, 389-392.
[2] Dean, E.J., *et al.* (2008). *PLOS Genet*. **4**, e1000113.

## The Arabidopsis RIBOSOMAL RNA PROCESSING7 nucleolar protein is required for 40S ribosome subunit biogenesis

Micol-Ponce, R., Sarmiento-Mañús, R., Ruiz-Bayón, A., Mora-Navarro, E., and Ponce, M.R.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

NF-kappa B activating protein (NKAP) is a multifunctional protein that acts in splicing and transcriptional repression in animals. The Arabidopsis ortholog of NKAP is MORPHOLOGY OF ARGONAUTE1-52 SUPPRESSED2 (MAS2), which is required for 45S rDNA transcription and 45S pre-rRNA processing [1]. Ribosomal RNA processing protein 7 (Rrp7) participates in the biogenesis of the 40S ribosomal subunit in *Saccharomyces cerevisiae*.

We identified RRP7, the Arabidopsis ortholog of Rrp7, in a yeast two-hybrid screen for MAS2 interactors. We found RRP7 localized at the nucleolus. Lack of RRP7 function causes nucleolar hypertrophy, 18S rRNA altered processing, and nucleolar retention of mature and precursor 18S rRNA species. The pleiotropic phenotype of *rrp7* mutants includes altered shoot phylotaxy, aberrant lateral organ venation pattern and ABA hypersensitivity at the seedling establishment stage.

The *RRP7* gene is coexpressed with genes encoding factors required for 45S pre-rRNA processing and ribosome subunit assembly, including *SMALL ORGAN4* (*SMO4*), which is required for 5.8S rRNA maturation. The Arabidopsis *NUCLEOLIN1* (*NUC1*) and *NUC2* redundant genes encode nucleolar proteins that participate in the epigenetic control of 45S rDNA expression [2]. We observed synergistic phenotypes in double mutant combinations of alleles of *RRP7* with alleles of *NUC1*, *NUC2*, *MAS2* or genes encoding components of the microRNA machinery. *rrp7* alleles seem epistatic to *smo4* alleles. The Arabidopsis genome contains hundreds of 45S rDNA genes, with four types of variants (*VAR*). *VAR* patterns of expression differ among accessions and developmental stages, and these patterns are altered in the *rrp7* and *smo4* mutants. Our results unveil the action and interactions of a key factor in 40S ribosome subunit biogenesis in Arabidopsis.

[1] Sánchez-García, A.B., *et al.* (2015). *Plant Cell* 27, 1999-2015.
[2] Pontvianne, F., *et al.* (2010). *PLOS Genet.* 6, e1001225

# Arabidopsis *SMALL ORGAN4* encodes a nucleolar and nucleoplasmic protein required for 5.8S rRNA maturation

Micol-Ponce, R., Sarmiento-Mañús, R., Fontcuberta-Cervera, S., and Ponce, M.R.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

MORPHOLOGY OF ARGONAUTE1-52 SUPPRESSED2 (MAS2) is the Arabidopsis ortholog of metazoan NF-kappa B activating protein (NKAP). MAS2 is required for 45S rDNA transcription and 45S pre-rRNA processing [1], and NKAP is involved in splicing and transcriptional repression. In *Saccharomyces cerevisiae*, Nucleolar protein 53 (Nop53) participates in the biogenesis of the 60S ribosomal subunit.

The Arabidopsis ortholog of Nop53 is SMALL ORGAN4 (SMO4) [2], which we identified as a MAS2 interactor in a yeast two-hybrid screen. In a genetic screen for Arabidopsis mutants with altered leaf shape, we identified *denticulata2* (*den2*), which we found to be an allele of *SMO4*. The morphological phenotype caused by *den2* is similar to, but stronger than that of *smo4* insertional alleles; all these mutants exhibit reticulate, pointed, and dentate vegetative leaves.

Null *smo4* alleles cause accumulation of 5.8S rRNA precursors, as already described for its yeast and human orthologs. We found the Arabidopsis SMO4 protein localized mainly to the nucleolus but also to the nucleoplasm. The *SMO4* gene is coexpressed with genes encoding factors required for 45S pre-rRNA processing and ribosome subunit assembly, including *RIBOSOMAL RNA PROCESSING7* (*RRP7*), which is involved in 18S rRNA maturation. Our results on the morphological, cytological and molecular phenotypes caused by the lack of function of SMO4 shed light on the role of this protein in 60S ribosomal subunit biogenesis and confirm its interaction with MAS2.

[1] Sánchez-García, A.B., *et al.* (2015). *Plant Cell* 27, 1999-2015.
[2] Zhang, X.R., *et al.* (2015). *J. Integr. Plant Biol.* 57, 810-818.

## Genetic and physical interactions between CAX INTERACTING PROTEIN4 and MORPHOLOGY OF ARGONAUTE1-52 SUPPRESSED2 in Arabidopsis

Aceituno-Valenzuela, U., Ruiz-Bayón, A., Sarmiento-Mañús, R., Micol-Ponce, R., and Ponce, M.R.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

The Arabidopsis ortholog of metazoan NF-kappa B Activating Protein is MORPHOLOGY OF ARGONAUTE1-52 SUPPRESSED2 (MAS2), an essential protein that seems to play a key role in the regulation of rRNA synthesis. By imaging of a MAS2:GFP fusion and fluorescence *in situ* hybridization, we found that MAS2 colocalizes with the 45S rDNA in nucleolar organizer regions. To identify MAS2 interactors, we performed a yeast two-hybrid screen; the most represented interactor found, in 23 of 55 positive clones, was CAX INTERACTING PROTEIN4 (CXIP4). This protein is of unknown function and was previously identified as interacting with the high-affinity vacuolar calcium antiporter CATION EXCHANGER1 (CAX1). CXIP4 is a plant-specific protein, with a conserved CCHC-type zinc finger motif (zinc knuckle), which is involved in DNA, RNA, and protein binding. In addition, the 30 N-terminal amino acids of CXIP4 show 70% similarity to mammalian Splicing regulatory protein, glutamine/lysine-rich 1 (SREK1)-interacting protein 1.

*CXIP4* is a single-copy essential gene in Arabidopsis, as shown by its insertional allele *cxip4-1*, which causes embryonic lethality. To circumvent such lethality, we constructed artificial microRNAs (*amiR-CXIP4*) targeting the *CXIP4* mRNA. The a*miR-CXIP4* plants displayed pointed and reticulate leaves, a phenotype that is characteristic of mutants affected in genes encoding ribosomal proteins and other genes involved in translation.

Additional transgenes were obtained to complement the lethality of homozygous *cxip4-1* plants, and to visualize the expression pattern of *CXIP4* and the subcellular localization of CXIP4. We are also studying the genetic interactions between *CXIP4* and *MAS2*, and between *cxip4* alleles and alleles of genes encoding components of the microRNA pathway and ribosomal factors. Our preliminary results suggest that CXIP4 is involved in ribosome biogenesis or the control of translation.

## Characterization of the Arabidopsis *MORPHOLOGY OF ARGONAUTE1-52 SUPPRESSED5* gene

Cabezas-Fuster, A., Micol-Ponce, R., Senent-Valero, Y., and Ponce, M.R.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

ARGONAUTE1 (AGO1) functions as part of the RNA-induced silencing complex and *ago1* mutations affect leaf development and polarity. To examine AGO1 function in leaf development, we carried out a screen for genetic suppressors. To this end, we used ethyl methanesulfonate to mutagenize the Arabidopsis *ago1-52* line, which carries a hypomorphic, viable and recessive allele of *AGO1*. We screened 37,000 M2 seeds, and isolated 23 viable double mutants exhibiting suppression of the leaf phenotype of *ago1-52*; the lines carrying second-site mutations were named *mas* (*morphology of argonaute1-52 suppressed*) [1].

This screen identified six alleles of *MAS5* and positional cloning of the *mas5-1* mutation allowed us to identify a G→A transition (Lys→Glu) in AT1G80070 (*PRE-MRNA PROCESSING8; PRP8*), which encodes a key splicing factor. Null alleles of *PRP8 (MAS5*) and its yeast and animal orthologs are embryonic lethal. Our *mas5* alleles might be neomorphic or antimorphic: they act as dominant suppressors of *ago1-52* but do not exhibit any visible phenotype in a wild-type genetic background.

The *ago1-52* allele carries a mutation that causes mis-splicing of its pre-mRNA, which finally produces a mixture of mutant and entirely wild-type AGO1 proteins; this aberrant splicing is not modified in the *ago1-52 mas5-1* double mutant plants. However, we observed a higher level of the wild-type AGO1 protein in *ago1-52 mas5-1* plants than in *ago1-52* plants.

We are studying the genetic interactions of *ago1-52* with *prp8* hypomorphic alleles obtained by other authors, and with mutations that cause mis-splicing, in order to ascertain the molecular nature of the suppression effect caused by *mas5* alleles on *ago1-52*.

[1] Micol-Ponce, R., *et al.* (2015). *Sci. Rep.* 4, 5533.

## The *api7* mutant reveals a role for Arabidopsis ABCE proteins in leaf development and venation patterning

Navarro-Quiles, C., Sosa-Domínguez, P., Mateo-Bonmatí, E., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

In a screen for Arabidopsis leaf morphological mutants induced by EMS, we isolated the *apiculata7* (*api7*) mutant, which has small, dentate and pointed leaves, with pale margins and aberrant venation pattern. The *api7* adult plants are also shorter than wild type in height. These phenotypes occur in many mutants with defects in components of the translation machinery. We combined linkage analysis and whole-genome, next-generation sequencing to identify the *api7* mutation, which we found to be a hypomorphic allele of *RNASE L INHIBITOR2* (*RLI2*). We obtained an insertional allele of *RLI2* from a public collection; this allele (which we named *rli2-2*) is null and recessive lethal. In all studied eukaryotes and archaea, null alleles or RNA interference lines of orthologs of Arabidopsis *RLI2* are lethal and hypomorphic alleles cause slow growth.

The *RLI2* gene encodes the Arabidopsis ABCE2 protein. The best-studied ABCE protein is yeast Rli1, which participates in ribosome biogenesis and recycling. Human and Arabidopsis ABCE proteins also function in RNA silencing. Consistent with its potential role in ribosome function, we observed synergistic phenotypes in double mutant combinations of *api7* and loss-of-function alleles of the *ASYMMETRIC LEAVES1* (*AS1*) and *AS2* genes, which encode transcription factors known to play a role in leaf dorsoventral patterning and polarity. To test the subcellular localization of RLI2, we constructed an $RLI2_{pro}$*:RLI2:GFP* translational fusion, which showed that RLI2 is a cytoplasmic protein, as expected from the absence of a predicted transmembrane domain. Although the aberrant venation pattern of *api7* leaves suggests a defect in auxin homeostasis, the transport and perception of auxin does not seem to be altered in *api7* roots. The *api7* mutation is the first viable mutant allele of *RLI2.* Our study of this allele revealed an unexpected role for ABCE proteins in leaf development and venation patterning.

# The *ANGULATA7* gene encodes a DnaJ-like zinc-finger-domain protein involved in chloroplast function and leaf development in Arabidopsis

Muñoz-Nortes, T., Pérez-Pérez, J.M., Ponce, M.R., Candela, H., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

The characterization of mutants with altered leaf shape and pigmentation has allowed the identification of nuclear genes that encode plastid-localized proteins with essential functions in leaf growth and development. A large-scale screen previously allowed us to isolate ethyl methanesulfonate (EMS)-induced mutants with small rosettes and pale green leaves with prominent marginal teeth, which were assigned to a phenotypic class that we dubbed Angulata. The molecular characterization of the *ANGULATA* genes should help us to advance our understanding of the relationship between chloroplast biogenesis and leaf morphogenesis.

Here we report the phenotypic and molecular characterization of the *angulata7-1* (*anu7-1*) mutant of Arabidopsis, which we found to be a hypomorphic allele of the *EMB2737* gene, previously known only for its embryonic-lethal mutations. *ANU7* encodes a plant-specific protein containing a domain similar to the central cysteine-rich (CR) domain of DnaJ proteins. DnaJ proteins normally function as chaperones, either alone or in combination with heat-shock protein 70, and have been proposed to participate in the folding, unfolding, assembly and degradation of other proteins.

We have found that ANU7 is necessary for the accumulation of photosynthetic pigments and the correct organization of the thylakoid membrane system. Our microarray and qRT-PCR expression studies show that many genes which normally function in the chloroplasts are upregulated in *anu7-1* rosettes, with a significant overrepresentation of those required for the expression of plastid genome genes such as many subunits of plastid transcriptionally active chromosome complexes (pTAC). The synergistic interaction between the *anu7-1* mutation and a loss-of-function mutation of *GENOMES UNCOUPLED1* (*GUN1*) points to a functional relationship between *ANU7* and *GUN1*, both of which have been isolated in the nucleoid fraction of plastids.

## Functions of chloroplast ribosome proteins revealed through characterization of the *crd* mutants of Arabidopsis

Robles, P., Ferrández-Ayela, A., Núñez-Delegido, E., and Quesada, V.

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202 Elche, Alicante, Spain.

The *crd* (*chloroplast ribosome defective*) mutants of *Arabidopsis thaliana* are loss-of-function alleles of four nuclear genes encoding different chloroplast ribosomal proteins: three of the small subunit (30S) and one of the large one (50S). Phenotypically, the *crd* mutants show a reduced growth and general paleness of green tissues. Cells of the pallisade meshophyll of vegetative leaves are not as densely packaged as in the wild type and their chloroplasts are abnormal. In order to determine if chloroplast function is perturbed in the *crd* mutants, we are performing different molecular analyses.

We have shown that paleness of *crd* leaves correlates with reduced levels of photosynthetic efficiency as determined by the ratio Fv/Fm. Through quantification of the different ribosomal RNA species, we have found changes in the 30S:50S ratio, being lower than the wild type in three *crd* mutants and higher in just one, which might suggest assembly and/or stability problems of their chloro-ribosomes. We have also studied by qRT-PCR whether, in addition to potential defects in chloroplast translation, the *crd* mutants show altered steady-state levels of plastid and/or nuclear gene transcripts. Our results revealed that the studied chloroplastic genes are upregualted, including those of genes involved in photosynthesis and control of gene expression in the organelle. Additionally, we found in the four *crd* mutants an increase in the transcript levels of the nuclear *CRD* genes, except in the one mutated in each of them.

In addition to the developmental effects produced by the reduction in chloro-ribosome function, we want to check if CRD gain of function has any effect at the morphological and/or molecular levels in the wild-type and mutant genetic backgrounds. We are making and characterizing CRD overexpression lines in which the transcription of these genes is constitutively driven by the CaMV 35S promoter.

## Functional characterization of the Arabidopsis mitochondrial transcription termination factors mTERF5 and mTERF9

Núñez-Delegido, E., Ferrández-Ayela, A., Robles, P., and Quesada, V.

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202, Elche, Alicante, Spain.

Plant genomes harbor a considerably larger number of mTERFs than animals. However, unlike animals, very little is known about its function in plants. To gain insight into the roles of plant mTERFs, we previously identified and characterized the *Arabidopsis thaliana mda1* and *mterf9* mutants, affected in the chloroplast-localized mTERF5 and mTERF9 proteins, respectively, which exhibited altered chloroplast morphology, reduced growth and pale pigmentation. We are carrying out different experimental approaches to further characterize *mTERF5* and *mTERF9* functions. The genomic sequence of *mTERF5* and *mTERF9* fused to the constitutive 35S promoter complement the *mda1-1* and *mterf9* mutant phenotypes, respectively. Besides, we have obtained *mTERF5* and *mTERF9*-overexpressing transgenic lines in a wild-type background, that we confirmed by qRT-PCR. We are currently studying them at the molecular and phenotypic levels.

To investigate the effect that impaired chloroplast biogenesis might have on *mTERF5* and *mTERF9* activity, we analysed their expression in several chloroplast defective mutants and found significant differences in *mTERF5* and/or *mTERF9* transcript levels for some of them. Our double mutant analysis reveals that the *sca3-2* mutation, affecting the RpoTp plastid RNA polymerase, is epistatic on *mda1-1*, while *mterf9* and *sca3-2* synergistically interact. This suggests that the affected genes participate in the same genetic pathway required for accurate chloroplast development. In this line, the maximum efficiencies of the photosystem II are reduced in *mda1-1* and *mterf9* compared with Col-0.

rRNA abundance is used as a proxy for the levels of the 50S and 30S ribosomal subunits. Hence, we quantified the levels of the different plastid rRNA species in *mda1-1* and *mterf9*. We found a differential accumulation of some plastid rRNAs in *mda1-1* and *mterf9* compared with Col-0, which is consistent with a defect in chloroplast ribosomal stability and/or assembly in the mutants.

# The characterization of the Arabidopsis *mterf6-5* mutant reveals a role for the *mTERF6* gene in organelle gene expression and plant development

Quesada, V., Ferrández-Ayela, A., and Robles, P.

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202 Elche, Alicante, Spain.

We performed a reverse genetics screen for mutations affecting the mitochondrial transcription termination factor (mTERF) family in *Arabidopsis thaliana.* One of the mutants identified in our screen proved to be a new allele not yet described of the *mTERF6* gene, that we have named *mterf6-5*. The *mterf6-5* mutant exhibits markedly reduced growth and developmental retardation, pale cotyledons, leaves, stems and sepals. Accordingly, photosynthetic efficiency is reduced in *mterf6-5* plants, as we determined by the $F_v/F_m$ ratio. The insertion of the T-DNA caused a strong reduction in *mTERF6* transcript levels in the *mterf6-5* mutant. We studied by qRT-PCR *mTERF6* expression pattern along development. We detected *mTERF6* transcripts in all the stages analysed, reaching their highest and lowest levels at the earliest and latest time points studied (7 and 20 days after stratification, respectively). mTERF6 was previously reported to be dually targeted to chloroplasts and mitochondria. Our qRT-PCR analysis shows that the expression of several characteristic plastid and mitochondrial genes is altered in the *mterf6-5* mutant, being most of them significantly upregulated. In addition, the transcript levels of nuclear genes encoding plastid and/or mitochondrial targeted proteins are also misregulated in *mterf6-5* plants, suggesting that defective chloroplast and/or mitochondrial function is signaled to the nucleus. In addition, we have performed a double mutant analysis and found that *mterf6-5* synergistically interacts with the chloroplast defective mutants *soldat10*, *mda1-1* and *mterf9* (affected in different *mTERF* genes) as well as with *sca3-2*, affected in the plastid RpoTp RNA polymerase. These results point to a functional relationship between *mTERF6*, other *mTERFs* and *RpoTp/SCA3*. Our analysis of the *mterf6-5* mutant reveals new functions for the *mTERF6* gene in plant development and its important role in organelle gene expression.

## Elucidating the interaction networks at work in the methyladenosine epitranscriptome

Rodríguez-Alcocer, E., Gómez-Peral, N., Hernández-Cortés, C., Ruiz, E., Burillo, E., Jover-Gil, S., and Candela, H.

Instituto de Bioingeniería. Universidad Miguel Hernández, 03202 Elche, Alicante, Spain.

Recent research on the reversible methylation of adenosine residues at their $N^6$ position, i.e. the most abundant internal modification present in the messenger RNA (mRNA) of eukaryotes, has led to the establishment of an entirely new field of study called epitranscriptomics. Despite this post-transcriptional modification has been known for about four decades, the study of adenosine methylation has emerged as a hot research topic only in the last few years, coinciding with the implementation phase of our grant, placing us in a privileged position to address frontier research questions in this field using *Arabidopsis thaliana* as a model organism.

Some key advances in this field have been: (i) the characterization of the multisubunit complex that methylates adenosine residues ("writer" proteins), (ii) the identification of enzymes with demethylase activity, such as the one encoded by the *FTO* gene, whose polymorphisms have been associated to obesity in humans ("eraser" proteins), and (iii) the identification of the YTH domain as the $N^6$-methyladenosine ($m^6A$) binding domain of some RNA binding proteins ("reader" proteins). As a continuation of our project, we are systematically performing yeast two-hybrid screens using proteins of the three functional categories (writers, erasers and readers) as baits, which have already yielded some promising protein-protein interactions. We are also setting up a novel, high-throughput RNA tagging protocol to identify mRNA molecules targeted by proteins from the three functional categories. Our ultimate goal is to make a significant contribution to this new field by identifying the protein-protein and RNA-protein interactions that shape the $m^6A$ epitranscriptome in *Arabidopsis thaliana*.

This work received support from Spain's Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF) ('Una manera de hacer Europa') [BFU2012-31719] and Generalitat Valenciana [ACOMP/2015/042]. E.R.-A.'s contract is also supported by a MINECO grant [PEJ-2014-A-21398].

## Impaired maintenance of the dNTP pool causes leaf reticulation in the *venosa4* mutants

Sarmiento-Mañús, R., Ruiz-Ramírez, J., González-Bayón, R., Quesada, V., Ponce, M.R., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

In the leaves of Arabidopsis *venosa* (*ven*) mutants, the leaf vascular network can be clearly distinguished as green reticulation on a paler lamina. We have previously shown that leaf reticulation may reveal alterations in the internal leaf architecture [1,2]. By positional cloning, we identified the ethyl methanesulfonate-induced *ven4-1* mutation as a new allele of a gene that encodes a putative phosphohydrolase of unknown function and is expressed in expanding leaves and flowers, roots, and stems. We obtained two insertional *ven4* alleles from public collections; these alleles exhibited a phenotype similar to that of *ven4-1*. The *ven4* plants have smaller palisade mesophyll cells and reduced fresh and dry weights, compared with wild type.

The VEN4 protein localizes in the nucleus, as described for its human ortholog, SAM domain and HD domain-containing protein 1 (SAMHD1), a dNTP triphosphohydrolase that is essential for dNTP catabolism. Consistent with the amino acid sequence similarity of human SAMHD1 and Arabidopsis VEN4, *ven4* mutants are hypersensitive to hydroxyurea; this substance alters the pool of dNTPs by inhibiting RIBONUCLEOTIDE REDUCTASE (RNR) activity. In addition, we found that *ven4-1* and *ven4-2* genetically interact with *tso2-1*, a mutant allele of *RNR2*.

We also observed synergistic phenotypes in the double mutant combinations of *ven4* alleles with *dov1* (*differential development of vascular associated cells 1*); *DOV1* encodes glutamine phosphoribosyl pyrophosphate aminotransferase 2 (ATase2), the enzyme that catalyses the first step in *de novo* purine biosynthesis [3]. Our results suggest that *VEN4* plays a key role in the maintenance of the dNTP pool in Arabidopsis, which is critical for DNA replication and repair.

[1] González-Bayón, R., *et al.* (2006). *J. Exp. Bot.* 12, 3019-3031.
[2] Mollá-Morales, A., *et al.* (2011). *Plant J.* 65, 335-345.
[3] Rosar, C., *et al.* (2012). *Mol. Plant* 5, 1127-1241.

# Artificial microRNAs targeting paralogous genes reveal new roles for transcription factors in Arabidopsis leaf development

Ruiz-Bayón, A., Jover-Gil, S., Aguilar-García, A.M., Micol, J.L., and Ponce, M.R.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

Plant genomes contain many gene families that include functionally redundant members; this can hamper analysis of individual gene functions. For example, the *Arabidopsis thaliana* genome contains ~2,000 genes encoding transcription factors (TFs) but a mutant phenotype has been described for only ~200 of these genes. To address the function of these genes encoding TFs, we obtained Arabidopsis transgenic lines expressing artificial microRNAs (amiRNAs), each of which targets a group of paralogous genes [1]. Several of these transgenic lines exhibit morphological alterations in leaves, which reveals a role for the gene families targeted by their amiRNAs in leaf development.

Here, we describe our analysis of transgenic lines carrying *amiR-TPL17* (targeting 2 members of the C2H2 TF family), *amiR-TPL36* (targeting 5 members of the NAC TF family), *amiR-TPL106* (targeting 2 members of the CCAAT-HAP5 TF family) and *amiR-TPL189* (targeting 2 members of the C2C2-CO-like TF family). In most cases, the transgenic plants were smaller than the wild-type Col-0, and their rosette and lamina area, and petiole length were reduced. The palisade mesophyll cells were also smaller than wild type in all the transgenic lines. These results suggest a role for the genes targeted by these amiRNAs in the control of mesophyll cell size, which contributes to the final size of the whole organ, and therefore in leaf development.

Since these lines were paler than Col-0, we also used confocal microscopy to determine whether the palisade mesophyll cells have fewer or smaller chloroplasts. Chloroplast size, chlorophyll *a* and *b* levels, and photosynthetic efficiency were reduced in *amiR-TPL17*, *amiR-TPL36* and *amiR-TPL189* plants. To confirm that the phenotypes of our *amiR-TPL* lines are caused by repression of their targets, we are producing artificial target mimics designed to inhibit the amiRNAs.

[1] Jover-Gil, S., *et al.* (2014). *Plant J.* 80, 149-160.

## How does CUC2 regulate leaf serration development in Arabidopsis?

Zhang, Z., Ravanelli, S., and Tsiantis, M.

Max Planck Institute for Plant Breeding Research. Department of Comparative Development and Genetics. Carl-von-Linné-Weg 10, 50829 Köln, Germany.

A key question in developmental biology is how developmental patterning generates the final form of an organ. *Arabidopsis thaliana* produces simple leaves bearing repeated marginal outgrowths termed serrations, providing a good opportunity to study this question. The NAC domain transcription factor CUC2 regulates serration formation by promoting the formation of PIN1 convergence points and auxin activity maxima along the leaf margin during leaf development (Bilsborough et al., 2011). However, how CUC2 regulates repeated formation of auxin maxima and serrations remains enigmatic. To address this question, we did an EMS-mutagenesis screen in a *cuc2-3* mutant background to identify novel components in regulating serration formation, specifically looking for suppressors that restore serration development.

Among the suppressors identified, we investigate #51 because it displays the strongest suppression effect and no other developmental defects. We found that #51 could restore auxin maxima and PIN1 convergence points along *cuc2* leaf margin. Currently we are trying to identify the molecular basis for the 51 mutation and understand how the gene defined by this mutation regulates the PIN1 convergence points and auxin maxima formation.

# A functional link between *DESIGUAL1* and cytokinins in early leaf development?

Navarro-Cartagena, S., Wilson-Sánchez, D., Nicolás-Albujer, M., Muñoz-Díaz, E., Alcañiz-Pascual, L., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Spain.

Bilateral symmetry is a striking property of many plants and animals. In bilaterally symmetric plant organs such as leaves, acquisition of symmetry requires properly regulated development on both sides of the midplane. However, how this occurs remains unclear at the molecular level. We are studying the Arabidopsis *DESIGUAL* (*DEAL*) gene family, whose members seem to be required for bilateral symmetry at very early stages of leaf organogenesis. The *deal1* mutants show defects in leaf bilateral symmetry and these morphological aberrations are visible at very early stages of leaf development, when cells proliferate but have not begun to expand. Therefore, DEAL1 seems to be involved in cell proliferation.

The DEAL1 protein localizes to the membrane of a sub-compartment of the endoplasmic reticulum. A split-ubiquitin membrane-based yeast two-hybrid screen for DEAL1 interactors identified, among other proteins, several components of the Very-Long-Chain Fatty Acid (VLCFA) elongation complex. VLCFAs negatively regulate leaf cell proliferation by inhibiting cytokinin biosynthesis. Specifically, VLCFAs inhibit the expression of *ATP/ADP ISOPENTENYLTRANSFERASE* (*IPT*) genes, which catalyze the first step of the cytokinin biosynthetic pathway [1].

To test the link between DEAL1 function and cytokinins, we analyzed the response of *deal1-1* to 6-benzylaminopurine, a synthetic cytokinin, which increased the penetrance and severity of the bilateral asymmetry phenotype. Cafenstrol, which inhibits the activity of the VLCFA elongation complex, reduced the penetrance and severity of the phenotype of *deal1-1*. Using quantitative PCR we found slightly increased the expression of several *IPT* genes in the *deal1-1* mutant. Therefore, our results indicate a potential link between DEAL1 function and cytokinin biosynthesis, possibly through VLCFAs.

[1] Nobusawa, T., *et al.* (2013). *PLoS Biol.* 11, e1001531.

## Cytokinins regulate Pi homeostasis in Arabidopsis roots

Silva-Navas, J., Navarro-Neila, S., Sáez, A., and del Pozo, J.C.

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

Phosphorous (Pi) is an essential macronutrient for plant growth. Pi is needed for the generation of ATP, nucleic acids, membrane phospholipids. In addition, Pi is involved in many metabolic and regulatory processes. Pi starvation is one of the most critical nutritional deficiencies that severely affects plant survival and reproduction. Using *in vitro* conditions, in which roots are normally grown in light conditions, Pi starvation strongly reduces Arabidopsis root growth, while increasing lateral root density.

Recently, our group have engineered a device, called D-Root, that allows the *in vitro* cultivation of plants with the aerial part exposed to normal photoperiodic conditions but the root system on darkness. Using the D-Root, we found that root system architecture under Pi deficiency significantly differs from the phenotype observed in light grown roots. Reduction on primary root growth in low Pi was minor (only 30% less than high Pi medium). Conversely to light grown roots, we found that Pi starvation decreases lateral root density in the D-Root. Different hormonal treatments combined with Pi starvation revealed that cytokinin significantly increases inorganic Pi concentration in roots. However, when cytokinin is applied to the double mutant *ahk3*/*cre1* this increase in inorganic Pi is not detected, pointing out a direct role of this hormone in Pi homeostasis. Moreover, cytokinin treatment reduces the expression of the Pi transporter PHO1, implicated in Pi mobilization from roots to shoots.

Taken together, our data indicate that light strongly influence Pi starvation response in roots. Furthermore, cytokinin controls Pi homeostasis and transport to shoots.

## A conserved carbon-starvation response underlies bud dormancy in woody and herbaceous species

Tarancón, C., González-Grandío, E., Oliveros, J.C., Nicolás, M., and Cubas, P.

Plant Molecular Genetics Department, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Spain.

Shoots are formed from axillary meristems and buds, whose activity is controlled by systemic and local signals that modulate growth and development. These signals convey information about nutrient and water availability, light quality, sink/source organ activity and other variables that determine the timeliness and competence to maintain development of new shoots. This information is translated into a local response, in meristems and buds, of growth or quiescence. Although some key genes involved in the onset of bud latency have been identified, the gene regulatory networks (GRN) controlled by these genes are not yet well defined. Moreover, it has not been determined whether bud dormancy induced by environmental cues, such as low red to far-red light ratio, shares genetic mechanisms with bud dormancy induced by other causes, such as apical dominance or a short-day photoperiod. The evolution and conservation of these genetic networks throughout angiosperms has not yet been studied. We have reanalyzed public transcriptomic datasets that compare quiescent and active axillary buds of Arabidopsis with datasets of axillary buds of the woody species *Vitis vinifera* (grapevine) and *Populus tremul*a x *Populus alba* (poplar) during the bud growthto-dormancy transition. Our aim was to identify potentially common GRN induced during the process that leads to para-, eco- and endodormancy in buds, and to study their biological significance. In Arabidopsis buds entering eco- or paradormancy, we identified four induced, interrelated GRN that correspond to a carbon (C) starvation syndrome, typical of tissues undergoing C depletion. This response is also detectable in poplar and grapevine buds before and during the transition to dormancy. In all eukaryotes, C limiting conditions are coupled to growth arrest and latency, like that observed in dormant axillary buds. Bud dormancy might in part be a consequence of the underlying C starvation syndrome triggered by environmental and endogenous cues that signal conditions unfavorable for sustained shoot growth.

## Developing Universal Synthetic Promoters driving specific expression in the Arabidopsis and maize leaf

Vercruysse, J.[1,2], Van Bel, M.[1,2], Storme, V.[1,2], Nelissen, H.[1,2], Vandepoele, K.[1,2], Gonzalez, N.[1,2,3], and Inzé, D.[1,2]

[1]Center for Plant Systems Biology, VIB, Technologiepark 927, 9052 Gent, Belgium.
[2]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Gent, Belgium.
[3]INRA, UMR1332 Biologie du fruit et Pathologie, INRA Bordeaux Aquitaine, CS20032, F-33882, Villenave d'Ornon cedex, France.

Synthetic promoters are artificial sequences that do not occur in nature and that can be used to precisely control gene expression. Here, we present the design of such synthetic promoters, using a combination of transcription factor binding sites (TFBSs) derived from Arabidopsis and maize gene expression data during leaf development.

In order to identify and compare the TFBS and binding TFs common or specific to Arabidopsis and/or maize, we conducted a large orthology study including leaf transcriptomic data from both organisms. We built an integrated network with all functional orthologs from both species, allowing us to determine expression conservation at gene level. Overall, we observed that the expression of genes involved in leaf growth is largely conserved, with some exceptions. The same tendency was observed for all known TFs. Exploring the conservation among TFs in both species will help us to understand how and, more importantly when, these TFs bind on TFBSs and regulate expression. With this information, we will contribute to building further the growth regulatory network in Arabidopsis and maize.

Based on this information, we inferred TFBSs that could be associated with a certain expression trend and designed synthetic promoters to control gene expression. To identify functional promoters, we used reporter constructs in a large screen for Arabidopsis. On the long term, we will study the universal character of the synthetic promoters by analyzing the expression specificity during leaf development in maize, one of the model organisms for monocots.

Additionally, we aim at improving yield and/or biomass by driving specific expression of known growth-promoting genes under the influence of a synthetic promoter.

## Advances in the genetic dissection of tomato leaf development from an enhancer trap mutagenesis program

Fonseca, R.[1], Yuste-Lisbona, F.J.[1], Pineda, B.[2], Pérez-Martín, F.[1], García-Sogo, B.[2], Atares, A.[2], Angosto, T.[1], Capel, J.[1], Moreno, V.[2], and Lozano, R.[1]

[1]Centro de Investigación en Biotecnología Agroalimentaria (BITAL), Universidad de Almería, 04120 Almería, Spain.
[2]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 46022 Valencia, Spain.

During the vegetative phase, the shoot apical meristem mainly produces leaves disposed following a specific phyllotaxis pattern. Many progresses have recently been made in the genetic dissection of leaf development and some regulatory genes have been discovered in *Arabidopsis thaliana*. However, genetic and molecular mechanisms determining leaf developmental pattern in crop species remain largely unknown. Indeed, leaf area and plant architecture are key traits in vegetable species since they are determinant factors for plant growth and productivity. Particularly, tomato (*Solanum lycopersicum* L.) develops unipinnate compound leaves (7-9 leaflets), which are positioned in a spiral phyllotaxis. In this work, we have screened a T-DNA insertion mutant collection of tomato with the aim to identify mutations that either increase or decrease the degree of leaf complexity.

Two mutant phenotypes, both inherited as monogenic traits and co-segregating with the T-DNA insertion, have been characterized and the tagged genes cloned. First, leaves of the mutant line 2477ETMM showed evident necrosis symptoms and consequently, a reduction of plant growth at early stages of development. Later, necrosis increased and leaves became curled and senescent. T-DNA mutation in the 2477ETMM line was located in the fifth exon of the *Solyc11g011960*, a gene coding for a UTP-glucose-1-phosphate uridylyltransferase involved in programmed cell death and leaf development. This finding means a novel gene function reported in tomato. Second, the mutant line 1381ETMM displayed a significant reduction of leaf size, giving rise to only one or two secondary leaflets; in addition, flower and fruit development were severely affected. 1381ETMM line only bore one T-DNA copy located in the sixth exon of the *LYRATE* gene, which codes for a lipase-like protein required to establish the

appropriate leaf morphogenetic pattern. Together, our results support that enhancer trapping is a valuable resource to identify novel gene functions regulating developmental pattern of tomato leaf.

## Simulation of whole-genome sequencing data to improve the design of mapping-by-sequencing experiments

Wilson-Sánchez, D., Sarmiento-Mañús, R., Ponce, M.R., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

During the past 25 years, we have obtained and studied hundreds of ethyl methanesulfonate (EMS)- and T-DNA-induced mutants that exhibit perturbations in leaf growth and development. To identify the causal mutations of the phenotypes of these mutants, we have moved from linkage analysis to mapping-by-sequencing using high-throughput, short-read next-generation sequencing technologies. To improve the design of these experiments, we simulated several experimental setups.

For EMS-induced mutants, we first evaluated which short-read technology is best suited to analyze the gene-rich genomic regions of Arabidopsis, and the minimum sequencing depth required to confidently call variants. Next, we simulated backcross mapping-by-sequencing experiments and determined how mapping population size and sequencing depth interact to affect mapping resolution. We also evaluated the viability of crossing two sibling non-allelic mutants to obtain a mapping population for simultaneously mapping of two recessive mutations. In addition, we compared different approaches to efficiently discriminate natural and EMS-derived single nucleotide polymorphisms; such discrimination is critical for localizing causal mutations in non-reference genetic backgrounds.

For T-DNA-induced mutants, we first tested a custom protocol to map insertions with paired-end Illumina-like reads. We then assessed the most cost-effective read depth and tested the viability of pooling several mutants. The results of these simulations proved useful for the design of real experiments.

## Easymap: a program to ease mapping-by-sequencing of large insertions and point mutations

Lup, S.D., Wilson-Sánchez, D., Andreu-Sánchez, S., and Micol, J.L.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain.

Forward genetic screens have identified many genes and continue to be powerful tools for the dissection of gene action and interactions in Arabidopsis and other plant species. Moreover, next-generation sequencing has revitalized the time-consuming genetic approaches to identify the mutation causing a phenotype of interest. Mapping-by-sequencing combines next-generation sequencing with classical mapping strategies and allows rapid identification of point mutations [1].

Currently, we have programs that can analyze whole-genome sequencing data to map the position of the causal mutations for a specific phenotype, but these programs are complicated to install or use, require additional software to perform complete analysis, or require the user to purchase expensive licenses. We are creating a program, called Easymap, which simplifies the data analysis workflow from raw reads to candidate mutations. Two main workflow types are available: bulked segregant mapping for point mutations, and tagged-sequence mapping for large insertions such as transposons or T-DNAs.

Easymap performs initial checks on user data, which are processed depending on the needs. Large insertions are mapped by a series of alignment and filtering steps, while single-nucleotide polymorphisms (SNPs) are analyzed based on their allelic frequencies in a phenotyped F2 mapping population. Auxiliary modules refine the output. The mutated genome is then compared with a fully annotated reference genome to detect the putative effects of the variation found (SNPs or large insertions) on gene function. Finally, all the relevant information for the user is presented in a unified report with graphical and text information. A module to simulate data is also included, which can be used to explore different experimental setups. We are currently testing Easymap as a tool to identify mutations in the Arabidopsis leaf mutants isolated in our laboratory.

[1] James, G.V., *et al.* (2013). *Genome Biol*. 14, R61.

## Cloning developmental genes using mapping-by-sequencing

Ruiz, E., Rodríguez-Alcocer, E., Jover-Gil, S., and Candela, H.

Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Spain.

Mapping-by-sequencing is a powerful approach that allows the rapid mapping and identification of the molecular lesion responsible for a mutant phenotype of interest. We have used this approach successfully to clone two developmental mutants of *Arabidopsis thaliana*. The first mutant causes albinism and lethality at the seedling stage. To identify the corresponding gene, we classified and pooled the individuals from an $F_2$ mapping population according to their phenotypes (wild-type or mutant). We purified genomic DNA from the plants of both pools, and the two samples were sequenced using the Illumina HiSeq 2500 next-generation sequencing platform. We have implemented an efficient bioinformatic pipeline to analyze the sequences from both pools. This pipeline has allowed us to identify a point mutation that damages a conserved residue at the acceptor site of an intron of the At2g04030 gene, which encodes a member of the Hsp90 family of heat-shock proteins in the genome of *Arabidopsis thaliana*. The second mutant exhibits abnormal fruit development, with fruits that consist of more valves (carpels) than those of the wild type. Following a similar approach, we have identified a non-synonymous substitution at a conserved amino acid residue of the ULTRAPETALA1 (At4g28190) protein, which is very likely responsible for the observed phenotype.

This work received support from Spain's Ministry of Economy and Competitiveness (MINECO) and the European Regional Development Fund (ERDF) ('Una manera de hacer Europa') [BFU2012-31719] and Generalitat Valenciana [ACOMP/2015/042]. E.R.-A.'s contract is also supported by a MINECO grant [PEJ-2014-A-21398].

## Relating wheat root architecture to nitrate uptake efficiency using computer simulations of CT data

Mellor, N., Griffiths, M., Wells, D., Schafer, E., Owen, M., and Bennett, M.J.

The Centre for Plant Integrative Biology, School of Biosciences, Sutton Bonington Campus, University of Nottingham, UK, LE12 5RD.

The FUTUREROOTS project aims to create a unique high throughput root phenotyping facility that exploits recent advances in µCT imaging, biological image analysis, wheat genetics and mathematical modelling to pinpoint the key genes that control root architecture and develop molecular markers and new crop varieties with improved nutrient and water uptake efficiency. As part of the Project we aim to use model-based phenotyping to assess the relative nitrate uptake efficiency of different wheat genotypes. We use non-invasive µCT to capture living root systems, grown in soil columns, of 10-day old wheat seedlings belonging to 8 genotypes from a Rialto-Savannah mapping population. After segmentation, skeletonisation and conversion to the RSML (Root System Markup Language) file exchange format, we then use Python scripts to convert the root system data into a format suitable for import into the newly open-source, 3D architectural plant root modelling platform OpenSimRoot (http://rootmodels.gitlab.io/). Using SimRoot we are then able to 'grow' root-systems in-silico matching the CT data, and make predictions of the nitrate uptake efficiency of each virtual plant, and thus compare between genotypes. In addition, by extracting parameter estimates relating to root growth and branching, we are able to extrapolate and predict future growth of the data derived root systems, and also simulate both partially and fully computer generated root architectures. Using these partially or fully computer generated systems it is posible to investigate to the impact of properties such as lateral root branching angle on nitrate uptake efficiency.

## Developing molecular tools for garlic breeding

Parreño, R.[1], Gallego, A.[1], Rodríguez-Alcocer, E.[1], Blasco-Espada, D.[1], Gómez del Castillo, F.[2], Castillo Martínez, P.[2], and Candela, H.[1]

[1]Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Spain.
[2]Coopaman, S.C.L., c/ General Borrero, s/n., 16660 Las Pedroñeras, Cuenca, Spain.

The genus Allium comprises several species of cultivated plants, such as garlic and onion, which are highly appreciated due to the commercial value of their bulbs. In order to develop resources for the molecular characterization of garlic, we have carried out the assembly of the chloroplast genome of a garlic cultivar, and have initiated the sequencing, de novo assembly and annotation of the garlic transcriptome using RNA samples derived from different tissues, including bulbs, bulbils, shoot apical meristems and vegetative leaves. The chloroplast genome of the studied cultivar is a circular, double-stranded DNA molecule with a total length of 153,131 base pairs, and contains 136 functional genes (including 90 protein-coding genes, 38 transfer RNA genes and 8 ribosomal RNA genes) and 6 pseudogenes. In order to identify genes involved in the pigmentation of garlic bulbs, we are characterizing a garlic variety that exhibits variegation, with bulbs containing white and purple sectors. The boundaries of these sectors are sharply defined and extend along the proximal-distal axis of the tunicas (modified leaves that ensheath the bulb). These sectors resemble those observed in clonal analysis studies carried out previously in monocotyledonous plants such as maize, suggesting that the distinct pigmentation is transmitted throughout cell divisions. Our transcriptome studies should help us to further understand bulb development and pigment synthesis pathways in this species.

# Growth and physiological responses of *Globba schomburgkii* Hook. f under soil and hydroponic conditions

Phantong, P.[1], Machikowa, T.[2], and Muangsan, N.[1]

[1]School of Biology, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima 30000 Thailand.
[2]School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000 Thailand.

*Globba schomburgkii* Hook. f (Zingiberaceae), a small perennial herb native to Thailand, is an ornamental plant and has high value in the national markets due to its beautiful inflorescence, called "Golden Dancing Girl". However, cultivation of this plant species is limited. This experiment aimed to compare growth and physiological responses of *G. schomburgkii* Hook. f under two conditions; soil and hydroponic conditions. *In vitro* plantlets (8 cm in height) were grown in hydroponics with the nutrient film technique in a greenhouse using SUT nutrient formula with electrical conductivities (EC) of 1.3 mS $cm^{-1}$ and pH between 5.5-6.5. In soil culture, the plantlets were grown in small pots containing sand: burned rice husk: peat moss (1:1:1 by volume). Growth and physiological characteristics were measured at 15, 30, 45 and 60 days after transplanting. The results showed that one hundred percent of the plantlets survived in both growth conditions. Plants grown in hydroponic culture revealed higher in shoot length, leaf area and stem diameter, except number of shoot than in soil culture. Plants grown in hydroponics had early inflorescence and many bulbils. For physiological response, hydroponic plants had higher photosynthesis rate, transpiration rate, and stomatal conductivity. These findings indicated the difference between the two conditions in term of leaf physiological indices. Hence, the result proved that *G. schomburgkii* Hook. f could adapt to the water-culture environment well and would provide a useful information for cultivators for developing a suitable method for propagating of *Globba* species and other related plant species.

# Synthesis of chiral alcohols; key intermediaries for the synthesis of bioactive molecules by Medlar; fruit grown in Algeria

Zeror, S., and Bennamane, M.

Laboratoire de Catalyse Asymétrique Ecocompatible. LCAE Université B. Mokhtar, Annaba, Algérie.

Stereoselective reductions of heteroaryl ketones containing furan, thiophene, chroman, and thiochroman moieties are of utmost importance in organic synthesis since the resulting chiral alcohols are used as antioxidants, or building blocks. [1] Asymmetric reduction of ketones with chemical catalyst or biocatalyst is a promising route for the production of enantio enriched alcohols. In the context of developing green and sustainable chemical processes, biotechnologies are attractive alternatives.

The biocatalytic reduction of ketones was performed using medlar *(Mespilus germanica. L)* fruit grown in large amounts in Algeria [2,3] Variety of heterocyclic aromatic ketones was reduced with medlar as catalyst in aqueous media. Prochiral ketones containing furan, thiophene, chroman, and thiochroman moieties are reduced with up to 98% ee. High enantioselectivities have been observed especially for the bioreduction of tetralone and thiochromanone with respectively 89% and 98% ee. These chiral benzylic alcohols are used as synthon-key in various syntheses of the many drugs.

In conclusion, Bio-reduction catalyzed by medlar fruits provides an attractive approach to access chiral alcohols with excellent enantiomeric excess. These results show that *medlar* fruits have enzyme system with ability to enantioselective reduction of ketone. Indeed, fruits represent an alternative source of “new”enzymes for use as catalysts in organic synthesis.

[1] Noyori, R., and Ohkuma, T. (2001). *Angew. Chem.* 40, 40.

[2] Bennamane, M., Zeror, S., and Aribi-Zouioueche, L. (2014). *Biocat. Biotrans.* 32, 327–332.

[3] Bennamane, M., Zeror, S., and Aribi-Zouioueche, L. (2015). *Chirality* 27, 205-210.

# Author index

# Participant list

| | |
|---|---|
| Aalen, Reidunn Birgitta | reidunn.aalen@ibv.uio.no |
| Aceituno-Valenzuela, Uri | uaceituno@umh.es |
| Adibi, Milad | adibi@mpipz.mpg.de |
| Ahmad, Zaki | zaki.ahmad.2015@live.rhul.ac.uk |
| Alaguero, Aurora | aalaguero@umh.es |
| Alcañiz-Pascual, Lourdes | lourdes.alcaniz@goumh.umh.es |
| Aljaiyash, Ahmed | aaljaiyash@yahoo.com |
| Alonso Díaz, Alejandro | alejandro.alonso@cragenomica.es |
| Altmann, Thomas | altmann@ipk-gatersleben.de |
| Amel, Benselama | amel1987amel@hotmail.fr |
| Andreu-Sánchez, Sergio | sergio_andreu@hotmail.es |
| Angosto, Maria Trinidad | tangosto@ual.es |
| Antón-López, Sofía | sofia.anton@goumh.umh.es |
| Azcona, Iñaki | iazcona@koppert.nl |
| Baekelandt, Alexandra | albae@psb.ugent.be |
| Belachew, Kiflemariam Yehuala | kiflemariam.belachew@helsinki.fi |
| Bennett, Malcolm | sbzmjb@exmail.nottingham.ac.uk |
| Betsch, Léo | leo.betsch@ens-lyon.fr |
| Blázquez, Miguel Angel | mblazquez@ibmcp.upv.es |
| Blein, Thomas | thomas.blein@ips2.universite-paris-saclay.fr |
| Boulmaali, Messaouda | maria-tasnim@hotmail.fr |
| Boulmaali, Mohamed Nadjib | nadjibmohamed57@hotmail.fr |
| Bretones, Sandra | s.bretonesamate@gmail.com |
| Bürstenbinder, Katharina | katharina.buerstenbinder@ipb-halle.de |
| Cabezas-Fuster, Adrián | acabezas@umh.es |
| Candela, Héctor | hcandela@umh.es |
| Capel, Carmen | ccapel@ual.es |
| Caperta, Ana | anadelaunay@isa.ulisboa.pt |
| Castañeda, Laura | ccl126@ual.es |
| Codesido, Verónica | v.codesido@phytoplant.es |
| Conceição, Sofia | sofia-conceicao@hotmail.com |
| Corneillie, Sander | sacor@psb.ugent.be |
| Costa-González, Tamara | tamara.gonzalez@goumh.umh.es |
| De Diego, Nuria | nuria.de@upol.cz |
| del Pozo, Juan Carlos | pozo@inia.es |
| Dubois, Marieke | marieke.dubois@ibmp-cnrs.unistra.fr |
| Efroni, Idan | idan.efroni@mail.huji.ac.il |
| Egea-Cortines, Marcos | marcos.egea@upct.es |
| Esteve-Bruna, David | destevebruna@gmail.com |
| Farahi-Bilooei, Sara | pcba002@rhul.ac.uk |
| Feijó, José | jfeijo@umd.edu |
| Fernandez, Lucie | lucie.fernandez@inra.fr |
| Fernández, Toñi | afl997@ual.es |
| Fischer, Urs | urs.fischer@slu.se |
| Fontcuberta-Cervera, Sara | sfontcuberta@umh.es |
| Fonseca, Rocío | rfr770@ual.es |
| Gonzaez, Nathalie | nagon@psb.vib-ugent.be |
| Grzegorek, Irmina | irmina.grzegorek@degruyteropen.com |
| Gutiérrez-Nájera, Nerea | nerea.gutierrez@alu.umh.es |
| Hulsmans, Sander | sander.hulsmans@kuleuven.be |
| Inzé, Dirk | nahau@psb.vib-ugent.be |

| | |
|---|---|
| Iversen, Vegard | vegardiv@student.ibv.uio.no |
| Juan-Vicente, Lucía | ljuan@umh.es |
| Justamante, María Salud | mjustamante@umh.es |
| Kierzkowski, Daniel | daniel.kierzkowski@gmail.com |
| Kumar Das, Prabir | genprabir106@gmail.com |
| Kunkowska, Alicja | a.kunkowska@gmail.com |
| Lemaire-Chamley, Martine | martine.lemaire@inra.fr |
| Lepiniec, Loïc | loic.lepiniec@inra.fr |
| Loudet, Olivier | olivier.loudet@inra.fr |
| Lozano, Rafael | rlozano@ual.es |
| Lup, Samuel Daniel | slup@umh.es |
| Madani, Tayeb | madani_tayeb@yahoo.fr |
| Maizel, Alexis | alexis.maizel@cos.uni-heidelberg.de |
| Mateo-Bonmatí, Eduardo | emateo@umh.es |
| Mellor, Nathan | nathan.mellor@nottingham.ac.uk |
| Micol, José Luis | jlmicol@umh.es |
| Micol-Ponce, Rosa | rmicol@umh.es |
| Mohammed, Binish | binish.mohammed@jic.ac.uk |
| Mora-Navarro, Eloy | emora@umh.es |
| Muangsan, Nooduan | nooduan@sut.ac.th |
| Muñoz-Díaz, Eduardo | eduardo.munoz01@goumh.umh.es |
| Muñoz-Nortes, Tamara | tmunoz@umh.es |
| Murray, Jim | murrayja1@cardiff.ac.uk |
| Musseau, Constance | constance.musseau@gmail.com |
| Nadi, Riad | rnadi@umh.es |
| Navarro-Cartagena, Sergio | s.navarro@umh.es |
| Navarro-Quiles, Carla | carla.navarroq@umh.es |
| Núñez-Delegido, Eva | eva.nunez@goumh.umh.es |
| Orman, Beata | beata.orman-ligeza@ulg.ac.be |
| Ortíz-Atienza, Ana | anaortiz@ual.es |
| Parreño, Ricardo | ricardo.parreno@graduado.umh.es |
| Penalva-Pérez, Pablo | pablo.penalva@goumh.umh.es |
| Peret, Benjamin | benjamin.peret@cnrs.fr |
| Perez Martin, Fernando | fpmartin@ual.es |
| Pérez-Pérez, José Manuel | jmperez@umh.es |
| Ponce, María Rosa | mrponce@umh.es |
| Quesada, Víctor | vquesada@umh.es |
| Quispe, Jorge L. | aristeo1310@hotmail.com |
| Robles, Pedro | probles@umh.es |
| Rodríguez-Cazorla, Encarnación | e.rodriguezc@umh.es |
| Rodríguez-Alcocer, Eva | eva.rodrigueza@umh.es |
| Rojo, Enrique | erojo@cnb.csic.es |
| Rolland, Filip | filip.rolland@bio.kuleuven.be |
| Rothan, Christophe | christophe.rothan@inra.fr |
| Ruiz-Bayón, Alejandro | alejandro.ruizb@umh.es |
| Ruiz-Ramírez, Jorge | jorge.ruizr@umh.es |
| Sarmiento-Mañús, Raquel | rsarmiento@umh.es |
| Schippers, Jos | schippers@bio1.rwth-aachen.de |
| Senent-Valero, Yaiza | yaiza.senent@goumh.umh.es |
| Shi, Chunlin | chunlin.shi@ibv.uio.no |
| Silva Navas, Javier | silva.javier@inia.es |

| | |
|---|---|
| Sinha, Neelima | nrsinha@ucdavis.edu |
| Sizani, Bulelani | bulelani.sizani@uantwerpen.be |
| Slovak, Radka | radka.slovak@gmi.oeaw.ac.at |
| Sosa-Domínguez, Pablo | pablo.sosa@goumh.umh.es |
| Spichal, Lukas | lukas.spichal@upol.cz |
| Takahashi, Naoki | naoki@bs.naist.jp |
| Takatsuka, Hirotomo | h-takatsuka@bs.naist.jp |
| Tarancón, Carlos | ctarancon@cnb.csic.es |
| Tena, Guillaume | guillaume.tena@nature.com |
| Timmermans, Marja | marja.timmermans@zmbp.uni-tuebingen.de |
| Tsiantis, Miltos | ciriacy@mpipz.mpg.de |
| Tsukaya, Hirokazu | tsukaya@bs.s.u-tokyo.ac.jp |
| Umeda, Masaaki | mumeda@bs.naist.jp |
| van der Knaap, Esther | esthervanderknaap@uga.edu |
| van Es, Sam | sam.vanes@wur.nl |
| Vera, Antonio | avera@umh.es |
| Vercruysse, Jasmien | jacru@psb.ugent.be |
| Vert, Gregory | gregory.vert@i2bc.paris-saclay.fr |
| Vissenberg, Kris | kris.vissenberg@uantwerpen.be |
| Volschenk, Chris | chrisvolschenk73@gmail.com |
| Wilson-Sánchez, David | dwilson@umh.es |
| Wuyts, Nathalie | nathalie.wuyts@psb.vib-ugent.be |
| Youssef, Chvan | chvan.youssef@inra.fr |
| Yuste-Lisbona, Fernando Juan | fyuste@ual.es |
| Zeror, Saoussen | saoussen_zeror@yahoo.com |
| Zhang, Zhongjuan | zhongjuan.zhang@mpipz.mpg.de |