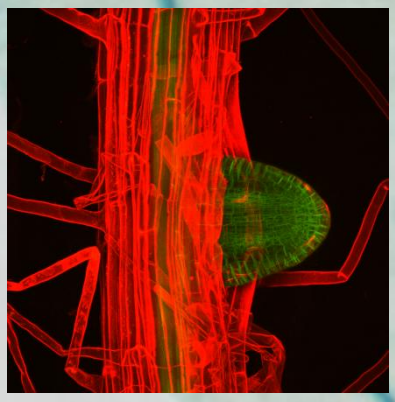
Plant Growth Biology and Modeling workshop

Covering the latest advances in the biology, modeling and automated phenotyping of

leaf and root development

Elche, October 14-16, 2009



AGRON OMICS

CPTB
The Centre for Plant Integrative Biology

UNIVERSITAS Miguel Hernández

SIXTH FRAMEWORK PROGRAMME

GENERALITAT VALENCIANA

Bancaja

MINISTERIO DE CIENCIA E INNOVACIÓN

turisme d'elx

Plant Growth Biology and Modeling Workshop

14-16 October 2009

Centro de Congresos "Ciutat d´Elx"
Elche, Spain

Local Organising Committee

Prof. José Luis Micol
Prof. María Rosa Ponce
Prof. José Manuel Pérez-Pérez
Dr. Héctor Candela
Dr. Silvia Rubio-Díaz
Dr. Rafael Muñoz-Viana

Scientific Committee

Prof. José Luis Micol (UMH, Elche, Spain)
Prof. Malcolm Bennett (CPIB, Nottingham, UK)
Prof. Charlie Hodgman (CPIB, Nottingham, UK)
Dr. Pierre Hilson (VIB, Ghent, Belgium)
Dr. Chiara Consonni (VIB, Ghent, Belgium)
Dr. Christine Granier (INRA, Montpellier, France)

Supporters

Agron-Omics
Bancaja
Centre for Plant Integrative Biology
Generalitat Valenciana
Instituto de Bioingeniería
Instituto Municipal de Turismo de Elche
Ministerio de Ciencia e Innovación
Universidad Miguel Hernández de Elche

Table of contents

Program

Wednesday 14th October

Session 1: Leaf biology and modeling

Chair: José Luis Micol, Universidad Miguel Hernández, Elche, Spain

8:45-9:00 Welcome and introduction to the meeting by José Luis Micol
9:00-10:00 Yuval Eshed, Weizmann Institute of Science, Israel
10:00-11:00 Hirokazu Tsukaya, Tokyo University, Japan
11:00-11:30 Coffee break and poster viewing
11:30-12:30 Miltos Tsiantis, Oxford University, UK
12:30-13:30 Short presentations
Stijn Dhondt, Ghent University, Belgium
Vera Matser, University of York, UK
Florent Pantin, INRA, Montpellier, France
Leila Kheibarshekan, Ghent University, Belgium
13:30-15:00 Lunch
15:00-16:00 Mary Byrne, John Innes Centre, UK
16:00-16:30 Robert Last, Michigan State University, USA
16:30-17:00 Coffee break and poster viewing
17:00-18:00 Thomas Berleth, Toronto University, Canada
18:00-19:00 Timothy Nelson, Yale University, USA
19:00-21:00 Refreshments and poster viewing

Thursday 15th October

Session 2: Root biology integrating modeling approaches

Chair: Malcolm Bennett, CPIB, Nottingham, UK

9:00-10:00 Malcolm Bennett, University of Nottingham, UK
10:00-11:00 Short presentations
Juan Carlos Del Pozo, CBGP-Madrid, Spain
Francine Perrine-Walker, IBIP-Montpellier, France
Jian Xu, National University of Singapore
11:00-11:45 Coffee break and poster viewing
11:45-12:45 Tobias Baskin, University of Massachusetts, USA
12:45-13:30 Short presentations
Kris Vissenberg, University of Antwerp, Belgium
Marios Nektarios-Markakis, University of Antwerp, Belgium
13:30-15:00 Lunch
15:00-16:00 Mark Estelle, University of California at San Diego, USA
16:00-16:40 Short presentations
Wolfgang Busch, Duke University, USA
Jose Guerra, Keygene, The Netherlands
16:40-18:00 Coffee break and poster viewing
18:00 Guided visit to Elche. Refreshments offered by the city council.

Friday 16th October

Session 3: Large-scale/automated phenotyping and phenotype data mining

Chair: Christine Granier, INRA, Montpellier, France

9:00-10:00 Christine Granier, INRA, Montpellier, France

10:00-11:00 Ulrich Schurr, Research Centre Jülich, Germany

11:00-11:30 Coffee break and poster viewing

11:30-12:30 Robert Last, Michigan State University, USA

12:30-13:15 Short presentations

Matthias Eberius, Lemna Tec GmbH, Germany

Katja Baerenfaller, ETH Zurich, Switzerland

Juliette Fabre, INRA, Montpellier, France

13:15-13:30 Meeting adjournment by Pierre Hilson

13:30-15:00 Lunch

Talks,
oral communications
and posters

SESSION 1: LEAF BIOLOGY AND MODELING

SESSION 2: ROOT BIOLOGY INTEGRATING MODELING APPROACHES

POSTERS

Abstracts

SESSION 1:
LEAF BIOLOGY AND MODELING

Florigen: a flowering hormone with general systemic roles in growth modulation of plant organs

Eshed, Y., and Lifschitz, E.

Dept. of Plant Sciences, Weizmann Institute of Science, Rehovot, 76100, Israel

The florigen paradigm implies a universal flowering-inducing hormone that is common to all flowering plants. We identified *FT* orthologs as universal originators of florigen and others implicated their polypeptides as the likely systemic agent. The developmental processes targeted by florigen remained, however, unknown. We analyzed the graft-transmissible impacts of florigen on different traits in the perennial shoot system of tomato. We show that florigen is imported by apices, leaves, stems and flowers, to modulate meristematic prepatterns that are supervised by local, innate balances between SFT, the tomato precursor of florigen, and SP, a potent SFT-dependent SFT inhibitor. By targeting local SFT/SP balances in tomato, florigen confers differential flowering responses of primary and secondary apical meristems, regulates the reiterative growth and termination cycles throughout development, and determines the complexity of compound leaves, the growth of stems and the formation of abscission zones. Various protein tags render only the autonomous effects of SFT, and production of systemic SFT require expression from specific cells. Developmental interactions between SFT and other regulators of the tomato shoot, with an emphasis on leaf development, will be discussed.

Integration of local information on leaf organogenesis

Tsukaya, H.

Graduate School of Science, The University of Tokyo; National Institute for Basic Biology, National Institutes of Natural Sciences, Japan

Leaves have a very simple shape, in particular in the case of a simple leaf. Mechanisms of leaf organogenesis are, however, not so simple. At the beginning of our study on the genetic control of leaf shape, we proposed that leaf blade expands through two axes: the longitudinal and the medio-lateral axes[1]. Further analyses have revealed two separate regulatory mechanisms of leaf length in arabidopsis: A control of polar cell expansion and another of polar cell proliferation[2]. Such two-dimensional expansion of leaf blade is known to depend on the establishment of dorsoventral polarities[3]. These models about the genetic control of leaf expansion seemed to be rather simple, but in recent years, a lot of data have accumulated to indicate that leaf-expansion control involves more complicated and integrative mechanisms (reviewed in Tsukaya 2008).

Although two-dimensional leaf expansion depends on dorsoventrality in many plant species, our recent analyses revealed that some monocot species expand their unifacial-type leaves in a dorsoventrality-independent manner (Yamaguchi and Tsukaya, in prep). Besides, we have noticed that “a whole reflects a part and a part reflects a whole”. In other words, information at individual-level is reflected into size and number of each leaf. For example, light condition perceived by old, mature leaves is reflected into size and number of cells in developing leaves[4]; and heteroblastic information controls cell size and cell number in each leaf[5]. In opposite, local information on cell proliferation in a part of leaf primordium is reflected into size of cells in the whole leaf. In this workshop, I will discuss on one of the latter organ-wide mechanisms: non-cell autonomous cross talk between cell-proliferation and cell-expansion control.

Accumulating evidences revealed that defects in cell proliferation in leaf primordia trigger excessive cell expansion in leaves, if it goes beyond some threshold level[6]: we call this phenomenon as “compensation”[7]. Curiously, excessive cell proliferation itself does not alter cell size; and excessive cell expansion does not decrease cell proliferation. Moreover, compensation depends on only a subset of genetic pathways for leaf-cell

expansion[8]. How are cell number and size integrated in a leaf primordium? We have developed a heat-shock inducible Cre-lox chimera system in which we can ON/OFF switch the expression of genes that control cell cycling in leaf blades, such as *ANGUSTIFOLIA3* (*AN3*) and *KPR2.* The loss-of-function of *AN3* or the over-expression of *KRP2* gene severely decreases cell proliferation and then triggers typical compensation phenotype. Does the compensation occur in the whole leaf when we reduce cell proliferation activity locally in a young leaf primordium? In other words, does compensation depend on cell-cell communication or not[4]? Our analyses on the inducible chimera system revealed that local defect in cell proliferation by the over-expression of the *KRP2* caused compensation only in a cell-autonomous manner, but interestingly, local loss -of-function of the *AN3* caused the compensation in a non-cell-autonomous manner (Kawade, Horiguchi and Tsukaya, unpublished). Based on our recent data, possible model of integration mechanisms between cell proliferation and cell expansion in a leaf primordium will be discussed.

References

1.- Tsuge, T., Tsukaya, H., and Uchimiya, H. (1996). Two independent and polarized processes of cell elongation regulate leaf blade expansion in *Arabidopsis thaliana* (L.) Heynh. *Development* **122,** 1589-1600.

2.- Tsukaya, H. (2006). Mechanism of leaf shape determination. *Ann. Rev. Plant Biol*. **57,** 477-496.

3.- Tsukaya H. (2008). Controlling Size in Multicellular Organs: Focus on the Leaf. *PLoS Biology* **6,** 1373-1376.

4.- Waites, R., and Hudson, A. (1995). *Phantastica*: a gene required for dorsoventrality in leaves of *Antirrhinum majus*. *Development* **121,** 2143–54.

5.- Fejani, A., Yano, S., Horiguchi, G., and Tsukaya, H. (2008). Control of leaf morphogenesis by long - and short -distance signaling: differentiation of leaves into sun or shade types and compensated cell enlargement. In: Plant Growth Signaling, Eds. by L.Bögre & G.T.S. Beemster (Plant Cell Monograph series, #10), pp. 47-62. Springer.

6.- Usami, T., Horiguchi, G., Yano, S., and Tsukaya, H. (2009). The *more and smaller cells* mutants of *Arabidopsis thaliana* identify novel roles for *SQUAMOSA PR OMO TER BINDING PROTEIN-LIKE* genes in the control of heteroblasty. *Development* **136,** 955-964.

7.- Fujikura, U., Horiguchi, G., Ponce, M.R., Micol, J.L., and Tsukaya, H. (2009). Coordination of cell proliferation and cell expansion mediated by ribosome-related processes in the leaves of *Arabidopsis thaliana*. *Plant J.* **59,** 499-508.

8.- Tsukaya, H. (2002). Interpretation of mutants in leaf morphology: genetic evidence for a compensatory system in leaf morphogenesis that provides a new link between Cell and Organismal theory. *Int. Rev. Cytol.* **217,** 1-39.

9.- Fujikura U., Horiguchi, G., and Tsukaya, H. (2007). Dissection of enhanced cell expansion processes in leaves triggered by defect in cell proliferation, with reference to roles of endoreduplication. *Plant Cell Physiol*. **48,** 278-286.

Using *Cardamine hirsuta* as a model system to understand diversification in leaf form

Tsiantis, M.

Department of Plant Sciences, University of Oxford, OX1 3RB, UK

A key problem in biology is to understand how diversity in organismal form is generated. To investigate this problem we study the genetic mechanisms underlying variation in form of the predominant photosynthetic organ of plants, the leaf. Leaf form can be classified as simple, where the leaf blade is entire as in the model organism *Arabidopsis thaliana*, or dissected where the blade is divided into distinct units called leaflets. Mechanisms that determine specification of dissected versus entire leaf shape and regulate the number, position and timing of leaflet production are poorly understood. To obtain an in-depth and unbiased understanding of these mechanisms we established *Cardamine hirsuta* - dissected leaf relative of *A.thaliana* - as a versatile experimental system where both forward and reverse genetics can be deployed for studying diversification of leaf form. This presentation will discuss how comparisons between *A.thaliana* and *C.hirsuta* have illuminated our understanding of processes underlying the evolution of form.

Image analysis tracks plant growth and supports in the phenotypic analysis of leaves

Dhondt, S.[1,2], Skirycz, A.[1,2], Maleux, K.[1,2], De Meyer, B.[1,2], Coppens, F.[1,2], Merks, R.[3,4], Beemster, G.T.S.[1,2,5], Hilson, P.[1,2], and Inzé, D.[1,2]

[1]Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB), Technologiepark 927, 9052 Gent, Belgium
[2]Department of Plant Biotechnology and Genetics, Ghent University, Technologiepark 927, 9052 Gent, Belgium
[3]NCSB-NISB, Kruislaan 318, Amsterdam, The Netherlands
[4]CWI, Science Park 123, Amsterdam, The Netherlands
[5]Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

Plant growth is the combined result of two processes: cell division and cell expansion. These processes are highly interconnected with many pathways including stress and hormone responses. Photosynthesis, water and mineral uptake, mobilization of starch and lipid reserves determine the resources available for growth. This multifactorial nature of plant growth makes it one of the most difficult biological processes to study.
Understanding the molecular mechanisms underpinning plant growth requires detailed and high throughput analysis methods to follow plant growth over time. Therefore, we developed automated platforms for both *in vitro* and *in vivo* rosette growth analysis of *Arabidopsis* plants, IGIS and WIWAM, respectively. IGIS (*In vitro* Growth Imaging System) allows to simultaneously monitor 10 petri dishes, containing growing plants. The plates are standing on a rotating disk and are photographed on a hourly basis. The WIWAM (Weighing, Imaging and Watering Machine) allows to monitor growth of 228 individual soil-grown plants under controlled watering conditions. Both platforms are supported by image and data analysis algorithms, allowing an automated extraction of the area and compactness of the rosettes. Extra image and data analysis algorithms were developed to elevate and speed up the more detailed analyses of vascular patterning and leaf growth kinematics on the cellular level. Vascular patterns can be extracted from dark field leaf images and several parameters as leaf lamina area, total length of the vascular pattern, number of branching and endpoints, areola area and vascular density can be measured. For the kinematic analysis of leaf growth we extract features like cell number, cell area and number of stomata from

microscopic drawings of the abaxial leaf epidermis, followed by a data analysis pipe line that calculates cell division rate, relative leaf expansion rate, cell area distributions and more. These platforms help to screen for growth phenotypes in various mutant backgrounds and support in the more detailed and elaborated phenotypic analysis of leaf growth kinematics and vascular patterning.

The role of class I TCP genes in determining leaf shape and size

Matser, V.[1], Davies, B.[2], and Waites, R.[1]

[1]University of York, UK
[2]University of Leeds, UK

The growth and development of leaves determines their final shape and size, and is a fundamental feature of plants, responding to environmental change and adapting to suit the physiological requirements of leaves. Using the LeafAnalyser and Leafpredictor software we have designed and built, we have quantified leaf shape and size variation in Arabidopsis with a library of more than 3,500 leaves. We have used this library to assess leaf shape and size in candidate plant lines, and aim to identify and characterize genes that have important roles in determining the final shape and size of leaves. Our interest has specifically fallen on INTERNODE SHORT1 (INS1) and INTERNODE SHORT 2 (INS2) members of the TCP family (TB1, CYC and PCFs), which are expressed in early leaf development affecting leaf shape. TCP genes are known to be involved in the regulation of a cell-cycle arrest front, travelling from the leaf tip to the base, and may influence the final shape and size of leaves at this early stage of development. My current research uses a combination of molecular genetics and morphometrics to examine the role of a small sub- family of class I TCP genes in leaf development. Single and double insertion lines are being phenotypically characterised, their leaf shape and size analysed, and histological assays conducted to characterise cell number, shape and size.

Hydraulics versus metabolics? A framework to reconcile determinisms of leaf growth

Pantin, F., Simonneau, T., and Muller, B.

LEPSE, UMR 759 INRA-SUPAGRO Montpellier, France

Leaf growth is controlled by several factors among which hydraulic and metabolic limitations emerge. Each limitation exerts a major influence when assessed separately. In this study, we aimed at comparing and grading the respective influences of hydraulic and metabolic limitations along leaf development in a wide range of environments and genotypes. A high throughput design was developed to monitor leaf growth on a day/night basis and to explore its responses to combinations of soil water deficits, evaporative demands and light conditions in a range of mutants altered in their hydraulic status (stomatal mutants) or starch metabolism. Using this experimental framework, we revealed two types of diel growth patterns and found that they were associated to either metabolic (reduced growth during nights) or hydraulic (reduced growth during days) limitation. Moreover, we found that during the course of leaf development, the prevailing control of leaf growth clearly switched from metabolics to hydraulics. The switching time was strongly modulated by environments and genotypes, namely earlier under water challenging conditions and delayed under conditions limiting starch availability. This suggests that hydraulic limitation establishes as leaf develops while metabolic limitation diminishes. We are now seeking for mechanistic causes of these growth limitations using complementary, modelling, histological and molecular approaches.

A Mathematical Model for Cell Division in the Arabidopsis Leaf Development

Kheibarshekan, L.[1,2], Dhondt, S.[2], Boudolf, V.[2], Beemster, G.T.S.[2,3,4], De Veylder, L.[2,3], and Govaerts, W.[1]

[1]Applied Mathematics and Computer Science, UGent, Belgium
[2]VIB Department of Plant Systems Biology, UGent, Belgium
[3]Department of Molecular Genetics, UGent, Belgium
[4]Department of Biology, University of Antwerp, Belgium

The lower leaf epidermis of the plant *Arabidopsis thaliana* consists of two cell types, stomatal guard cells and pavement cells. Stomata are small pores on the surface of leaves whose aperture is controlled by two guard cells. When these guard cells are open, the stomata allow gas exchange, mainly CO_2 for photosynthesis and H_2O, between the leaf and the atmosphere. In this study we concentrate on the development of Stomata in Arabidopsis leaves to investigate the role of cell size and cell age in cell division and cell differentiation. We also study the evolution of the cell cycle duration during development.

We concentrate on the initiation and regulation of precursor cells that form guard cells and pavement cells and this study will restrict primarily to the number and sizes of pavement and guard cells.

We build a computational framework to describe the development of the leaf epidermis. The model includes a number of parameters, among which the cell cycle duration (L) and the growth rates of pavement and guard cells (g_{PC} and g_{GC}) , as well as two thresholds (T_{PC} and T_{GC}) for the area of pavement and guard cells. Two other parameters are p_1, the fraction of pavement cells with area below T_{PC} that are in the process of dividing into pavement cells and p2, the fraction of pavement cells with area below T_{PC} and $2T_{GC}$ that will divide into guard cells. The model is based on a map from the density functions of pavement and guard cells on a given day to the density functions on the next day. To estimate the parameters, optimization methods have been used.

An important and rather unexpected result is that the cell cycle duration is nearly constant during leaf development. Another unexpected result is that there is no evidence for the existence of a threshold T_{PC}.

A role for the ribosome in plant growth and patterning

Byrne, M.E.

John Innes Centre, Norwich, UK

Plants establish two stem cell populations early in embryogenesis. The stem cell population at the base of the embryo forms the root meristem, which contributes to the root system. The stem cell population in the shoot meristem, at the apex of the embryo, ultimately gives rise to organs and axillary meristems of the shoot system. Shoots are characterized by reiterative production of organs from an indeterminate shoot apical meristem. Early in development lateral organs, such as leaves, establish dorsoventral polarity. Outgrowth of the leaf lamina depends on signaling from the meristem to the initiating leaf, as well as concerted interactions between adaxial and abaxial domains of the leaf. Control of these patterning events and plant growth involves a network of regulatory gene interactions that require precise coordination of gene expression. We have identified new players in this network as ribosomal proteins. We propose a role for the ribosome and translation in control of growth as well as in regulation of patterning events in plant development.

The iPlant Collaborative: an overview

Last, R.

Department of Biochemistry and Molecular Biology and Department of Plant Biology, Michigan State University 301 Biochemistry, East Lansing, MI 48823, USA

The Plant Science Cyberinfrastructure Collaborative (PSCIC) program is intended by NSF to create a new type of organization – a cyberinfrastructure collaborative for the plant sciences - that would enable new conceptual advances through integrative, computational thinking. I will give a brief overview of the project, from the point of view of a member of the Board of Directors.

Control of leaf vascular patterning

Berleth, T.[1], Scarpella, E.[2], and Marcos, D.[1]

[1]Dept. of Cell and Systems Biology, University of Toronto, 25 Willcocks Street, Toronto, Canada M5S3B2.
[2]Department of Biological Sciences, University of Alberta, CW-405, Edmonton T6G 2E9, USA

Feedback-regulated auxin flows have been implicated in an amazing number of plant patterning processes and have become subject to mathematical modeling. To this end, improved genetic dissection, experimental interference and novel visualization tools have to be integrated with computer simulations towards increasingly precise theoretical predictions and experimental quantifications.

Auxin Response Factors have critical, partially overlapping functions in controlling the expression of AtPIN auxin-efflux associated proteins and can be used as genetic tools to locally manipulate auxin signal transduction and auxin transport. We have used genetic and experimental interference tools as well as live visualization to dissect the formation of *Arabidopsis* leaf venation patterns. During leaf development networks of procambial cells, the precursors of all mature vascular cell types, emerge from homogeneous subepidermal tissue. A crucial member of the AtPIN family of auxin efflux proteins, AtPIN1, is expressed prior to preprocambial and procambial cell fate markers in domains that become restricted toward sites of procambium formation. Subcellular AtPIN1 polarity indicates that auxin is directed to distinct “convergence points” in the epidermis, from where it defines the positions of major veins. Integrated polarities in all emerging veins indicate auxin drainage toward pre-existing veins, but veins display split polarities as they become connected at both ends. Auxin application and transport inhibition reveal that convergence point positioning and AtPIN1 expression domain dynamics are self-organizing, auxin transport-dependent processes. Our results suggest that epidermal “convergence points” are part of a more general developmental module defining not only the positions of major leaf veins, but also the positioning of lateral shoot organs. Live visualization of individual pre-procambial domains demonstrates the dynamic nature of the process selecting procambium cells.

Using the monocot leaf cellular gradient as a developmental timeline and map

Nelson, T.[1], Gandotra, N.[1], Tausta, S.L.[1], Brutnell, T.[2], Li, P.[2], Kebrom, T.[2], Ponnola, L.[2], Turgeon, R.[3], Reidel, E.[3], van Wijk, K. [3], Majeran, W.[3], Sun, Q.[3], and Liu, P.[4]

[1]Yale University, New Haven CT 06511, USA
[2]Boyce Thompson Institute, Ithaca NY 14853, USA
[3]Cornell University, Ithaca NY 14853, USA
[4]Iowa State University, Ames IA 50011, USA

The leaves of many monocots are formed from base-to-tip files of cells produced largely by cell divisions at the leaf base. Cell files within an immature leaf display zones of cell division at the base followed by progressive stages of cell expansion and differentiation. Developmental events such as the source-sink transition can be mapped to points or zones along this physical gradient. This permits a systems analysis of the leaf cells that correspond to particular developmental events, with the aim of revealing the molecular networks producing the feature.

We have obtained systems data (transcriptomes, proteomes, metabolites) corresponding to 4 developmental zones of maize leaves. The same leaf zones were characterized at anatomical, ultrastructural, and physiological levels. The modeling of the data as regulatory and interaction networks is in early stages, but initial views of coexpression patterns via pathway graphics and genome/proteome browsers reveal the sequence of molecular events for many processes. As predicted, the transcripts, proteins, and metabolites corresponding to the C4 carbon fixation pathway are dramatically distributed between bundle sheath and mesophyll cells. We are obtaining a reference leaf zone dataset from rice, a C3 monocot, to compare systems at the times that maize differs by forming anatomical features in support of C4 biology.

SESSION 2: ROOT BIOLOGY INTEGRATING MODELING APPROACHES

Phenotyping root growth & development at the Centre for Plant Integrative Biology

French, A., Wells, D., Lucas, M., Voss, U., Mooney, S., Pridmore, T., and Bennett, M.

Centre for Plant Integrative Biology, University of Nottingham, UK

The Centre for Plant Integrative Biology (CPIB) at the University of Nottingham aims to create a *virtual root* which will serve as an exemplar for using Integrative Systems Biology (ISB) to model multi-cellular systems. CPIB brings together biologists, engineers, mathematicians and computer scientists to generate new data, biological resources and virtual models of plant roots that will aid understanding of how they grow and develop. The output of the programme will be quantitative observational data, validated models constituting the prototype "virtual root" and proofs of concept which will form the basis for further research programmes. Generating new and innovative approaches to characterise root growth and development is critical to delivering this output. I will describe several approaches recently developed at CPIB to characterise root systems architecture at the molecular, cellular, tissue and organ scales.

Identification of novel genes involved in lateral root formation

del Pozo, J.C.[1], Manzano, C.[1], Casero, P.[2], Casimiro, I.[2], De Rybel, B.[3], and Beeckman, T.[3]

[1]Centro de Biotecnología y genómica de plantas (CBGP). Campus de Montegancedo. Autovía M-40 Km38 28223 (Pozuelo de Alarcón) Madrid. España.

[2]Universidad de Extremadura. Facultad de Ciencias. Dpto. de Anatomía y Biología Celular y Zoología, Avda. de Elvas s/n, Badajoz 06071, Spain

[3]Department of Plant Systems Biology, VIB, Technologiepark 927, B-9052 Gent, Belgium

Plant root growth and development can be adapted to many environmental conditions. The root system depends on the number and position of the lateral roots (LR) along the main root axis. Lateral root initiation is an essential and continuous process that is a highly regulated by auxin. However, the molecular and genetic components that determine the position of lateral roots are unknown.

SKP2D gene encodes an F-box protein that is cell cycle regulated and is expressed in dividing areas (root and shoot meristems) and during early stages of lateral root formation. Interestingly, *SKP2D* is expressed along the main root in discrete patches that correspond to founder cells (we called Lateral Root Initiation Points, **LRIP**). We did not find any anticlinal cell division in the stained patches closest to the root tip, suggesting that this gene is a good marker to study pericycle cell specification events. Indeed, this promoter is being useful to identify novel mutants and genes involved in lateral root primordia formation.

We have conducted a mutagenesis with EMS in *SKP2D*::GUS plants. We have carried out a genetic screening to identified mutants with altered expression pattern of *SKP2D* in the LRIP. Two mutants of these mutants, called *sdrel50* and *sdrel53* (*skp2d root expression less*), have an altered lateral root development. The *sdrel50* mutant has a shorter root and develops more lateral roots than wild-type plants per centimetre of main root. The *sdrel53* mutant develops fewer emerged LR than wt roots, but shows a significant amount of arrested root primordia in different stages, suggesting that *SDREL53* gene is critical for LR formation and emergence. However, *sdrel53* roots responds to auxin treatments since

this mutant forms LR after application of the hormone. Cloning and characterization of *SDREL53* gene will give new insights on lateral root formation.

In addition, we have developed a transgenic plant that harbours the *SKP2D* promoter region fused to GFP reporter gene. To identify molecular components that may control founder cell specification, we carried out a transcriptomic analysis of root fluorescent-activated cell sorting using SKP2D::GFP root protoplasts. We have identified 200 genes that are expressed in higher level in these founder cells than in the rest of the pericycle cells. The reproducibility of the expression profiles, the resolution of our experimental approach, and the potential involvement in lateral root development will be evaluated with overexpressing and mutants of some of these identified genes.

The role of Nitrate transporter NRT1.1 in *Arabidopsis* lateral root development

Perrine-Walker, F.[1], Krouk, G.[1], Mounier, E.[1], Gojon, A.[1], Bennett, M.[2], and Nacry, P.[1]

[1]Biochimie et Physiologie Moléculaire des Plantes, UMR 5004 CNRS/INRA/SupAgro-M/UM2. Institut de Biologie Intégrative des Plantes. Place Viala, 34060 Montpellier Cedex 1, France

[2]Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, U.K.

Nitrate is both a nitrogen source for higher plants, and a signal molecule regulating their development. In *Arabidopsis thaliana*, the NRT1.1 nitrate transporter is crucial for nitrate signalling governing root growth, and has been proposed as a nitrate sensor. However, the sensing mechanism is unknown. Evidence suggests that NRT1.1 strongly affects auxin distribution in the root system. Auxin promotes lateral root development, and mutation of *NRT1.1* enhances both auxin accumulation in lateral roots and growth of these roots at low, but not at high, nitrate concentration. Thus we propose that the role of NRT1 .1 is to repress lateral root development at low nitrate (or low N) availability by preventing auxin accumulation in those roots. To determine how NRT1.1 modifies auxin levels in lateral roots, we characterised NRT1.1 protein expression and localization in primordia and emerged lateral roots under various growth conditions. This is compared with the expression and localization of various auxin carriers (PINs, AUX1 and LAX3). We propose a modified model in which NRT1.1 plays an important role in the signalling pathway during lateral root emergence.

Deciphering molecular genetic and cellular mechanisms of root development in plants

Xu, J.

Department of Biological Sciences, National University of Singapore

Root development of the model eudicot plant *Arabidopsis thaliana* and the model monocot crop *Oryza sativa* (rice) differs in both overall architecture and the anatomy of individual roots. Mechanisms underlying the evolution and formation of diverse root morphologies and structures have been largely unexplored. In the Arabidopsis root, a framework of genes has emerged to explain stem cell formation and root patterning at the cellular level. Now, the issue arises to what extent molecular genetic and cellular mechanisms have been conserved between Arabidopsis and rice and to whether some as-yet unidentified general mechanisms are at work in stem cell formation and root patterning during plant evolution. To address this we have been developing molecular genetic tools and bio-imaging techniques that will allow developmental and molecular cell biology research in rice. We are investigating conserved and divergent aspects of Arabidopsis and rice root patterning genes in 1) cell division and differentiation; 2) stem cell formation and tissue layering; and 3) architecture and growth control; to discover novel stem cell factors, gene regulatory networks and developmental mechanisms; and to establish root architectural design principles in plants.

The mechanism of root twisting

Baskin, T.I., and Cobb, A.

Biology Department, University of Massachusetts, Amherst, USA

Under certain environmental conditions and in certain genetic backgrounds, arabidopsis roots twist as they grow. The twisting results in the epidermal cell files forming helices. For seedlings on the surface of agar medium, within Petri dishes held vertical, twisting causes the roots to grow at an angle to gravity, a phenomenon often termed "root skewing". A remarkable feature of root twisting is that it is asymmetric. For a given condition or genotype, the helices formed are almost exclusively of one handedness (e.g., right handed) with individuals adopting the other handedness being rare, or absent entirely.

Root twisting has been conclusively linked to cortical microtubules. First, twisting almost always arises from moderate interference with microtubule function, either by means of low concentrations of inhibitors or mutants with small effect. Second, when cortical microtubule orientation changes form transverse to helical at the shoot-ward side of the root's growth zone, nearly all of the arrays form right-handed helices. Third, in a collection of two dozen mutants in α- or β-tubulin, the angle of root twisting was inversely correlated with the pitch angle of the cortical microtubule helix. While these results establish a role for microtubules in causing roots to twist, they do not explain the mechanism by which the usual linear growth pattern of the root becomes helical.

To investigate this, we are taking a three-fold approach. First, we are determining to what extent an engineering model that accounts successfully for twisting in single cell filaments can be scaled up to account for twisting in a multicellular structure, such as a root. Second, we are carrying out a kinematic analysis of growth in three-dimensions to discover the local (or cellular) basis for the helical growth form. Third, we are quantifying the orientation of cellulose microfibrils as a function of position in the root to determine whether helical microfibrils participate in helical growth. These three lines of inquiry are on-going and, in the seminar, I will present our progress on each of them. To date, the results support a model whereby cellulose microfibrils become helical in the shoot-ward half of the elongation zone, an asymmetric mechanical organization that drives helical growth.

References
1.- Sellen, D.B. (1983). The response of mechanically anisotropic cylindrical cells to multiaxial stress. *J. Exp. Bot.* **34,** 681-687.
2.- Liang, B.M., Dennings, A.M., Sharp, R.E., and Baskin, T.I. (1996). Consistent handedness of microtubule helical arrays in maize and arabidopsis primary roots. *Protoplasma* **190,** 8-15.
3.- Ishida T, Kaneko Y, Iwano M, Hashimoto T (2007). Helical microtubule arrays in a collection of twisting tubulin mutants of *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **104,** 8544-8549.

Arabidopsis root cell elongation and its control

Vissenberg, K.

Univ. Antwerp, Biology Dept., Plant Growth and Development, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

The model plant *Arabidopsis thaliana* offers one of the best experimental systems to study root developmental processes of higher plants. The whole genome is sequenced, its development occurs in a highly predictive and precise way and cellular development can be easily monitored with a microscope. Along the root axis, cells are formed by division in the meristem, they elongate in the adjacent elongation zone and take up their final functions in the differentiation zone.

During cell elongation the cooperation of a set of enzyme families enables the wall to grow without loosing its strength.

Xyloglucan endotransglucosylase/hydrolases (XTHs) are believed to be involved in this 'wall loosening'. They break and rejoin xyloglucan chains, allowing the cellulose microfibrils to move apart driven by protoplasmic pressure. Of this enzyme family we have studied the expression of several of its genes, we have localized its endotransglucosylase (XET) action *in muro*, we have heterologously expressed several proteins and characterised their properties in detail and have looked at the effect of overexpression of these genes on root development.

To date, several approaches are used in the lab to define genes that also play a role in the mechanism of cell elongation. They involve enhancer trap-plants, T-DNA mutants and even the growth of the etiolated hypocotyl. We have furthermore performed a micro-array experiment on roots that were treated with 1-aminocyclopropane-1-carboxylic acid (ACC), the precursor of ethylene. Upon this ACC-induced inhibition of cell elongation 240 genes were differentially expressed between treated roots and control ones. The role of several identified genes in the mechanism of elongation and its control is under study. Recent progress in these studies will be presented.

Characterization of genes involved in the elongation of the Arabidopsis root and its response to ACC

Nektarios-Markakis, M., Verbelen, J.P., and Vissenberg, K.

Univ. Antwerp, Biology Dept., Plant Growth and Development, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

The root of *Arabidopsis thaliana* is used as a model system to unravel the molecular nature of cell elongation and its arrest. Application of the precursor of ethylene, 1-aminocyclopropane-1-carboxylic acid (ACC), decreases the maximally reached cell length (from 130 µm to 55 µm) in the elongation zone within minutes of treatment[1]. Plant own and environmental cues such as osmotic and salt stress can phenocopy this fast growth response. We have performed a micro-array experiment on roots that were treated with ACC and have identified 240 genes that were differentially expressed between treated roots and control ones. At present the role of several selected genes in the mechanism of elongation and its control is under study. Besides a 'reverse genetics' approach, where knock-out or knock-down plants are phenotypically analysed, the effect of overexpressing a selected gene on the development of the root is monitored. In addition, promotor::GUS or GFP fusions learned us the exact timing and position of gene expression and the effect of hormone treatments. In order to reveal the exact location of the gene products within the cells, protein-GFP fusions are used. The progress in the study of several genes will be presented.

References

1.- Le, J., Vandenbussche, F., Van Der Straeten, D., and Verbelen, J-P. (2001). In the early response of Arabidopsis roots to ethylene, cell elongation is up and down regulated and uncoupled from differentiation. *Plant Physiol.* **125,** 519–522.

The complexity of auxin signaling

Estelle, M., Calderon-Villalobos, L.I., Castillejo, C., Choe, G., Chapman, E., Ge, L., Greenham, K., Lavy, M., Prigge, M., Shao, R., and Yu, H.

Cell and Developmental Biology, The University of California, La Jolla CA., USA 92093.

Auxin regulates a bewildering array of processes during plant growth and development. This complexity is belied by the apparent simplicity of the auxin-signaling pathway. Auxin regulates transcription via the TIR1/AFB-Aux/IAA-ARF pathway. The hormone directly promotes Aux/IAA action of $SCF^{TIR1/AFB}$ Degradation the Through thus permitting ARF-dependent transcription. In the case of the TIR1/AFB proteins, recent results indicate that different members of the family have distinct activities both with respect to auxin binding and Aux/IAA interaction. We are currently exploring the possibility that these differences contribute to the complexity of auxin response.

In vivo quantification of dynamic gene expression in the Arabidopsis root

Busch, W., Twigg, R.W., Mace, D.L., Ohler, U., and Benfey, P.N.

Department of Biology and Institute for Genome Sciences and Policy, Duke University, Durham, NC 27708, USA

Gene expression is a dynamic continuous phenomenon. To describe the expression of a gene it is therefore important to quantitatively capture spatio-temporal patterns of gene expression under defined conditions. As for many other quantitative experiments, it is essential to acquire measurements from multiple individuals. As yet, it is almost not possible to perform life imaging with cellular resolution on developing organs in multiple replicates and under different environmental conditions.

We developed a microfluidics device, called the root array, which enables such studies. It permits more than 60 roots to be grown in parallel and to be imaged in short time intervals by laser confocal microsopy. The design of the root array allows for rapid exchange of growth media to alter environmental conditions and to observe subsequent alterations of gene expression. Our pipeline includes fully automated image acquisition and gene expression quantification. We currently use promotor:GFP as well as GFP fusion protein based reporter lines to systematically capture expression patterns in the developing root. To assess the dynamics of gene expression, time courses and perturbations with changing media composition are conducted. The use of the root array can be extended to a variety of purposes that involve measurements at cell type resolution. For instance, we use molecular FRET sensors to conduct *in vivo* sensing of metabolites in the developing root in different growth media.

Non destructive root phenotyping in tomato

Guerra, J., Volpin, H., van Schriek, M., Speckmann, G-J., de Regt, B., Dixit, S., and Verstegen, H.

Keygene N.V. Agro Business Park 90 6708 PW Wageningen, The Netherlands

The importance of the root system has long been recognized as crucial to cope with different environmental conditions such as drought conditions. Root phenotyping in tomato is laborious and prone to errors. We used a conveyor belt system combined with a image processing unit acquired from Lemna tec company to investigate the possibility of screening root mass non destructively. Five different genotypes were used from the introgression library from Daniel Zamir http://zamir.sgn.cornell.edu/Qtl/Html/home.htm and grown with transparent pot technology. A total of 35 samples were used, to digitally recognize root traits via image processing. The correlation between the digital traits extracted from the pictures and the actual root weight measured destructively was 93%, $P<0.001$. We consider these results promising and therefore will expand the developed image processing protocol to a set of 200 individuals for further validation.

SESSION 3: LARGE-SCALE/AUTOMATED PHENOTYPING AND PHENOTYPE DATA MINING

A high-throughput phenotyping approach to disentangle the relationships between leaf expansion, cell division, cell expansion and endoreduplication in a leaf.

Granier, C., Tisné, S., Massonnet, C., Fabre, J., Wuyts, N., Vile, D., and Dauzat, M.

INRA-LEPSE, Montpellier, France

Leaf area expansion is affected by many environmental conditions including incident light, soil water content, and day-length. At the cellular level, these changes are associated with differences in cell number and/or cell size, but also with differences in the extent of endoreduplication. The functional relationships between cellular processes and leaf area expansion have been evaluated by mutational analysis and the study of transgenic lines. A few studies have shown that the regulation of leaf size could be disrupted by alterations in genes involved in cell division, cell expansion or endoreduplication, but many attempts to increase leaf size by modifying cell division or expansion have failed. A multi-scale high-throughput phenotyping and modeling approach was used in our group to determine how these cellular processes interact with the regulation of leaf area expansion both in collections of accessions, populations of recombinant inbred lines and selected mutants affected either in endoreduplication, in cell cycle regulation or in cell expansion. Both the quantitative genetics and statistical modelling approaches lead to the conclusion that these three cellular processes are controlled, at least to some extent, by whole leaf and whole plant developmental processes. As a consequence, their impact on leaf growth itself is expected to be limited which is consistent with many experimental results.

Phenotyping: Quantitative Analysis of Structure and Function of Plants

Schurr, U.

Forschungszentrum Jülich, ICG-3: Phytosphere, 52425 Jülich, Germany

Plant phenotyping is often a bottleneck for the identification of gene function. The talk will illustrate, how modern technologies can be used to quantify plant growth, transport, photosynthesis and exchange processes at a mechanistic, high-throughput and finally on a field level. An integrated concept of plant phenotyping of dynamic plant function and structure based on defined genetic material, adequate environmental simulation and monitoring as well as novel, mostly non-invasive sensors will be presented. Integration into automated systems increases throughput and accuracy of the results. Systematic approaches for the development of phenotyping technologies and protocols as well as their integration into databases will be presented. The role of heterogeneous and dynamic environments will be highlighted as one of the crucial aspects of plant phenotyping for the identification of relevant functions. An outlook will be presented on future technologies and the integration of non-invasive and invasive analysis of plants.

Matrix Genetics: The Chloroplast 2010 Project

Last, R.

Department of Biochemistry and Molecular Biology and Department of Plant Biology, Michigan State University 301 Biochemistry, East Lansing, MI 48823 USA

Rational engineering of plants for improved productivity, environmental stress tolerance and increased healthfulness will require a predictive understanding of plant metabolism. The plant chloroplast is the master chemist of the plant cell, and an attractive target for metabolic engineering. In addition to serving as the site of carbon sequestration through photosynthesis, this organelle produces many nutrients essential to the human diet, including amino acids, different antioxidant vitamin classes and lipids. The Chloroplast 2010 Collaborative (www.plastid.msu.edu) is using the extensive functional genomics resources available in Arabidopsis to speed up the process of discovering new genes that contribute to chloroplast function. The long-term goal of this project is a predictive understanding of the structure and function of the *Arabidopsis thaliana* chloroplast. The centerpiece of this approach is the testing of thousands of knock-out mutant lines for large numbers of metabolic and developmental phenotypes. The unique features and novel results of this 'matrix genetics' approach will be discussed, as will possible biotechnological outcomes from the project.

This work is funded by the National Science Foundation 2010 Project.

High throughput plant phenotyping - bridging the data bottleneck for plant growth and development data needed for modeling

Eberius, M., Schwartz, S., Vandenhirtz, D., Vandenhirtz, J., Schunk, R., and Radermacher, M.

LemnaTec GmbH, Schumanstr. 18, 52146 Wuerselen, Germany

Dealing with the variability within and between different lines, genetic backgrounds, growth conditions and stress treatments of plants makes it necessary to assess growth and development of high numbers of plants. Thus biologically significant data for modeling or validation of models can be provided. Assessing high numbers of plants within short time periods comprehensively needs a high degree of automation and reproducibility to allow relevant comparisons between plants. Especially the reaction of plants on temporary stress, which is often a question of hours, makes fast data acquisition indispensable.

Particularly modeling growth and development on plant and groups of plants forces any measurement system to deal with more global parameters like leaf areas and e. g. leaf orientation distributions at least in addition to single leaf approaches. Such more global parameters can be measured very effectively by automated image-based plant phenotyping looking at plants from different directions. In all cases where new, only technically assessable parameters are measured, it is of high importance to identify the correlation (or noncorrelation) to manually measured parameters. While multiple-parameter statistics will in many cases produce well defined correlations much better than single parameter correlations, some image based parameters can provide easily a set of information which is very difficult to assess by manual measurements. Such information may even be easily assessed by breeders in a qualitative or semi-quantitative way. Looking beyond the visible range or radiation image based phenotyping opens new windows to other wavelength ranges like NIR or IR allowing e. g. to follow non-destructively changes in the water status of plants.

Besides image analysis based phenotyping, highly automated systems additionally allow measurement of physical parameters like individual water loss per plant and the simulation of specific water stress profiles for each plant individually while eliminating variability which

would result from individual plant size or position in the greenhouse or growth chamber.

The pep2pro *Arabidopsis thaliana* proteome database: an avenue to biological insights

Baerenfaller, K.[1], Hirsch-Hoffmann, M.[1], Svozil, J.[1], Hull, R.[2], Russenberger, D.[1], Lu, Q.[1], Gruissem, W.[1], and Baginsky, S.[1]

[1]Institute of Plant Sciences, ETH Zurich, Universitaetstrasse 2, 8092 Zurich, Switzerland
[2]Faculty of Life Sciences, University of Manchester, Brunswick Street, Manchester M13 9PL, UK

We expanded the characterization of the *Arabidopsis thaliana* proteome map and integrated proteome data from different resources into the pep2pro database. Pep2pro is specifically designed for flexible and comprehensive proteome data integration and allows for comparative quantitative analyses. With pep2pro a first set of proteomics AGRON-OMICS LGB (leaf growth baseline) experiments was analysed. Preliminary results indicate that proteins, which are more abundant in fully expanded leaves than in leaves at earlier growth stages, are primarily associated with stress responses.

Altogether, pep2pro now contains more than one million high quality spectra that were assigned to peptides with high confidence. We used the high coverage and density of the proteome map for reliable protein quantification by normalized spectral counting, and extracted organ-specific biomarkers and quantitative information on diverse biological processes.

With the pep2pro database we also investigated the peptide features that distinguish detected from non-detected peptides, and benchmarked our dataset against a tool for the prediction of proteotypic peptides. Here, we found substantial disagreement between predicted and detected proteotypic peptides. In addition, experimental spectra are very useful in selecting good transitions for SRM (selected reaction monitoring). This suggests that large-scale proteomics data are essential for setting up the targeted proteomics surveys that will generate the quantitative proteome data required for systems biology approaches.

An information system for the phenotyping platform PHENOPSIS

Fabre, J.[1], Granier, C.[1], Neveu, P.[2], Tireau, A.[2], and Dauzat, M.[1]

[1]Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux (LEPSE), INRA-AGRO-M, UMR 759, 2 Place Viala, 34060 Montpellier Cedex 1 France

[2]Laboratoire d'Analyse des Systèmes et Biométrie (LASB), INRA-AGRO-M, UMR 729, 2 Place Viala, 34060 Montpellier Cedex 1 France

The PHENOPSIS platform consists in three automated growth chambers, each designed to grow more than 500 *Arabidopsis thaliana* plants in highly homogenous and reproducible micro-meteorological conditions with controlled soil water stati.

This system produces meteorological data (4 variables), data concerning the irrigation of the plants (~10 variables), daily automated pictures of the plants (infra-red or in the visible), and data measured on the plants by the experimentaters (~10-20 variables issued from destructive or non-destructive analyses), which represents at least 200Go of data. This huge amount of data needed tools to store, browse, extract, control and analyse data and meta-data.

An entire information system was thus built consisting in a database on MySQL, an automated process of data transfer, and a Web interface (http://bioweb.supagro.inra.fr/phenopsis) linking users to data and providing applications like statistics programs with R software and image analysis with ImageJ software.

We present here the database, the Web interface and the applications built on it.

POSTERS

A search for mutations that suppress the morphological phenotype of an *argonaute1* allele

Aguilera, V., Quinto, P., Micol-Ponce, R., Ece, S., Albolafio, S., Jover-Gil, S., Micol, J.L., and Ponce, M.R.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

The main component of the miRNA-mediated silencing complex (RISC) is encoded by the *ARGONAUTE1* (*AGO1*) gene in the model plant *Arabidopsis thaliana*. Mutant *ago1* alleles alter leaf morphogenesis and many other developmental processes, often causing lethality or sterility.

With a view to identify novel genes involved in miRNA-guided gene silencing, we have mutagenized seeds of the viable and fertile *ago1-52* line, which had been isolated in our laboratory. In a screening of 36,810 M2 seeds, we have identified 17 lines in which the phenotype of *ago1-52* is partially or almost completely suppressed. We are positionally cloning these suppressor mutations.

Role of auxin in the early *DcAUX1* expression during rooting

Agulló-Antón, M.A.[1], Alonso, A.[2], Jiménez, J.A.[2], Nicolás, C.[2], Sánchez-Bravo, J.[1], and Acosta, M.[1]

[1]Departamento de Biología Vegetal. Facultad de Biología. Universidad de Murcia. Campus Universitario de Espinardo. 30100 Espinardo (Murcia), Spain

[2]Departamento de Fisiología Vegetal. Facultad de Farmacia. Universidad de Salamanca. Plaza de los Doctores de la Reina s/n. 37007 Salamanca, Spain

Auxins are plant hormones necessary for rooting of cuttings. It has been shown that endogenous auxins need special transporters to go from cell to cell until they reach the organ in which they act. The gene codifying for one of these transporters in carnation cuttings is *DcAUX1* (Oliveros-Valenzuela et.al. 2008). Exogenous auxin treatment of carnation cuttings previous to plantation for rooting is a usual procedure in carnation plant commercialization and in carnation flower production. One of the physiological questions that we may do is how auxin treatment affects the endogenous auxin transport.

To answer this question, just excised carnation cuttings (cv Master) were planted for rooting after being hydrated with or without an auxin treatment (control). *DcAUX1* expression was measured using Northern-blot in the basal stem of cuttings before the treatment and in different periods during the early stages of rooting (until 72 hours), and also one week after planting.

Control cuttings presented a continuous oscillation of *DcAUX1* expression with a maximum 48 hours after planting. The transporter showed the same level of expression at 72 h and 1 week after insertion. In cuttings treated with auxins, also oscillations were observed, but levels of expression were lower than in control cuttings until 72 hours, when the expression of *DcAUX1* started to be sensitively bigger in treated cuttings.

Our results show an inhibition of the expression of *DcAUX1* caused by the presence of exogenous auxins, which demonstrates that *DcAUX1* expression is continuously adapted to auxin levels in favour to the plant physiological economy.

Probing mechanical properties of *Arabidopsis thaliana* root cells using Atomic Force Microscopy

Fernandes, A.N.[1], Walker, J.J.[2], Wells, D.[1], Chen, X.[3], Everitt, N.[2], Roberts, C.J.[3], and Scotchford, C.[2]

[1]Centre of Plant Integrative biology, School of Biosciences, University of Nottingham, England, LE12 5RD.
[2]Division of Materials, Mechanics and Structures, Faculty of Engineering, University of Nottingham, England, NG7 2RD.
[3]Laboratory of Biophysics and Surface Analysis, School of Pharmacy, University of Nottingham, England, NG7 2RD.

Root growth is accompanied by changes in cell wall properties. The knowledge of mechanical properties of cell walls could be vital to understand how these properties of the plant cells interact with other aspects of plant cell biology to effect root growth.

The inherent surface mechanical properties of *Arabidopsis thaliana* whole roots were studied at the nanoscale using the technique of Atomic Force Microscopy (AFM). This novel experimental application was developed in the initial instance and experiments were conducted to investigate the stiffness patterns along the length of the root. The development of the technique involved the challenge of keeping the root alive and properly positioned. Besides, it had to be compatible to the attached surface, for a suitable period of time to facilitate and execute the indentation process. Rigorous characterisation work has been carried out to establish that the sample does not degrade over time.

Root (epidermal) cells are assumed to be pressurised cylinders and indentation experiments were carried out by varying turgor pressure and tip geometry. These experiments helped infer the contribution from the plant cell wall itself and the turgor pressure to the measured mechanical properties. Force-Indentation curves were generated along the length of the root and these were analysed to understand the indentation process in the root epidermal tissue and its corresponding effects. Interestingly, the force-indentation curves were found to be different when the turgor pressure was varied.

Application of conventional mechanical models to cases, in nanometer regimes tends to increase error margins to a large extent. Hence the force-indentation curves were used in preliminary analysis. The work done during loading and unloading phases of the force

measurements were determined separately and were expressed in terms of “Index of plasticity”, η, which characterises the relative plastic/elastic behaviour of the material when it undergoes deformation by an external force.

Genetic characterization of some leaf mutants with abnormal mesophyll growth

Candela, H., Galiana-Briones, Y., Muñoz-Nortes, T., Sánchez-García, A.B., Sempere-Ferrández, A., Casanova-Sáez, R., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, 03202 Elche, Alicante, Spain

Despite their apparent structural simplicity, the leaves of *Arabidopsis thaliana* comprise several cell layers, each of them with a diversity of differentiated cell types. A mutational approach is ideal to identify genes that are important for the differentiation, patterning and/or proliferation of such cell types.

We will present our advances toward the map-based cloning of the *APICULATA1* (*API1*), *ANGULATA8* (*ANU8*), *ANU11*, *ANU12*, and *EROSA3* (*ERO3*) genes of *Arabidopsis thaliana*. Preliminary observations suggest that mutant alleles of these genes (which were induced by ethyl methanesulfonate) cause defective growth or differentiation of leaf mesophyll cells. The identification of the corresponding genes will help us to gain insight into the mechanisms that regulate the proliferation of mesophyll cell layers, coordinating their growth with that of other leaf tissues.

Positional cloning of 27 genes required for leaf morphogenesis in *Arabidopsis thaliana*

Casanova-Sáez, R., Candela, H., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

Leaves are energy factories that produce chemical energy from sunlight to support plant life and most food chains on land. The shape and size of plant leaves are critical for their functions, which include gas exchange, light harvesting, and photosynthesis. Leaf growth and development depend on multiple developmental processes, whose genetic bases are not completely understood.

To further our understanding of the mechanisms of leaf morphogenesis in *Arabidopsis thaliana*, a screen for mutants with abnormal leaf shape was initiated in 1993 in the laboratory of J.L. Micol. This screen led to the identification of 153 EMS and 28 fast neutron-induced mutants, which fell into 93 and 8 complementation groups, respectively. The systematic map-based cloning and molecular characterization of these genes should allow us to elucidate the network of genetic mechanisms that contribute to leaf architecture. For this purpose, we are fine mapping 27 genes from our collection using polymorphisms between Landsberg *erecta* and Columbia-0. These map positions will ultimately allow us to clone and characterize the corresponding genes at the molecular level.

Role of disease resistance gene in shaping genetic incompatibility in *Arabidopsis thaliana*

Chae, E., Laitinen, R., Bomblies, K., and Weigel, D.

Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany

Previous work suggested a Dobzhansky-Muller type, two-gene interaction is the basis of poor performance in necrotic F1 hybrids of wild strains of Arabidopsis thaliana[1]. I will discuss a case of hybrids in which two independent systems cause incompatibility. One system is due to an epistatic interaction between two linked loci on chromosome 3; one of these maps to the *RECOGNITION OF PERONOSPORA PARASITICA 1* (*RPP1*) cluster. This complex locus, where TIR-NBS-LRR type of disease resistance genes highly diverge both in sequence and number in different strains, is emerging as a cause for multiple cases of hybrid incompatibilities[1,2].

The role of this class of R genes, which can trigger autoimmune responses in hybrids, will be discussed in the context of epistatic interaction with the closely linked locus. The second incompatibility system appears to map to a single locus, *ACCELERATED CELL DEATH* 6 (*ACD6*), which suggests that accumulation of divergent alleles even at one gene can cause genetic incompatibility.

This heterozygous disadvantage case seems to underlie several other necrotic hybrids as well. Implication of divergent alleles of disease resistant gene in adaptation and speciation will be discussed.

References

1.- Bomblies, K., Lempe, J., Epple, P., Warthmann, N., Lanz, C., Dangl, J.L., and Weigel, D. (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. *PLoS Biol.* 5(9):e236.

2.- Alcázar, R., García, A.V., Parker, J.E., and Reymond, M., (2009) Incremental steps toward incompatibility revealed by Arabidopsis epistatic interactions modulating salicylic acid pathway activation. *PNAS* **106,** 334-339.

Genetic analysis and positional cloning of *incurvata13*, a new mutant allele of *AUXIN RESISTANT6*

Esteve-Bruna, D., Pérez-Pérez, J.M., Lozano, F.M., Ponce, M.R., and Micol, J.L.

Division de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

To build an almost flat wild-type leaf lamina, the growth of the different tissue layers must be tightly coordinated. Mutations affecting the genes involved in such coordination should produce uneven leaves. We aimed to test our hypothesis by studying a large collection of viable and visible mutations disrupting the shape of *Arabidopsis thaliana* leaves. Leaf incurvature or hyponasty is a deviation from flatness found in most of these mutants[1]. We positionally cloned the *INCURVATA13* (*ICU13*) gene and found it to encode AUXIN RESISTANT6 (AXR6), a core subunit of the SCF complex of E3 ubiquitin ligases[2]. The *icu13* mutation affects mRNA splicing and is predicted to truncate the AXR6 protein. Functional complementation of the *icu13* mutant with the C-terminal domain of AXR6 is ongoing. *icu13* and *eta1* (*enhancer of tir1-1 auxin resistance*) mutants are allelic and, in addition to their Incurvata leaf phenotype, they display simpler venation patterns and are defective in auxin signaling. To fully understand the role of AXR6 during leaf growth and vein patterning, we are analyzing the genetic interactions of *icu13* and *eta1* with available mutations affecting other components of the SCF^{TIR1} pathway.

References

1.- Berná, G., Robles, P., and Micol, J.L. (1999). A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana*. *Genetics* **152,** 729-742.

2.- del Pozo, J.C., and Estelle, M. (2000). F-box proteins and protein degradation: an emerging theme in cellular regulation. *Plant Mol. Biol.* **4,** 123-128.

Arabidopsis *TCU2* is required for leaf bilateral symmetry

Ferrández-Ayela, A., Lapeña-Muñoz, A., Ponce, M.R., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

Leaves of the *transcurvata2-1* (*tcu2-1*) mutant are folded downwards in a slightly asymmetrical manner relative to the midvein, and exhibit a venation with reduced length, density and number of bifurcations. Mesophyll cell size heterogeneity, stem length and flower size are increased compared with the wild type. *tcu2-1* also shows early flowering and delayed anther dehiscence. The first two leaves are fused in 10% of the seedlings. The siliques are short and thick, and many are three-valved.

We positionally cloned the *TCU2* gene, which encodes a protein of unknown function. We are making constructs for the phenotypic rescue of the mutant, constitutive expression of the *TCU2* gene, visualization of its spatial expression pattern, and the subcellular localization of the TCU2 protein. We are also conducting microarray and double mutant analyses in order to study the genetic interactions of *TCU2* and its role in leaf and whole-plant development.

A gene that affects *Arabidopsis thaliana* primary root, rosette leaves and floral stem development

García-López, M.C.[1], Jiménez-Ruiz, J.[1], Macías, D.[1], Fernandez-Ocaña, A.[2], Barroso, J.B.[1], Belaj, A.[3], de la Rosa, R.[3], Corpas, F.J.[4], and Luque, F.[1]

[1]Departamento de Biología Experimental, Universidad de Jaén, Jaén, Spain
[2]Departamento de Biología Animal Vegetal y Ecología, Universidad de Jaén, Jaén, Spain
[3]Centro de Alameda del Obispo, IFAPA, Córdoba, Spain
[4]Estación Experimental del Zaidín, CSIC, Granada, Spain

Our group is interested in the study of the juvenile-to-adult transition in olive trees as a model for this phase transition in woody plants. In a previous work we identified the *JAT* gene, which is presumably involved in the juvenile-to-adult transition in olive. To confirm this hypothesis we performed two different experimental approaches.

First, we carried out a prospective analysis of the *JAT* expression in 30 olive plant saplings from a cross of "*Jabaluna*" x "*Picual*" cultivars as well as in five wild olive saplings. The plants were followed-up until for a four years time period after seeded and determined the first flowering season. Eight of the 30 cross saplings had been flowered at the end of the four-years follow-up, as well as the five wild olive saplings. The average *JAT* mRNA level was determined by Q-RT-PCR in buds early at the spring. The flowered cross saplings had nearly five-fold higher *JAT* mRNA level than the non-flowered ones, $p<0.01$, and the same to the wild olive saplings. This result confirms that a high expression of the *JAT* gene is required to get a non-delayed juvenile-to-adult phase transition in olive trees.

The second approach was to study the inactivation of the *Arabidopsis thaliana* probable orthologue of *JAT* by T-DNA mutagenesis. Thus, *A. thaliana* transgenic plants with T-DNA inserted in the *AtJAT* gene were analysed in search for any phenotypic alteration in the plant development. For this purpose, seeds of the *A. thaliana* Line 951G12 that carries a T-DNA inserted in the 5'UTR of the *AtJAT* mRNA (*T-DNA: :AtJAT*) was obtained from GABI-Kat FST population. Self-pollinated seeds from homozygous mutant and wild *AtJAT* plants were collected and grown

under a long-day condition. Although all seeds, wild and mutant, germinated at the same time, a defect in the early growth of the primary root was observed in the *T-DNA: :AtJAT* plants. Roots of *A. thaliana T-DNA: :AtJAT* plants grew slower in the first days than did those of wild plants ($p<0.001$ at 11 days) but finally reached the same length. The floral stem also appeared at the same time in both wild and mutant plants, but at first the *T-DNA::AtJAT* mutant stem grew more slowly than did the wild one ($p<0.01$ at 21 days), to reach the same length at the later stages. The number of flowers and siliques were lower in the *T-DNA::AtJAT* mutant plants ($p<0.05$ in no of flowers and $p<0.001$ in no of siliques at 32 days) and the rosette leaves continue to grow in the mutant plants to produce abnormally larger leaves than in wild plants ($p<0.001$ at 41 days). These abnormal rosette leaves grow together with the slower growth of the stem and the lower number of flowers and siliques suggest a kind of partial or uncompleted phase change from the juvenile to adult plant. Therefore, the *T-DNA: :AtJAT* mutant plant maintains juvenile characters even after adult traits have already appeared.

A dynamical system model of the shade avoidance trigger in *Arabidopsis thaliana*

Hersch, M.[1], Lorrain, S.[2], Fankhauser, C.[2], and Bergmann, S.[1]

[1]University of Lausanne, Lausanne, Switzerland and Swiss Institute of Bioinformatics, Switzerland
[2]University of Lausanne

At growth onset, plants such as *Arabidopsis Thaliana*. have the ability to evaluate the quality of the light they perceive and thus determine whether or not they are in a shaded environment. In the shade, a particular growth pattern is triggered, skotogenesis, characterized by a strong hypocotyl elongation and a reduced leave growth. This enables the plant to rise above the neighbouring plants that were putatively shading it and come to the light, thus providing the plant with a competitive advantage. The molecular pathways underlying this behavior are still largely unknown, but qualitative models of the triggering mechanism have been suggested in the literature. Those models involves the phytochromes, the DELLA proteins, the "Phytochrome Interactig Factors" (PIFs), gibberellin and "Long Hypocotyl in Far-Red 1" (HFR1) proteins. In this work, we unite two existing models into a differential equation model of the skotogenesis triggering mechanism. This model includes the effect of hypocotyl elongation, and shows that in the case of skotogenesis, molecular and macroscopic effects have to be integrated, thus creating a closed loop between molecular processes, plant morphological changes and the environment. We show that the environment (here expressed by the light quality) has a direct influence on the position of the attractor of our dynamical system, thus making it a robust triggering mechanism. The model also makes several predictions, which could then potentially be tested experimentally.

Establishment of zones of canker diseases risk caused by *Fusarium circinatum* and *Diplodia pinea* in conifer forest based on *RESISTANCE LEVEL* and environmental factor

Iturritxa, E.[1], Garcia-Serna, I.[1], Gordon, T.R.[2], Clark, S.L.[2], Mesanza N.[3], Renobales, G.[3], and Ganley, R.[4]

[1]NEIKER. Granja Modelo - Arkaute. Apdo. 46. 01080 Vitoria-Gasteiz, Spain
[2]Department of Plant Pathology, University of California, Davis, California
[3]Facultad de Farmacia. Universidad del País Vasco, Spain
[4]ENSIS Te Papa Tipu Innovation Park. 49 Sala Street. Private Bag 3020 Rotorua, New Zealand

Pitch canker and Diplodia shoot blight are fungal diseases caused by the fungal species *Fusarium circinatum* and *Diplodia pinea* respectively. These diseases are the main cause of damages in nurseries and plantations of *Pinus radiata* in the northern area of Spain and they are the principal health problem associated with this forest tree specie. The disease, caused by the fungus *Fusarium circinatum*, was first reported in California in 1986 and it is also present in the south-eastern United States, Mexico, South Africa, Haiti, Japan, Spain, France, Italy and Chile, where it has caused substantial damage to a variety of both exotic and native species of *Pinus* genus, especially Monterey pine.

Symptoms caused by the invasion of *Fusarium circinatum* and *Diplodia pinea*, both of them widely spread in Spanish plantations of Pinus species, have several aspects in common, they can include reduced seed germination, seedling blight, dieback, cankers, root disease or crown wilt and in some cases, may result in the death of the plant (Correl et al 1991; Gordon et al. 2001; Storer et al. 1997, 1998, 2002; Gadgil et al. 2003; Chou 1987; Palmer and Nicholls 1985; Peterson 1981; Wingfield 1980).

Increased susceptibility to these pathogens can occur from a variety of factors such as drought, physical damage or other environmental stresses (Owen & Adams, 2001; Bernard & Blakeslee, 2006; Dwinell et al. 1985; Blodgett, Kruger, and Stanosz 1997; Chou 1987; Nicholls and Ostry 1990; Paoletti, Danti, and Strati 2001; Wingfield 1980). Symptoms can include reduced seed germination, seedling blight, dieback, whorl cankers, root disease or crown wilt and in some cases, may result in the death of the plant Control, prevention and eradication of *Fusarium circinatum* are complicated as the fungi are capable of surviving in the

needles, branches, shoots, wood and pinecones over an extended period of time (OEPP/EPPO, 2005; Storer et al. 1998) the management results actually difficult and highly expensive.

The objective of this study was to evaluate the susceptibility of conifer species grown in Spain to *Fusarium circinatum* and *Diplodia pinea* to facilitate risk infection predictions for forestry and preservation purposes in the areas of Spain were these disease are a real problem.

Role of multiple myrosinases in *Arabidopsis thaliana*

Chen, K., Bejai, S., and Meijer, J.

Department of Plant Biology & Forest Genetics, Uppsala Biocenter, Box 7080, Swedish University of Agricultural Sciences, SE75007 Uppsala, Sweden

Myrosinases are β-thioglucoside glucosidases present in *Capparales* plants. These enzymes defend plants against attackers through breakdown of the secondary metabolites glucosinolates into toxic products. It has also been speculated that the myrosinaseglucosinolate system has a role in development. Myrosinases are present in gene families but the properties and roles of different forms are not well understood. *Arabidopsis thaliana* seems to contain at least seven myrosinase genes (*TGG1-TGG6, PEN2*). We have recently expressed and characterised three recombinant proteins for enzymatic properties, which revealed some differences. Expression analysis of transcripts showed that *TGG1* and *TGG2* are only present in above-ground tissues while *TGG4* and *TGG5* are expressed in roots only. Reporter lines transformed with constructs containing myrosinase promoters driving GUS or GFP confirmed this division in localization between above-ground and below-ground tissues. Since both glucosinolates and myrosinases may be systemically transported in the plant and secreted we are very much interested in the dynamics during plant development and stress. In addition, we want to study how this system is regulated if used both for defense as well as for growth and development.

The study is supported by FORMAS and Perssons fund.

The microRNA pathway participates in proximal-distal polarity and venation pattern establishment in *Arabidopsis thaliana* leaves

Jover-Gil, S., Aguilera, V., Robles, P., Candela, H., Barrero, J.M., Micol, J.L., and Ponce, M.R.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

We have isolated mutants carrying novel alleles of the microRNA (miRNA) pathway genes *ARGONAUTE1* (*AGO1*), *HYPONASTIC LEAVES1* (*HYL1*) and *HUA ENHANCER1* (*HEN1*). Characterization of these mutants shows that a number of shared phenotypic traits are always associated with the loss of function of components of the miRNA pathway. These traits include a reduced rosette size, abaxial trichomes in the first pair of leaves, a gradual transition between lamina and petiole, compact inflorescences, moderately low fertility, slow growth, and late flowering. A closer examination, carried out with scanning electron microscopy, uncovered epidermal cells similar to those of the lamina in the petiole of all these mutants. In addition, the vascular patterns of cotyledons and leaves were less complex in *dcl1-9*, *hen1-13* and *hyl1-12* mutants than in the wild type. These observations point to the involvement of the miRNA pathway in the establishment of proximal-distal leaf polarity and in vascular patterning.

Neither leaf nor sepal: the complex nature of the epicalyx in the mallow family (Malvaceae)

Martínez-Asperilla, A., Bello, M.A., Nieto-Feliner, G., Álvarez, I., and Fuertes-Aguilar, J.

Plant Evolutionary Biology Group. Real Jardín Botánico, CSIC. Plaza de Murillo 2, 28014 Madrid, Spain

The epicalyx is a transitional structure located on the flowering branches between the bracteoles and the calyx. Among the different species of *Lavatera-Malva* lineage, the epicalyx displays 1-3 free or fused lobes and leaf traits. Interpreted as sterile inflorescence bracts resultant from floral reductions in Malvales, the epicalyx displays a sepal-like morphology and seems to mimic other leafy structures. However, its shape, position and whorled nature makes it different from leaves, stipules, bracts, bracteoles and sepals, making this structure unique within the suit of appendages of the flowering branches. As part of an ongoing evo-devo project, our study aims to discuss homology of the epicalyx from a morphological and developmental genetics context, studying the floral development, anatomy and the expression patterns of genes associated to either sepal/meristem determinancy (e.g. class A *MADS box* genes) or leaf-like traits in selected species of *Lavatera* and *Malva*. Here we compare the floral development of two sister species with contrasting epicalyx phenotypes: *Malva hispanica* with 1-2 free bracteoid pieces and *Lavatera trimestris* with three fused pieces. Our results suggest that although the epicalyx initiates at the pedicel before the differentiation of the flower bud, its development is independent and cannot be considered a floral whorl.

Probing phenotype and molecular profile reproducibility: a comparison of Arabidopsis leaf growth across ten laboratories

Massonnet, C., and the AGRON-OMICS consortium

INRA-LEPSE, Montpellier, France

A major goal of the life sciences is to understand and model how molecular processes control phenotypes. Because the study of biological systems relies on the work of multiple independent research groups, biologists commonly assume that organisms with the same genetic makeup will display similar phenotypes when grown in comparable conditions. We investigated to what extent the growth and molecular phenotypes of identical Arabidopsis genotypes can be reproduced in different laboratories adhering to a standardized protocol. The results were obtained in a pan-European experiment performed in ten locations across six different countries. First, we determined the appropriate environmental conditions and a minimum set of leaf growth variables marking the contrasts between three selected Arabidopsis accessions: Col, L*er* and Ws. We then shared a detailed protocol among all laboratories with the aim to assess the reproducibility of leaf phenotype, and of metabolite and transcript profiles extracted from the same leaf samples. The statistical analysis of the data revealed significant differences between measurements obtained from distinct locations, sometimes resulting in a change of genotype ranking in terms of growth performance. Our findings underscore that the challenge of describing, monitoring and precisely controlling environmental conditions is generally underestimated. However, we also demonstrate that independent growth and molecular profile datasets can be used to distinguish between genotypes when produced with particular attention for environmental parameters. This comparative analysis pinpoints likely variables that account for differences in separate laboratories.

Could the extent of cell division, cell expansion and endoreduplication in a leaf be controlled by leaf expansion itself ?

Massonnet, C., Tisné, S., Vile, D., Fabre, J., Wuyts, N., Dauzat, M., and Granier, C.

INRA-LEPSE, Montpellier, France

Leaf area expansion is affected by many environmental conditions including incident light, soil water content, and day-length. At the cellular level, these changes are associated with differences in cell number and/or cell size, but also with differences in the extent of endoreduplication. The functional relationships between cellular processes and leaf area expansion have been evaluated by mutational analysis and the study of transgenic lines. A few studies have shown that the regulation of leaf size could be disrupted by alterations in genes involved in cell division, cell expansion or endoreduplication, but many attempts to increase leaf size by modifying cell division or expansion have failed. A multi-scale high-throughput phenotyping and modeling approach was used in our group to determine how these cellular processes interact with the regulation of leaf area expansion both in collections of accessions, populations of recombinant inbred lines and selected mutants affected either in endoreduplication, in cell cycle regulation or in cell expansion. Both the quantitative genetics and statistical modelling approaches lead to the conclusion that these three cellular processes are controlled, at least to some extent, by whole leaf and whole plant developmental processes. As a consequence, their impact on leaf growth itself is expected to be limited which is consistent with many experimental results.

Characterization of root and leaf development in *Arabidopsis thaliana* plants over-expressing XTHs

Miedes-Vicente, E.[1], Mellerowicz, E.[2], and Vissenberg, K.[1]

[1]University of Antwerp, Biology dept. Plant Growth and Development, Groenenborgerlaan 171, 2020 Antwerp, Belgium
[2]Department of Forest Genetics and Plant Physiology, Umea Plant Science Center, SLU, S901 83 Umea, Sweden

Studies on developmental processes of higher plants often use the model plant *Arabidopsis thaliana* as it offers significant advantages. We studied the growth and development of the roots and leaves to evaluate the effect of the over-expression of 5 xyloglucan endotransglucosylase/hydrolase (XTH) genes (*At*XTH17, 18, 19, 20 and *Ptt*XTH16-34). These genes have been linked to both the primary growth (*At*XTH17, 18 and 19) and with secondary growth (*At*XTH20 and *Ptt*XTH16-34)[1,2]. During growth it is believed that XTHs are involved in `wall loosening' since they break and rejoin xyloglucan chains. This potentially allows cellulose microfibrils to move apart driven by protoplasmic pressure.

In a first set of experiments, we measured the expression levels of the individual genes in the roots and the leaves by semi-quantitative RT-PCR. All the genes were indeed found to be over-expressed. However, as expression levels don't tell anything on protein levels and enzymic activities, the total XET activity in roots and leaves was measured by use of a very specific radiometric test[3]. Higher levels of XET activity were demonstrated.

In a next set of experiments the effect of the higher XET activity levels on the development of the root and the leaves was investigated. Root growth was followed during 14 days and the length and growth rate was recorded. In a parallel set of experiments, the development (size) of the leaves was followed. Using propidium iodide staining and confocal microscopy the boundaries of the epidermal cells of the leaves were visualized. These images were then used and analyzed by a new and fast protocol designed with ImageJ.

The methodology and results of these analyses will be presented.

References
1.- Vissenberg K. *et al.,* (2005). Differential expression of AtXTH17, AtXTH18, AtXTH19 and AtXTH20 genes in Arabidopsis roots. Physiological roles in specification in cell wall construction. *Plant and Cell Physiology* **46,** 192-200.
2.- Bourquin *et al*., (2002). Xyloglucan Endotransglycosylases Have a Function during the Formation of Secondary Cell Walls of Vascular Tissues. *The Plant Cell* **14,** 3073-3088.
3.- Fry *et al*., (1992). Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. *Biochem J.* **282,** 821- 828.

The *ven3* and *ven6* reticulate mutants are defective in carbamoyl phosphate synthesis

Mollá-Morales, A.[1], Sarmiento-Mañús, R.[1], González-Bayón, R.[1], Robles, P.[1], Quesada, V.[1], González-Torres, P.[1], Hannah, M.[2], Willmitzer, L.[2], Pérez-Pérez, J.M.[1], Ponce, M.R.[1], and Micol, J.L.[1]

[1]División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

[2]Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

The Arabidopsis *venosa* (*ven*) mutants exhibit reticulate leaves, whose vascular network is seen as a green reticulation on a paler lamina, a trait indicative of altered internal leaf architecture. To gain insight into the processes that pattern epidermal, mesophyll and vascular tissues, we have characterized one *ven6* and four *ven3* semidominant mutants, all of which exhibited reduced palisade mesophyll cell density.

A metabolomic analysis of *ven3* and *ven6* leaves showed altered levels of some intermediates of the arginine biosynthesis pathway: ornithine was increased, and arginine and citrulline were reduced. Supplementation of the growth medium with citrulline completely suppressed their mutant phenotypes. Consistent with this, the mutants were more sensitive than the wild type to the inhibition of growth in ornithine-supplemented medium.

We positionally cloned the *VEN3* and *VEN6* genes, which were found to encode the large and small subunits of carbamoyl phosphate synthetase (CPS). In *Escherichia coli*, CPS is a heteromultimer consisting of four large and four small subunits that catalyzes the conversion of glutamine into glutamate and carbamoyl phosphate, which in turn condenses with ornithine to produce citrulline. Our genetic and molecular analyses of the *ven3* and *ven6* mutants indicate that CPS function is essential for mesophyll cell development in the interveinal tissues of Arabidopsis vegetative leaves.

The carbon status of the *Arabidopsis thaliana* rosette is improved when plants are exposed to water deficit; an integrated perspective using growth, metabolite, enzyme and gene expression analysis

Hummel, I.[1,4], Pantin, F.[1], Sulpice, R., Piques, M., Rolland, G.[1], Dauzat, M.[1], Granier, C.[1], Stitt, M.[1], Gibon, Y.[2,3], and Muller, B.[1]

[1]INRA, UMR759 LEPSE, Montpellier, France
[2]MPI-MP, Golm, Germany
[3]present address INRA, UMR 619, Bordeaux, France
[4]present address INRA, UMR 1137, Nancy, France

Because growth and carbon fluxes are severely altered in plants exposed to soil water deficit, it has been suggested that water-stressed plants would suffer from carbon shortage. In this study, we tested this hypothesis in *Arabidopsis thaliana* by providing a broad view on responses of growth, C balance, metabolites, enzymes of the central metabolism and a set of sugar responsive genes. The results show that in response to soil water deficit, (i) rosette relative expansion rate is more reduced than photosynthesis on a DW basis leading to a more positive C balance under drought, (ii) several soluble metabolites accumulate in response to soil water deficit and K^+ and organic acids are the main contributors to osmotic adjustment. Moreover, (iii) all C metabolites measured (not only starch and sugars but also organic acids and amino acids) show a diurnal turnover that increases under water deficit suggesting these metabolites are readily available and used, (iv) most enzymes activities are increased by water deficit, especially on the long term and enzymes known to respond to sugar starvation from earlier studies show opposite response to water deficit, (v) water deficit induces a shift of the expression level of a set of sugar responsive genes towards a more favorable status by days in the wild type and during nights in the severely C starved *pgm*. This set of data converge to show that soil water deficit improves the carbon status of the plant and thus suggest that the growth of droughted plants, at least under our conditions is not likely to be C limited.

A reverse genetics approach to the analysis of leaf development

Muñoz-Viana, R., Rubio-Díaz, S., Pérez-Pérez, J.M., Ponce, M.R., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

Because of their photosynthetic activity, leaves are the ultimate source of most of the oxygen that we breathe and of the food that we eat. Yet the processes by which these organs grow are poorly understood. Previous forward genetics studies yielded a large number of mutants affecting leaf growth, shape or size. However, none of these previous attempts reached genome saturation[1]. The group of Prof. Ecker at the Salk Institute is obtaining a large collection of sequence-indexed homozygous T-DNA insertion mutants that will cover 25,000 genes of the Arabidopsis genome[2].

To identify novel genes required for leaf growth regulation, we have begun a reverse genetics screen using T-DNA insertion lines in 6,800 Arabidopsis genes, available from the ABRC. These lines are grown *in vitro* and those displaying developing leaves with aberrant phenotypes are documented and kept for further studies. In order to saturate the genome with viable and fertile leaf mutations, we plan to screen the entire Salk homozygous T-DNA insertion collection for visible mutations affecting leaf organogenesis.

We present here the identification of novel leaf mutants resulting from a high-throughput screen of more than 5,000 sequence-indexed homozygous T-DNA insertion lines

References

1.- Berná, G., Robles, P., and Micol, J.L. (1999). A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana*. *Genetics* **152**, 729-742.

2.- Alonso, J.M., *et al* (2003) .Genome-wide insertional mutagenesis of *Arabidopsis thaliana. Science* **301**, 653-657.

High-throughput analysis of leaf mutant phenotypes in *Arabidopsis thaliana*

Pérez-Pérez, J.M.[1], Rubio-Díaz, S.[1], Hernández-Romero, D.[1], Sánchez-Soriano, J.[2], Dhondt, S.[3], Beemster, G.T.S.[3], Ponce, M.R.[1], and Micol, J.L.[1]

[1]División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain
[2]Centro de Investigación Operativa, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain
[3]Department of Plant Systems Biology, Flanders Institute for Biotechnology (VIB), Technologiepark 927, 9052 Gent, Belgium

Little is known about how cell growth and cell division are coordinated within a growing primordium to develop into a laminar structure of determinate size, such as the leaf. To genetically dissect the mechanisms underlying leaf growth, we have analyzed a representative collection of 111 *Arabidopsis thaliana* mutants displaying abnormally shaped or sized leaves. The semi-automated quantitation of several parameters at the organ, tissue and cellular levels from first- and third-node leaves in these mutants has allowed us to determine a quantitative framework for leaf trait variation in *Arabidopsis thaliana*, which should be useful for the classification of the leaf phenotypes observed in genome-wide gene-indexed collections of T-DNA insertional mutants.

Study of stem cells pool size in the Arabidopsis shoot meristem by functional characterization of the AMP1 pathway

Pitorre, D., Marizzi, C., Huang, W., and Sieberer, T.

Department for Microbiology, Immunobiology and Genetics, Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria

AMP1 (*ALTERED MERISTEM PROGRAM 1*) encodes a putative glutamate carboxypeptidase with sequence similarity to the mammalian N-acetyl α-linked acidic dipeptidases of the glutamate carboxypeptidase II protein family[1]. In mammals, members of this enzyme class have been shown to cleave the neuropeptide N-acetylaspartylglutamate[2] and polyglutamyl folates for intestinal uptake[3]. Whether the biochemical function of AMP1 is identical to animal counterparts or adapted divergent activities is not resolved to date.

Several *amp1* mutant alleles in Arabidopsis have been previously identified and studied[4-9]. Mutations in *AMP1* generate a combination of phenotypes including an increased rate of leaf initiation, more branching, an increase in shoot apical meristem (SAM) size and elevated levels of cytokinin[6,10]. We are currently further investigating AMP1 function by a combination of different approaches. First, we are generating several two-component lines in the *amp1-1* background to determine in which areas of the plant AMP1 is necessary and sufficient to overcome the SAM enlargement. Second, we are investigating the role of cytokinin in the *amp1* phenotype, to determine if the increase in hormone level is responsible or a consequence of the SAM enlargement. Furthermore, we identified and characterized two independent Arabidopsis knock-out mutant alleles of *AMP1 RELATED PROTEIN 1 (AMR1*). *AMR1* also encodes a putative glutamate carboxypeptidase and shares 39% identity, 56% similarity to AMP1 (1). While single mutant *amr1* plants present no obvious phenotype, the *amp1 amr1* double mutants display an exaggeration of *amp1* related phenotypes suggesting a redundant function of AMP 1 and AMR1.

References

1.- Helliwell, C.A., Chin-Atkins, A.N., Wilson, I.W., Chapple, R., Dennis, E.S., and Chaudhury, A. (2001) *Plant Cell* **13,** 2115-2125.

2.- Robinson, M.B., Blakely, R.D., Couto, R., and Coyle, J.T. (1987) *J Biol Chem* **262,**

14498-14506.
3.- Halsted CH, Ling EH, Luthi-Carter R, Villanueva JA, Gardner JM, & Coyle JT (1998) *J Biol Chem* **273,** 204 17-20424.
4.- Hou Y, Von Arnim AG, & Deng XW (1993) *Plant Cell* **5,** 329-339.
5.- Saibo NJ, Vriezen WH, De Grauwe L, Azmi A, Prinsen E, & Van der Straeten D (2007) *Planta* **225,** 83 1-842.
6.- Chaudhury AM, Letham SC, Craig S, & Dennis ES (1993) in *The Plant Journal*, pp. 907-916.
7.- Conway LJ & Poethig RS (1997) *Proc Natl Acad Sci U S A* **94,** 10209-10214.
Mayer U, Ruiz R, Berleth T, Misera S, & Jurgens G (1991) *Nature* **353,** 402-407.
8.- Mordhorst AP, Voerman KJ, Hartog MV, Meijer EA, van Went J, Koornneef M, & de Vries SC (1998) *Genetics* **149,** 549-563.
9.- Chin-Atkins AN, Craig S, Hocart CH, Dennis ES, & Chaudhury AM (1996) *Planta* **198,** 549- 556.

Identification of new genetic functions chloroplast-localized and involved in Arabidopsis development

Quesada, V., Micol, J.L., and Robles, P.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

Chloroplasts are the plant organelles where photosynthesis, as well as many intermediary metabolic pathways, take place. This organelle has its own genome, as a consequence of its symbiotic origin, and its development seems to be related to important plant developmental processes, such as the proper differentiation of certain cell types or tissues, such as the leaf mesophyll. Pioneering bioinformatics predictions have estimated that the number of proteins contained in the chloroplasts ranges from 2000 to 3000. Taking advantage of recent challenges achieved in the genomics and proteomics fields in Arabidopsis, we are trying to identify new genetic functions that are nucleus-encoded and chloroplast localized and whose perturbation gives rise to visible abnormal developmental phenotypes. We are selecting genes for which no insertional alleles have been described to date, and focusing on those that encode proteins involved in transcription and translation inside the chloroplasts, in order to determine how the regulation of the flux of genetic information occurs in these organelles and how it relates to plant development. We are screening insertional alleles of these genes in several publicly available knock-out collections aimed to find mutations whose characterization sheds some new light in chloroplast function.

Growth responses of Zinnia to different Organic media

Riaz, A., Farooq, U., and Khan, M.A.

Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan

Zinnia is a beautiful summer annual flower, which is gaining popularity for its variety of colorful flowers. Its flowering pattern is greatly affected by the growth media. The present research was thus conducted to evaluate the growth and flowering responses of Zinnia to different organic growing media. Growth and flowering of three different varieties of Zinnia (tall, medium and dwarf) were studied using different organic media including coconut coir + soil, composted leaves + silt, farm yard manure + silt and composted leaves alone were compared with common soil as a control. Seeds were grown in peat moss and then transplanted to pots containing the different growth media. Pots were arranged according to a CRD (Completely Randomized Design). Growth parameters that were evaluated included plant height, stem diameter, time of flower initiation, flower duration, number of flowers/plant, flower quality, flower head size, number of ray florets/head, number of disc florets/head, number of lateral branches, internodal distance, number of leaves/plant, leaf area and root length. Results showed that, among the plant varieties, the dwarf variety showed the best overall response to the organic media, while the tall variety did not respond well to the organic growing media treatments. Despite its relatively poor growth response, the tall variety had the highest survival rates among all varieties. Among the different media evaluated for growing Zinnia, a mixture of coconut coir + soil and of leaf manure + silt showed the best results for all the varieties used in this experiment.

Genomic and functional characterization of the gene family of the mitochondrial transcription termination factors (mTERFs) in *Arabidopsis thaliana*

Robles, P., Micol, J.L., and Quesada, V.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

In metazoans, it has been discovered that some proteins encoded by the nuclear genome are able to bind to the mitochondrial DNA terminating transcription [mitochondrial transcription termination factors (mTERF)]. These transcription factors belong to a large family of proteins. Members of this family have recently been found in monocotyledonous and dicotyledonous plants, as well as in the moss *Physcomitrella patens*, but are apparently absent in fungi and prokaryotes. The mTERF proteins studied so far are involved in the control of mitochondrial transcription, some of which being required not only for transcription initiation, but also for its termination. Experimental results recently obtained in mice demonstrate that some *mTERF* genes are essential for life since loss of their function proves lethal very early in development. Given the unawareness of the function of these proteins in plants, we are carrying out a genome-wide *in silico* analysis of the mTERF-transcription factor gene family in plants, mainly focusing on *Arabidopsis thaliana* and rice. We are conducting an extensive functional analysis of the mTERF proteins in *Arabidopsis thaliana* by characterizing at the genetic, phenotypic and molecular levels, T-DNA tagged knockout mutations in different *mTERF* genes whose perturbation causes a mutant phenotype. We have already identified several *mTERF* loss-of-function insertional mutants displaying developmental phenotypes which will be presented at the meeting. Our results will contribute to elucidate the function that the *mTERF* family of genes plays in plant development.

Positional cloning of mutations affecting leaf growth

Rubio-Díaz, S., Muñoz-Viana, R., Pérez-Pérez, J.M., Hernández-Romero, D., Antón-Bolaños, N., Ponce, M.R., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain

We have isolated several hundreds of viable and fertile Arabidopsis mutants with abnormal leaf morphology[1]. More than forty of them have already facilitated the molecular identification of genes required for leaf organogenesis[2]. In our large-scale screen, we identified dozens of mutants with small leaves[3], and assumed that their reduced leaf size indicates loss of function of genes required for leaf growth.

The *exigua* (*exi*) mutants display small, dark green vegetative leaves. In the frame of the AGRON-OMICS project, the Exi phenotypic class is being used in our lab to understand how cell expansion mechanisms are coordinated during leaf growth. We have positionally cloned several *EXI* genes and found them to encode different subunits of the cellulose synthase that functions in secondary cell wall expansion. For the positional cloning of additional growth regulatory genes, we are also studying mutants belonging to the Ondulata (Ond), Serrata (Sea), Orbiculata (Orb), Angusta (Anu) and Apiculata (Api) phenotypic classes, which also exhibit reduced leaf size. One gene that has been cloned recently is *OND3*, which encodes the ARC6 protein, required for chloroplast division.

References

1.- Pérez-Pérez, J.M., Candela, H., Quesada, V., Robles, P., Ponce, M.R., and Micol, J.L. (2009). Lessons from a search for Arabidopsis leaf mutants. *Int. J. Dev. Biol.*, in press.

2.- Micol, J.L. (2009). Leaf development: time to turn over a new leaf? *Current Opin. Plant Biol.* **12**, 9-16.

3.- Berná, G., Robles, P., and Micol, J.L. (1999). A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana*. *Genetics* **152**, 729-742.

RUG1 encodes the Arabidopsis porphobilinogen deaminase

Sarmiento-Mañús, R., Quesada, V., Giménez-Molina, Y., Ponce, M.R., and Micol, J.L.

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Spain

The vegetative leaves of the Arabidopsis *rugosa1* (*rug1*) mutant display a protruded lamina and are smaller and more irregular in shape than those of the wild type. The most conspicuous trait of *rug1* is the spontaneous development of lesions in its vegetative leaves, which appear as patches of necrotic tissue. Such phenotype is reminiscent of the lesion-mimic mutants, which develop lesions in the absence of pathogens. The internal leaf structure is altered in the *rug1* mutant, as shown by light and confocal microscopy. Expression of several cytological and molecular markers associated with disease resistance responses was detected in the damaged areas of *rug1* leaves.

We positionally cloned the *RUG1* gene, which was found to encode phorphobilinogen deaminase (PBGD), also known as hydroxymethyl bilane synthase. PBGD is chloroplast-localized and catalyzes the fifth enzymatic step of the tetrapyrrole biosynthesis pathway, which in higher plants produces chlorophyll, heme, siroheme and phytochromobilin. A microarray analysis showed that nearly 300 nuclear genes are differentially expressed between *rug1* and the wild type, about a quarter of which encode proteins involved in plant defense. Our results suggest that impairment of the porphyrin pathway by defective PBGD activates plant defense mechanisms and alters normal leaf development in Arabidopsis.

QTLs underlying the response of leaf expansion to drought in *Arabidopsis thaliana* highlight different processes by which leaf area can be maintained or increased

Tisné, S.[1], Vile, D.[1], Dauzat, M[1]., Pervent, M.[1], Reymond, M.[1], Koornneef, M.[2], and Granier, C.[1]

[1]LEPSE INRA Montpellier, France
[2]MPIZ Cologne, Germany

Understanding the physiological and genetic bases of plant performance under drought is an important challenge in the context of global climate change.

Changes in leaf area caused by drought was analysed in a population of recombinant inbred lines derived from a cross between two *Arabidopsis thaliana* accessions, L*er* and An- 1, at two day-lengths. Quantitative trait loci (QTLs) controlling responses of leaf production and expansion to drought were identified and characterised by developmental and cellular processes.

A set of QTLs that conferred a maintain or an increase in leaf area in response to drought was identified. A combination of 3 alleles increased both leaf production and expansion but despite a spectacular effect on the response of rosette area to drought, this pathway only functioned in short days. A QTL conferred a low reduction in leaf expansion in response to drought via a low reduction both in epidermal cell area and cell number. Additionally, two QTLs conferred a low reduction in leaf expansion but just because leaf expansion was reduced in well-watered conditions, without a specific effect of drought.

Our findings highlight the values of quantitative genetic approaches for exploring processes regulating plant responses to drought and open perspectives for genetic engineering of plant performance under drought.

Genetic regulation of “unifacial leaf” development in *Juncus*

Tsukaya, H.[1,2], and Yamaguchi, T.[2]

[1]Graduate School of Science, The University of Tokyo, Tokyo, Japan
[2]National Institute for Basic Biology, Okazaki, Japan

In angiosperms, leaves generally develop as a bifacial structure with distinct adaxial and abaxial identities. On the other hand, “unifacial leaves, in which leaf blades have only the abaxial identity, have evolved in a number of divergent species in monocots. Unifacial leaves provide a unique chance to study the mechanism of repeated evolution, the phenomenon widely observed during organismal evolution. In addition, unifacial leaves could be a useful “mutant” for developmental studies of leaf axis formation in monocots, because leaf polarities in unifacial leaves are distinct from those in bifacial leaves (Yamaguchi and Tsukaya: DOI 10.1007/s10265-009-0255-3). Furthermore, it is also interesting many unifacial leaf species develop flattened leaf blades, even though they lack adaxial-abaxial polarity. This fact is quite interesting, since it is widely believed that the juxtaposition between adaxial and abaxial identities promotes lateral outgrowth of the leaf blade, which lead to the formation of the dorsoventrally flattened leaf structure.

To clarify the mechanism of unifacial leaf development and evolution, we are studying the genus *Juncus* (Juncaceae) as a model. *Juncus* contains species suitable for molecular genetic analysis and their leaf forms are highly diversified. We revealed that the leaf blade in unifacial leaves is completely abaxialized at the gene expression level, and that genetic mechanism, which promotes abaxial identity, works predominantly in unifacial leaves. We will also present the genetic mechanism controlling the blade outgrowth in unifacial leaves, independent of the leaf adaxial-abaxial polarity. We identified a transcription factor, whose expression patterns correlates with blade outgrowth in unifacial leaves, and show genetic evidence it indeed promotes blade outgrowth in unifacial leaves.

A model based on quantitative gene expression describing the relationships between stem cell, meristem identity, cell division and floral primordia initiation

Weiss, J.[1], Amat-Plata, S.[2], Pietras-Lebioda, A.[3], Delgado-Benarroch, L.[1], and Egea-Cortines, M.[1]

[1]Genetics, Dept. of Agricultural Science and Technology, ETSIA, Universidad Politécnica de Cartagena, UPCT, 30203, Cartagena, Spain
[2]Dept. of Applied Mathematics, ETSII, Universidad Politécnica de Cartagena, UPCT, 30203, Cartagena, Spain
[3]Pracownia Analiz DNA, Uniwersytet Wrocławski ul. Sienkiewicza 21, 50-335 Wrocław, Poland

The shoot apical meristem (SAM) is a self-maintaining structure producing cells that end up forming lateral organs. Three functions are responsible for SAM activity and output. One is the maintenance of stem cells in the centre of the meristem depending on expression of *Wuschel* in Arabidopsis and orthologs in other species i.e. *Rosulata* in *Antirrhinum*[1]. A second one is the so-called meristem identity which is a totipotent state typical of cells that form the dome of the meristem. Usually these cells express orthologs of *Shoot-meristemless* like *Hirzina* in *Antirrhin um*[2]. Cells displaced from the dome by continuous growth get recruited to form lateral primordia. Cell division can be detected by *Histone H*4 that is expressed in all dividing cells, *CYCD3b* that is present in the dome[3], and *CYCD3a* that is expres sed only in newly forming primordia . We have performed a thorough study of *Antirrhinum* SAMs measuring *ROA*, *HIRZ*, *H4*, *CYCD3a* and *b* under different growth conditions that modify the number of flowers produced per plant. Large changes in meristem output is accompanied by very modest alterations in genes related to stem cell maintenance or meristem identity, confirming data obtained by GFP analysis[4] in Arabidopsis, but do not support model-based conclusions about changes in stem cell homeostasis[5] . In contrast cell division is strongly affected. Using the gene expression data we have built a model that describes for the first time the quantitative relationships between stem cells (marked by *ROA*), meristem identity (marked by *HIRZ*) formation of primordia (obtained as the number of flowers produced per SAM) and cell division in the different regions (complete SAM *H4*, dome *CYCD3b* and lateral primordia *CYCD3a*). We are

testing the model under conditions that cause increases in floral number per plant.

References

1.- Kieffer, M. et al. (2006). Analysis of the transcription factor WUSCHEL and its functional homologue in Antirrhinum reveals a potential mechanism for their roles in meristem maintenance. *Plant Cell* **18**, 560-573

2.- Golz, J.F., Keck, E.J. and Hudson, A. (2002). Spontaneous mutations in KNOX genes give rise to a novel floral structure in Antirrhinum. *Current Biology* **12**,515-522.

3.- Gaudin, Y. et al. (2000). The expression of D-cyclin genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the cycloidea gene. *Plant Physiology* **122**, 1137-1148

4.- Reddy, G.Y. & Meyerowitz, E.M. (2005). Stem-cell homeostasis and growth dynamics can be uncoupled in the Arabidopsis shoot apex. *Science* **310**, 663-667.

5.- Geier, F. et al. (2008). A quantitative and dynamic model for plant stem cell regulation. *PLoS One* **3**, e3553

Modulation of plant growth via brassinosteroid signalling

Zhiponova, M.[1], Ugrinova, M.[1], Pizon, J.[1], Inzé, D.[1], Reuzeau, C.[2], and Russinova, E.[1]

[1]Flanders Institute for Biotechnology Department of Plant System Biology, University of Gent (VIB PSB, U-Gent).
[2]CropDesign N.V.-a BASF Plant Science Company

Plant growth is regulated by developmental programs modified by environmental cues acting through plant hormones. Brassinosteroids (BRs) are important for agriculture as they determine important agronomic traits including biomass, crop yield, senescence time, plant stature, flower architecture, and stress adaptation. Mainly genetic modifications of BR biosynthesis were attempted for yield increase. However BR biosynthesis is complexly regulated and its manipulation often had negative effects on important crop traits. Alternatively the use of BR signal transduction has renewed interest in engineering the BR pathway in crops by increasing the feasibility of generating plants without defects in reproductive development. By performing a microarray expression analysis of *Arabidopsis* plants treated with BRs and a newly identified chemical compound, bikinin, that inhibits a key regulator of BR signaling, the BIN2 glycogen-synthase kinase-3 (De Rybel *et al*., 2009), we identified several putative regulators of the BR signaling. The role of 20 selected candidate genes in plant growth and development is currently explored by overexpression analysis in *Arabidopsis* and rice. Among the primary transformants several phenotypic categories were observed including plants with a larger size. Currently a detailed phenotypic characterization of the transgenic *Arabidopsis* and rice plants is carried out. In summary, the analysis of the candidate genes in *Arabidopsis* will not only increase our knowledge for regulation BR signaling but will also contribute to the relatively fast identification of genes for crop improvement.

Genetic determinants of diversity in non-aberrant stomata patterns in Arabidopsis

Delgado, D.[1], Triviño, M.[1], Martín, C.[1], de Marcos, A.[1], Alonso-Blanco, C.[2], Mena, M.[1], and Fenoll, C.[1]

[1]Facultad de Ciencias del Medio Ambiente, Universidad de Castilla-La Mancha, Toledo E-45071, Spain
[2]Centro Nacional de Biotecnología, CSIC, Campus de la Universidad Autónoma de Madrid, Spain

Stomata differentiation in Arabidopsis photosynthetic organs takes place as a continuous process during plant development. The gradual differentiation of these bi-celled epidermal structures occurs, as organs grow, through the interaction of environmental factors with stereotyped genetic programmes that involve cell divisions and cell fate acquisitions, resulting in distinct and variable stomata numbers and distribution patterns in the mature organs. This implies that overlapping and probably redundant developmental pathways, involving many genes and set to integrate responses to various environmental conditions, must operate to allow a diversity of stomata patterns and numbers while guaranteeing their functionality. Dissecting such gene circuits has only started, and in the last few years, a number of positive and negative regulators of stomata differentiation have been identified genetically and molecularly, and gene functions have been ascribed mostly based on severe, aberrant phenotypes produced by strong (often knock-out) alleles. However, little is known as yet on how diversity in functional, non-aberrant stomata patterns could be set. To approach this question, we have used a double strategy studying quantitative traits in natural and induced Arabidopsis mutants. On one hand, through measurement of the quantitative parameters Stomatal Index (SI) and Density (SD) in a large sample of Arabidopsis accessions, we have shown that Arabidopsis displays a broad –though subtle- diversity for both characters that we have explored through QTL analyses. On the other hand, chemical mutagenesis allowed us to identify mutant alleles of stomata differentiation genes that produce weak phenotypes which are both suited for quantitative analyses and for assigning novel functions to known genes. We will present the progress made in our laboratory with the two strategies.

Towards high-throughput cell type specific analysis in *Arabidopsis* leaves

Grønlund, J.[1], Kumar, S.[1], Dhondt, S.[2], Beemster, G.[3], Hilson, P.[2], Beynon, J.[1] and Buchanan-Wollaston, V.[1]

[1]Warwick HRI, University of Warwick, UK.
[2]VIB, Department of Plant Systems Biology, Ghent University, Belgium.
[3]Department on Biology, University of Antwerp, Belgium.

Although our lives are dependent on plants, our understanding of how they grow and how different levels of organisation (*i.e.* whole plant, organ, cell, molecular module and molecule) are linked is still not understood. Therefore, the AGRON-OMICS consortium is using existing and novel tools to collect data that enable us to model the growth of the *Arabidopsis* leaf under non-limiting and limiting environmental conditions (*e.g.* drought).

After initiation of the leaf primordial, biomass accumulation is controlled mainly by cell proliferation and expansion in the leaves. However, the *Arabidopsis* leaf is a complex organ made up of around 20 individual cell types and 11 structures. At the same time, the growing leaf contains cells at different stages of development with the cells furthest from the petiole being the first to stop expanding and undergo senescence. Sampling entire leaves can therefore give a distorted view of what is going on in only a subset of the cells.

To facilitate studies in single cell types or structures, or of cells at specific stages of development in the *Arabidopsis* leaf we are generating a set of fluorescently labeled *Arabidopsis* lines using the LhG4/*pOp6* trans-activation system. We are then coupling these lines with recent technical advances in analysis of specific cell types in *Arabidopsis* roots. The method we are establishing consists of protoplasting tissue, followed by fluorescence-activated cell sorting (FACS) to obtain cell fractions highly enriched for fluorescently labeled cells. One drawback of this method is the prolonged period in which protoplasting takes place (≤3h), and the transcriptional changes in the cells in response to this treatment. To examine and address this problem, we have performed a microarray experiment in which the effect of protoplasting has been examined in the absence or presence of specific inhibitors.

Author index

Participant list

Abbassi, B.H.	bhabbasi@qau.edu.pk
Aguilera-Díaz, V.	vaguilera@umh.es
Agulló-Antón, M.A.	maagullo@um.es
Akinsipe, A.	bayoakins1@yahooco.uk
Andújar-Pastor, A.	alfonsoandujar@yahoo.es
Baerenfaller, K.	bkatja@ethz.ch
Baskin, T.	baskin@bio.umass.edu
Bennett, M.	malcolm.bennett@nottingham.ac.uk
Berleth, T.	berleth@csb.utoronto.ca
Beynon, J.	jim.beynon@warwick.ac.uk
Buchanan-Wollaston, V.	vicky.b-wollaston@warwick.ac.uk
Busch, W.	wolfgang.busch@duke.edu
Byrne, M.	mary.byrne@bbsrc.ac.uk
Candela, H.	hcandela@umh.es
Casanova-Sáez, R.	rcasanova@umh.es
Chae, E.	eunyoung.chae@tuebingen.mpg.de
Consonni, C.	chcon@psb.ugent.be
Coppens, F.	frcop@psb.vib-ugent.be
del Pozo, J.C.	pozo@inia.es
Dhondt, S.	stdho@psb.vib-ugent.be
Draper, J.	jhd@aber.ac.uk
Eberius, M.F.E.	matthias.eberius@lemnatec.de
Egea-Cortines, M.	marcos.egea@upct.es
Eshed, Y.	yuval.eshed@weizmann.ac.il
Estelle, M.	mestelle@mail.ucsd.edu
Esteve-Bruna, D.	desteve@umh.es
Fabre, J.	juliette.fabre@supagro.inra.fr
Fenoll, C.	carmen.fenoll@uclm.es
Fernandes, A.N.	anwesha.fernandes@nottingham.ac.uk
Ferrández-Ayela, A.	aferrandez@umh.es
Fiorani, F.	evelyn.debue@cropdesign.com
Fuertes-Aguilar, J.	jfuertes@rjb.csic.es
Galan-Estella, F	fernando.galan@uam.es
Garcia-Lopez, C.	cgarlo@ujaen.es
González-Reig, S.	santiagogr@gmail.com
Granier, C.	granier@supagro.inra.fr
Grønlund, J.	j.t.gronlund@warwick.ac.uk
Gruissem, W.	wgruissem@ethz.ch
Guerra, J.	jgu@keygene.com
Hersch, M.	micha.hersch@unil.ch
Hilson, P.	pihil@psb.ugent.be
Hodgman, C.	Charlie.Hodgman@nottingham.ac.uk
Iturritxa, E.	eiturritxa@neiker.net
Jover-Gil, S.	sjover@umh.es
Kheibarshekan-Asl, L.	leila.kheibarshekanasl@ugent.be

Khoshakhlagh, F.	f41farzad@yahoo.com
Last, R.	roblast1@gmail.com
Lydon, S.	susie@cpib.ac.uk
Matser, V.	vam502@york.ac.uk
Meijer, J.	johan.meijer@vbsg.slu.se
Mena, M.	montana.mena@uclm.es
Micol, J.L.	jlmicol@umh.es
Miedes-Vicente, E.	eva.miedesvicente@ua.ac.be
Mollá-Morales, A.	a.molla@umh.es
Motiejunas, D.	info@cropdesign.com
Muller, B.	muller@supagro.inra.fr
Muñoz-Nortes, T.	tmunoz@umh.es
Muñoz-Viana, R.	rmunoz@umh.es
Murray, J.	murrayja1@cardiff.ac.uk
Nektarios-Markakis, M.	markmarios@gmail.com
Nelson, T.	timothy.nelson@yale.edu
Osmont, K.S.	kosmont@unil.ch
Pantin, F.	pantin@supagro.inra.fr
Pérez-Pérez, J.M.	jmperez@umh.es
Perrine-Walker, F.	perrine@supagro.inra.fr
Pitorre, D.	delphine.pitorre@univie.ac.at
Ponce, M.R.	mrponce@umh.es
Quesada, V.	vquesada@umh.es
Riaz, A.	atiff23@gmail.com
Robles, P.	probles@umh.es
Rodríguez-Cazorla, E.	e.rodriguezc@umh.es
Rubio-Díaz, S.	silvia.rubio@umh.es
Runions, A.	runionsa@cpsc.ucalgary.ca
Russinova, J.	eurus@psb.ugent.be
Sánchez-García, A.B.	ana.sanchezg@umh.es
Sarmiento-Mañús, R.	rsarmiento@umh.es
Schurr, U.	u.schurr@fz-juelich.de
Schwartz, S.	stefan.schwartz@lemnatec.com
Shafiq, M.	shafiqnibge@gmail.com
Sundaram, S.	shanthy_s@rediffmail.com
Tsiantis, M.	miltos.tsiantis@plants.ox.ac.uk
Tsukaya, H.	tsukaya@biol.s.u-tokyo.ac.jp
Umbreen, S.	sumbreen@rbge.org.uk
Vandenbroucke, K.	filipa.debastos@bayercropscience.com
Vissenberg, K.	kris.vissenberg@ua.ac.be
Walsh, S.	secretariat@bsse.ethz.ch
Wilson, M.	michael.wilson@nottingham.ac.uk
Xu, J.	dbsxj@nus.edu.sg

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes

Notes