

WORKSHOP ON GROWTH PHENOTYPING AND IMAGING IN PLANTS

Two days of conferences on Tue 17 - Wed 18 July 2007

Training activities on Thu 19 July 2007

Venue : INRA-SUPAGRO- Place Pierre Viala - Montpellier- France

General Theme : High-throughput phenotyping for complex integrated traits such as growth involves methodological and technical choices which determines eventually the quality of the phenotyping process. Many groups have developed tools, techniques and frameworks of analyses to analyse growth in a quantitative and reproducible way. The aim of these 3 days workshop is to gather together groups for sharing expertise in this domain.

Four topics will be covered during the three days with a combination of lectures, posters, visits of HTP phenotyping platforms and practical sessions the last day. The 4 topics are:

- I- Leaf Growth Phenotyping.
- II- Root Growth Phenotyping.
- III- Imaging Growth in Plants.
- IV- Plant Growth Modelling.

Organizers :

The workshop is organized by Christine Granier (INRA-LEPSE) in the framework of the AGRON-OMICS European Integrated Project (www.agron-omics.eu).

Scientific Program Committee:

Dr Christine Granier (LEPSE, Montpellier), Dr Fabio Fiorani (PSB, Gent), Dr Pierre Hilson (PSB, Gent)

Local Organizing Committee:

Dr Christine Granier (LEPSE, Montpellier), Dr Catherine Massonnet (LEPSE, Montpellier), Mrs Juliette Fabre (LEPSE, Montpellier).

The workshop is co-sponsored by :



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www.agron-omics.eu



School of Agronomy
www.supagro.fr



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Research www.inra.fr



Solutions for automation
www.optimalog.com

PROGRAM

Monday 16 th

18.00 - 19.00 Registration- Poster mounting
Hall d'Honneur- Amphi Philippe Lamour - Montpellier SUPAGRO

Tuesday 17th

8.30 - 9.00 Registration- Poster mounting
Hall d'Honneur- Amphi Philippe Lamour- Montpellier SUPAGRO

9.00 - 9.15 Welcome (Pierre Hilson, Christine Granier)

9.15 - 13.30 Morning Session : Leaf Growth Phenotyping
Chair : Fabio Fiorani (PSB, Gent)



9.15 - 9.55 : Christine Granier (INRA, Montpellier)
'Compensations between leaf growth variables mask non visible phenotypes'

9.55 - 10.35 : Hendrik Poorter (Utrecht University, Utrecht)
"Leaf growth, the third dimension "

10.35 - 10.55 : Rhonda Meyer (IBB, Postdam-Golm)
'Biomass and leaf area in the study of Heterosis in Arabidopsis thaliana'

10.55 - 11.40 : *Coffee break-Posters*

11.40 - 12.00 : Monica Kavanova (University of Munich)
'Variations on the same theme : effects of nitrogen and phosphorus deficiency on cell proliferation and cell growth in grass leaves.'

12.00 - 12.40 : Gerrit Beemster (PSB, Gent)
"Leaf phenotyping from whole rosette down to the molecular level"

12.40 -13.20 : Andrew Fleming (US, Sheffield)
"Leaf growth: All cells are equal, but are some cells more equal than others?"

Lunch : 13.30 – 14.50

14.50 - 16.30

1st Afternoon Session : Genetic and Leaf Growth Phenotyping

Chair : Christine Granier (INRA, Montpellier)

14.50 - 15.20 :

José Manuel Pérez-Pérez (UMH, Alicante)

'A reverse genetic approach to leaf development'

15.20 - 15.40

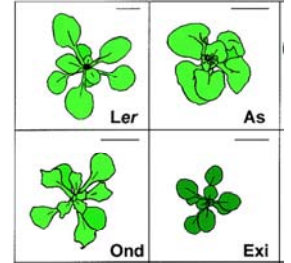
Gorou Horiguchi (University of Tokyo)

'Establishment and use of genetic resources for characterization of leaf size/shape regulation with an emphasis of compensation syndrome'

15.40 - 16.00

Bjorn Pieper (MPI, Koln)

'Genetic architecture of plant growth and related traits in Arabidopsis thaliana.'



16.10 - 18.30

2nd Afternoon Session : Root Growth Phenotyping

Chair : François Tardieu (INRA, Montpellier)

16.10 - 16.50 :

Bertrand Muller (INRA, Montpellier)

'Phenotyping root growth and architecture - Variables, interactions and model parameters'

16.50 - 17.20 :

Coffee break-Posters

17.20 - 18.00 :

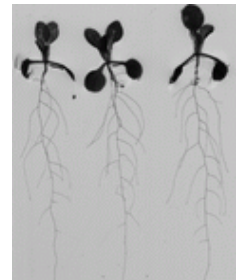
Verônica Grieneisen (Utrecht University, Utrecht)

'Control of root development and growth by polar auxin transport'

18.00 - 18.40 :

Malcolm Bennett (CPIB, Nottingham)

'Dissecting the hormonal control of cell elongation in roots'



18.40 - 20.00 :

Wine and Posters

Wednesday 18th

8.45 - 13.20 : **Morning Session : Modelling Plant Growth**
Chair : Gerrit Beemster (PSB, Gent)

9.00 - 9.40 : Jan Traas (ENS, Lyon)
"Towards a virtual flower."

9.40 - 10.20 : Enrico Coen (JIC, Norwich)
" Growth, Genetics and geometry of leaf shape "

10.20 - 11.00 : Roeland Merks (PSB, Gent)
*"Modelling formation of Auxin transport channels during leaf development :
a traveling-wave hypothesis*

11.00 - 11.40 : Coffee break - Posters

11.40 - 12.20 : François Tardieu (INRA, Montpellier)
*' The phenotyping platform Phenodyn :
model-assisted analysis of the genetic variability of tolerance to environmental stresses'*

12.20 - 13.00 : Jérémie Lecoeur (SUPAGRO, Montpellier)
'Use of biophysical plant modelling in determination of complex phenotypic traits'

13.00 – 13.20 : Christophe Reuzeau (Crop-Design, Gent)
"TRAITMILLTM: A genomic tool for modelling gene function in plant development."

Lunch : 13.20 – 14.40



14.40 - 18.00

Afternoon Session : Imaging in Plants
Chair : Bertrand Muller (INRA, Montpellier)

14.40 - 15.20: Volker Backer (MRI-CNRS, Montpellier)
'Automatic measurement of plant features using ImageJ and MRI Cell Image Analyzer'

15.20 - 16.00 : Achim Walter (ICGIII, Jülich)
"Quantitative imaging of leaf and root growth at the Jülich Plant Phenomics Center".

16.00 - 16.20 : Xavier Draye, Aurélie Babé (Université de Louvain)
'Smartroot : interactive analysis and annotation of root morphology in time series of difficult root system images'

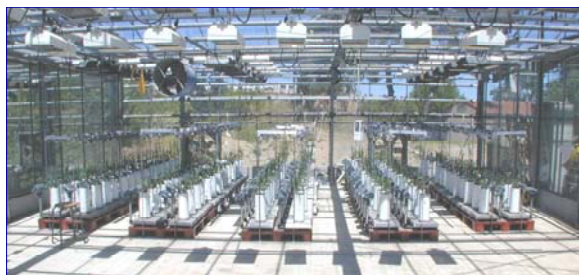
16.20 - 16.50 : Coffee break - Posters

16.50 - 17.10 : Aurélie Urbain (IJPB, Versailles)
'Chemical Imaging of growth of root using Fourier transform Infra-Red spectroscopy'

17.10 - 17.30 : Karine Lee (JIC, Norwich)
'Visualising plant growth in four dimensions using Optical Projection Tomography.'

17.30 - 18.10 : Kris Ver Donck (Maïa Sc, Geel)
'Towards High Speed Image Analysis tools for leaf rosette, leaf cells and plant cells in culture '- Demonstration of the eaZYX imaging software

18.15 – 19.15 Visits of PHENOPSIS and PHENODYN:HTP phenotyping platforms



Thursday 19th

Practical course on Leaf Growth Phenotyping

(1 computer room, 40 participants max.)

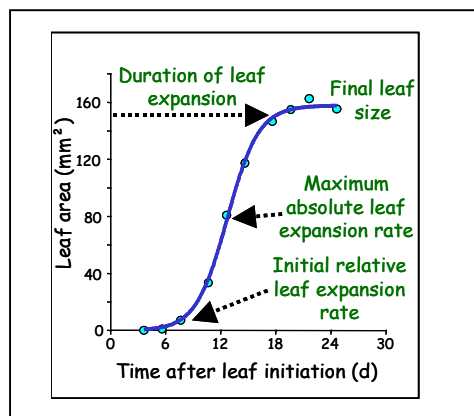
9.30 – 17.30 : Training activity on computer (software = Excel)

(with lunch break : 13:15-14:15)

Recording leaf growth phenotypes and its plasticity in response to environmental conditions from cells to whole rosette in *Arabidopsis thaliana*

(François Tardieu, Bertrand Muller, Hendrik Poorter, Christine Granier)

- Leaf production : estimations of plastochron, phyllochron
- Leaf expansion : estimation of relative growth rate, relative and absolute leaf expansion rates, duration of leaf expansion
- Cell expansion and cell division : estimation of cell division rate, cell doubling time, duration of cell cycle, cell expansion rate
- Temperature and leaf growth
- Soil water deficit and leaf growth
- Evaporative demand and leaf growth



17.30-18.00 : Concluding remarks

AGRON-OMICS European Integrated Project www.agron-omics.eu.



CONFERENCES ABSTRACTS

COMPENSATIONS BETWEEN LEAF GROWTH VARIABLES MASK NON VISIBLE PHENOTYPES.

Christine Granier

Laboratoire d'Ecophysiologie des Plantes sous Stress Environnementaux UMR INRA-SUPAGRO- Place Viala 34060 Montpellier, France

Natural occurring genetic variability in *Arabidopsis thaliana* and a collection of leaf growth mutants were used to analyse plasticity of leaf development in response to various environmental conditions. The presence of any relationships between the leaf growth variables was investigated in a range of day-lengths, incident light and soil water contents. A few genotypes did not reduce significantly their final leaf area in response to environmental stresses but, for all genotypes, environmental stresses had opposite effects on rate and duration of leaf expansion as well as on epidermal cell number and cell size. The balance between cell number and cell size in response to environmental stresses was not due to differences in endoreduplication. A strong QTL associated with this balance was identified on chromosom 2 in the *Ler* * An-1 population.

The responses of different genotypes to environmental conditions have the potential to reveal some of the components of the genetic variability associated with the adaptation of growth to environmental conditions.

LEAF GROWTH: THE THIRD DIMENSION

Hendrik Poorter

Inst. Environmental Botany, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands

Much attention in this workshop will focus on the expansion of leaf area over time, and the factors controlling this process. However, leaves also grow in the third dimension, which is easily characterized by the LMA, the leaf mass invested per unit area. In this talk I will show how important this parameter is in determining the photosynthetic capacity of the leaf, and thereby its' carbon gain under various conditions. However, I will also show that not necessarily the plants with the highest photosynthetic capacity will also show the highest growth rate. The genetic regulation of LMA in *Arabidopsis* will also be discussed.

BIOMASS AND LEAF AREA IN THE STUDY OF HETEROSIS IN *ARABIDOPSIS THALIANA*

Rhonda C. Meyer^{1,4}, Hanna Witucka-Wall¹, Hanno Scharr², Achim Walter², Michael Melzer³, Thomas Altmann^{1,4}

¹ Department of Genetics, Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam-Golm, Germany

² ICG-III, Research Centre Jülich, 52428 Jülich, Germany

³ Leibniz Institute of Plant Genetics and Crop Plant Research, 06466 Gatersleben, Germany

⁴ Max-Planck-Institute of Molecular Plant Physiology, 14476 Golm, Germany

The widespread occurrence of heterosis in the model plant *Arabidopsis th* opens the possibility to investigate the genetic basis of this phenomenon using the tools of genetical genomics. We analysed two divergent accessions, C24 and Col-0, which in previous studies exhibited heterosis and transgressive segregation for biomass. Significant differences in shoot weight between parents and hybrids can be detected as early as 6 days after sowing (6 DAS) and almost the full degree of heterosis is manifested at 10 DAS.

We carried out a detailed morphological analysis at 1, 4, 6, and 10 DAS with germinating seeds and developing seedlings of the parental genotypes and the reciprocal F1 hybrids. Organ sizes (length and area of cotyledons, length of hypocotyls), number of epidermis and mesophyll cells per area and per cotyledon, and epidermis and mesophyll cell sizes were calculated. Microscopic analyses revealed that the enhanced seedling size of the F1 hybrids versus the parents occurs through an initial increase in cell size (at 4 DAS) followed by the formation of an increased number of cells per cotyledon (at 6 DAS).

We investigated a possible relationship between leaf area or rosette diameter versus shoot weight, which is a prerequisite for non-destructive analysis of biomass heterosis. Leaf area appeared to be the better indicator for shoot weight than rosette diameter. There was significant correlation between heterosis for shoot biomass and heterosis for leaf area. This led us to develop an imaging system to monitor plant growth.

VARIATIONS ON THE SAME THEME: EFFECTS OF NITROGEN AND PHOSPHORUS DEFICIENCY ON CELL PROLIFERATION AND CELL GROWTH IN GRASS LEAVES

Monika Kavanová, Fernando A. Lattanzi, Agustín A. Grimoldi & Hans Schnyder

Chair of Grassland Science, Department of Plant Sciences, Technical University Munich Am Hochanger 1, 85350 Freising, Germany

Nitrogen and phosphorus are the two mineral nutrients required in highest quantities by plants, and thus many studies have focused on the effects of their deficiency on plant functioning. Yet, the cellular bases of the growth response to different nutrient availabilities are not resolved. Here we use a kinematic analysis – a method to transform the spatial pattern of growth variables along the leaf axis into the developmental history of an individual cell – to analyze the cellular parameters underlying the leaf growth reduction in the grass *Lolium perenne* growing under phosphorus and nitrogen deficiency. The reduction in the leaf growth rate under both nitrogen and phosphorus deficiency was due to decreases in the cell division and mitotic growth rates, which led to a reduction in the cell production rate; and postmitotic elongation rates, which led to a reduction in the final cell length. No other parameters responded to nutrient status, be it the size of meristematic cells, the number of division cycles meristematic cells went through, or the distance from leaf base where postmitotic elongation started and stopped. But, both nutrient deficiencies prolonged the total residence time of a cell in the leaf growth zone and delayed its maturation. All these effects were strikingly similar between nitrogen- and phosphorus-deficient plants, suggesting that at the cellular level, leaf growth responds equally to both major nutrients.

LEAF PHENOTYPING FROM WHOLE ROSETTE DOWN TO THE MOLECULAR LEVEL

Gerrit Beemster

Department of Plant Systems Biology, VIB, Technologiepark 927, 9052 Ghent, Belgium

The regulation of plant growth comprises a complex regulatory network the behavior of which is determined by external (environmental) variables as well as internal, genetically determined characteristics. The complexity of the system largely stems from both the huge number of (interacting) components that are involved as well as the integration across different organizational levels. One can readily distinguish the molecular, cellular, organ and whole plant level processes, which to a large extent have been studied separately. Current developments of genomic tools, microscopy and image analysis, particularly for model species like *Arabidopsis*, allow us to start analyzing the links between different regulatory levels. Indeed this aim is the basic challenge of the AGRONOMICS project.

Using an *in-vitro* system, we are developing an integrated set of approaches to accurately quantify growth. These encompass: 1. **Time-lapse imaging** to measure the growth dynamics of the rosette and to some extent individual leaves. 2. **Kinematic analysis** to measure the dynamics of cell division and cell expansion during the growth of individual leaves. A crucial part of this analysis is the accurate measurement of cell size, which is still a laborious largely manual task. However, we are working towards automation of this task. 3. **Flow cytometry** to measure nuclear DNA content distribution. 4. **Molecular profiling techniques**. 5. **Promotor activity** analysis using reporter genes.

This presentation will give an overview of our developments of these analyses, as well as some of the progress made at the integration of these data.

LEAF SHAPE: ALL CELLS ARE EQUAL, BUT ARE SOME CELLS MORE EQUAL THAN OTHERS?

Andrew Fleming

Department of Animal and Plant Sciences University of Sheffield Western Bank Sheffield S10 2TNUK

The study of the proportional growth rate differences that underpin shape change during development is termed allometry. The genetic basis underpinning allometry has long been described and progress has been made in the identification of the genetic factors influencing leaf form. However, the cellular mechanism underpinning these genetically defined allometric relationships remains very unclear. As has been pointed out, gene products cannot directly encode shape. Rather, they must encode components of a shape defining mechanism so that altered temporal or spatial activity of these components leads to a reproducible and robust output in terms of leaf form.

Classical observations invoked the presence of a marginal meristem in the leaf whose activity was predicted to control lamina growth via the extent of cell proliferation in this region. Clonal analysis in the 1980's showed that this was not the case and the concept of the marginal meristem fell into disrepute. Nevertheless, a number of data suggest that the leaf margin does somehow influence leaf development. Taking an enhancer trap strategy, we set out to identify genes specifically expressed in this tissue. Our analysis identifies brassinolide activity in the leaf margin as an input to the allometric relationship between leaf length and width. Thus, the leaf margin appears to be a key component of the leaf shape control mechanism. The mechanism by which the leaf margin influences form and the genetic control of leaf margin identify will be discussed.

A REVERSE GENETICS APPROACH TO LEAF DEVELOPMENT

Hernández-Romero, D.*, Rubio-Díaz, S.*, Pérez-Pérez, J.M.*, Geysen, J.¹, Ponce, M.R., and Micol, J.L.

* These authors contributed equally to this work

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

¹*MAIA Scientific, Ciplastraat 3, B-2440 Geel, Belgium*

Not few developmental studies based on forward genetics approaches only allowed the isolation of mutants with conspicuous and viable phenotypes. This was the case of several attempts of saturation of the *Arabidopsis* genome with mutations affecting leaf shape and size. Over 350,000 independent T-DNA insertion events have been mapped into the *Arabidopsis thaliana* genome and about 10,000 lines homozygous for single T-DNA insertions have been produced (<http://signal.salk.edu/>), which makes *Arabidopsis thaliana* a model suitable for a reverse genetics approach to leaf development. We performed a morphometric analysis of several rosette and leaf parameters in 138 lines previously identified as leaf mutants, what would allow us to establish a numeric framework for leaf phenotype comparisons and their tabulation. A similar approach will be used for the analysis of a large collection of sequence-indexed homozygous insertion mutants.

ESTABLISHMENT AND USE OF GENETIC RESOURCES FOR CHARACTERIZATION OF LEAF SIZE/SHAPE REGULATION WITH AN EMPHASIS OF COMPENSATION SYNDROME

Gorou Horiguchi¹, Ali Ferjan¹, Ushio Fujikura¹, Ken Usami², and Hirokazu Tsukaya^{1,2}

¹*The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan*

²*National Institute for Basic Biology, 38 Nishigo Naka, Myodaiji-cho, Okazaki, Aichi, 444-8585, Japan*

Size and shape of leaves are characterized by the number and size of constituent cells and by the spatial distribution of cells. We isolated and characterized a number of leaf size/shape mutants of *Arabidopsis thaliana* in relation to total cell number, average cell size, and cell numbers along the length and width of the leaf blade. Such measurements allowed us to identify several unique mutant classes. oligocellula (oli) and extra-small sisters (xs) are specifically defective in cell proliferation and cell expansion, respectively. angustifolia3 (an3) and fugu are mutants showing “compensated cell enlargement” in which a reduction of cell number is accompanied by an increase in cell size, while a group of mutants shows the phenotype opposite to the an3/fugu class. By using these and other mutants, we characterized developmental aspects of compensated cell enlargement to elucidate how cell proliferation and cell expansion are coordinated in the context of leaf development. Major conclusions obtained from these analyses are that 1) compensation is induced post-mitotically in response to a substantial decrease in cell proliferation activity, 2) only a subset of cell expansion pathways is linked to AN3-dependent cell proliferation pathway, 3) there are at least three different compensation modes and 4) endoreduplication plays only a partial role in the compensated cell enlargement. We will present plausible models that explain the occurrence compensated cell enlargement.

GENETIC ARCHITECTURE OF PLANT GROWTH AND RELATED TRAITS IN *ARABIDOPSIS THALIANA*

Pieper B, Eilts B, Koornneef M, Reymond M.

Max Planck Institute for Plant Breeding Research Dept. Plant Breeding and Genetics Carl-von-Linné-Weg-10 50829 Cologne Germany

The genetic basis of plant growth and related traits is being studied using naturally occurring variation in *Arabidopsis thaliana*. Two independent recombinant inbred line (RIL) populations derived from the crosses *Landsberg erecta* x *Shakdara* and *Landsberg erecta* x *Kashmir-2* were analyzed in a single experiment. The genetic maps of both RIL populations were anchored with a set of common markers. Plant growth was quantified by measuring the projected rosette area (PRA) at various points during rosette expansion until a maximum was reached using digital image analysis. Additionally, length, width and area of the largest leaf of every plant were measured and flowering time was also scored including the numbers of rosette leaves and cauline leaves. QTL have been detected for all traits including the relative growth rate (RGR) and rosette compactness that were calculated from the PRA. Comparison of QTL mapping results obtained from both RIL populations indicated that plant growth and related traits have a unique genetic determinism in each mapping population. On the other hand, flowering time and related traits appeared to be mostly under the control of the same loci in both populations. Selection of near isogenic lines (NILs) is in process in order to validate the major QTL for plant growth. The ultimate objective is to clone these QTL and to elucidate their molecular basis. Furthermore, various models are fitted to the dataset to better describe the genetic variability of growth.

PHENOTYPING ROOT GROWTH AND ARCHITECTURE : VARIABLES, INTERACTIONS AND MODEL PARAMETERS

Bertrand Muller

LEPSE – INRA / SUPAGRO – UMR759 – Montpellier – France

Plant roots have crucial functions in water, mineral acquisition and plant anchorage. The ecologist, the breeder or the molecular genetist want to better understand the role of root growth and architecture in the fitness of species in different habitats, in the production of a crop in the field or conversely, the role of a gene on root growth and architecture. For each of them, a key issue is thus to be able to quantitatively describe root growth and architecture in order to draw quantitative and robust relationships between these descriptors and those they address. However, this description is not straightforward for the following reasons:

- (i) Root growth and architecture are highly plastic when the plants are exposed to various environments in the root region, either globally or locally
- (ii) Root growth and architecture are the subject of ontogenic variations. They follow inherent changes that accompany plant development.
- (iii) Root growth and architecture are strongly pruned by the internal status of the plant (carbon and minerals). Any condition that modifies the availability of these inner resources will alter root growth and architecture.
- (iv) Points (i) to (iii) inevitably interact

For all these reasons and except in very simple cases, instantaneous variables (e.g. root length) will be poor descriptors of the root system. By contrast, model based phenotyping can help to take into account the environment of the root, the ontogenic variations and the internal status of the plant. Models should also be able to account for the interactions between them.

In this paper, I will give a brief survey of root growth and architecture variables, show evidences for points (i) to (iv), and finally review few simple models whose parameters can be used to phenotype root growth and architecture.

CONTROL OF ROOT DEVELOPMENT AND GROWTH BY POLAR AUXIN TRANSPORT

Verônica A. Grieneisen

Theoretical Biology/Bioinformatics, Utrecht University Padualaan 83584 CH Utrecht The Netherlands

In plant development, the phytohormone auxin plays a key-role, controlling cell identity, cell division and cell expansion. Interestingly, in both distal regions of plant roots and shoots, characteristic auxin maxima have been found which correlate with these developmental outputs. It is also known that auxin export facilitators (PINs) are associated with auxin maxima. Combining the above empirical knowledge, we present a model that spans molecular and cellular levels describing diffusion and PIN-facilitated auxin transport in and across cells within a structured tissue layout. The modelling results solve two major issues in plant development: it pinpoints the necessary elements for the formation and maintenance of an auxin-maximum, and also explains how roots change growth patterns. Thus we explain both robust auxin maximum formation and development processes that occur over the time-scales of weeks. To generalize the concepts that occur in this system, the model further permits us to connect internal PIN-topology of the root and macroscopic properties such as auxin-capacitance, auxin gradient properties and timescales. We will further discuss the gained modelling insights in light of the latest discoveries on PLETHORA genes.

DISSECTING THE HORMONAL CONTROL OF ROOT CELL ELONGATION

Susana Ubeda-Tomas, Ranjan Swarup, Malcolm Bennett

Centre for Plant Integrative Biology, University of Nottingham, UK.

Genetic and physiological studies have revealed that at least six different classes of plant hormones regulate *Arabidopsis* root growth. Identifying which root tissues perceive each phytohormone represents an essential prerequisite to understanding how these signals collectively control root growth. We recently demonstrated that auxin regulates differential root growth by inhibiting epidermal cell elongation. This unprecedented level of cellular resolution was achieved by expressing a dominant form of the auxin repressor IAA17 termed *axr3-1* in selected root tissues. We have recently employed this approach to identify which root tissue(s) perceive several other phytohormone signals (s) that control root growth. I will describe how we are using this novel information to create multiscale models of root growth in the new BBSRC Centre for Plant Integrative Biology at the University of Nottingham.

THE VIRTUAL MERISTEM: ARTEFACT OR REALITY?

J. Traas¹, S. Stoma², J. Chopard², O. Hamant¹, C. Godin²

¹*Laboratoire de Reproduction et Développement des Plantes, UMR INRA/CNRS/ENS, 46 allée d'Italie, 69364 Lyon Cedex 7, France*

²*Team Virtual Plants UMR DAP CIRAD, TA A-96/02, 34398 Montpellier Cedex 5, France*

During the last decade an impressive body of knowledge on meristem function has been generated. This concerns information on the genes involved, their expression patterns, cell differentiation, cell division patterns, etc. The complexity of these data is such, that an integrated view of our current knowledge on meristem function is no longer possible. Therefore, we are currently developing adapted mathematical and informatics approaches to integrate the knowledge and to advance the level of understanding in the field. Our efforts are oriented in two directions. First we are trying to link the gene regulatory and signalling networks to specific morphogenetic events. Although much is known on the overall composition of these networks, almost nothing is known on the way in which they interact with the structural elements of the cells and tissues to create particular shapes. It is therefore important to link specific gene activities to precisely quantified growth characteristics of the cells and tissues in which they are expressed.

In parallel to this largely descriptive effort, we are also developing modelling tools. To formulate and test hypotheses on spatial aspects such as flows of signalling molecules, strain within tissues, and the role of gene products in the spatial control of cell proliferation, we are creating a model in the form of a virtual meristem. This model will integrate as much spatial, dynamic and quantitative information as possible.

GROWTH, GENETICS AND GEOMETRY OF LEAF SHAPE

Enrico Coen

John Innes Centre, Norwich Research Park, Colney Lane, Norwich, NR4 7UH, UK

Much progress has been made recently in our understanding of how genes control patterns of cell types or regional identities within an organism during its development. However, the link between this process of patterning and growth or morphogenesis is much less well understood. Bridging this gap requires a quantitative understanding of how genes modify growth of multicellular tissues in 3D space. We have been addressing this problem using a combination of genetic, morphological, computational and imaging approaches in collaboration with Andrew Bangham (University of East Anglia) and Przemyslaw Prusinkiewicz (Calgary). The results provide new insights into how genes interact with patterns of growth at various scales to modify shape. The talk will illustrate how integrating biological and computational methods may lead to a quantitative mechanistic framework for development and evolution.

MODELING FORMATION OF AUXIN TRANSPORT CHANNELS DURING LEAF DEVELOPMENT: A TRAVELING-WAVE HYPOTHESIS

Roeland M.H. Merks, Yves Van de Peer, Dirk Inzé, and Gerrit T.S. Beemster
Department of Plant Systems Biology, VIB, Technologiepark 927, 9052 Ghent, Belgium
Molecular Genetics, Ghent University, Technologiepark 927, 9052 Ghent, Belgium

In 1969 Tsvi Sachs published his seminal hypothesis of vascular development in plants: the canalization hypothesis. A steady, uniform flux of the phytohormone auxin from the shoot to the root would canalize progressively into discrete channels, the future vascular tissue. Sachs envisaged a mechanism requiring cells to measure auxin fluxes: a positive feedback loop between the flux of auxin and the cells' auxin transport capacity would drive such auxin canalization. Recent experimental studies have confirmed the central role of polar auxin flux in plant vasculogenesis, but it is unclear if and by which mechanism plant cells could respond to auxin flux. We will argue that known auxin sensing mechanisms more likely respond to auxin concentrations than to auxin flux. We will present a computer model of an alternative mechanism for polar auxin channeling, which is more consistent with recent molecular observations. The mechanism assumes PIN1-localization and PIN1-expression depend on local measures of auxin concentration. The model generates traveling waves of polar auxin transport and PIN1 expression, producing auxin channels, and correctly reproduces the main experimental manipulations of polar auxin transport. Thus our work proposes an alternative hypothesis for the formation of polar auxin channels in plant development.

THE PHENOTYPING PLATFORM PHENODYN : MODEL-ASSISTED ANALYSIS OF THE GENETIC VARIABILITY OF TOLERANCE TO ENVIRONMENTAL STRESSES

François Tardieu
INRA, UMR 759 LEPSE, 2 place Viala, 34060 Montpellier cedex 01, France

Each genotype has its own set of functional responses (e.g. cell cycle, expansion, photosynthesis) to each environmental condition (e.g. temperature, nutrient or water availabilities). The rank of genotypes for biomass or leaf area therefore varies between experiments, depending on the precise scenario of environmental conditions. The proposed method consists in genetic analyses of responses (common for all scenarios) of studied functions to environmental conditions, in the platform Phenodyn which deals with 400 plants simultaneously over a large range of environmental conditions. Leaf growth rate and transpiration are recorded every 15 minutes, together with soil water status and micrometeorological conditions. In maize, responses of leaf growth rate to temperature, evaporative demand and water deficit were common to all experiments, and are the base of a model simulating the time courses of transpiration and leaf elongation rate¹. In this way, we identified QTLs of sensitivities to water deficit and evaporative demand in 3 mapping populations², or dissected the complex phenotypic consequences of genetic transformations of ABA biosynthesis [Parent's abstract]. The combined genetic-ecophysiological model predicted the time courses of leaf growth in genotypes known by their alleles only, and reproduced the complex pattern of responses to ABA. Interfaced with a model coordinating the developments of all leaves of a plant, it simulated plant leaf area in a network of field experiments [Chenu's abstract]. This combination of methods allows progress in the simulation of performances of virtual genotypes in changing environmental scenarios

¹ Sadok et al (2007) PCE 30, 135–146

² Welcker et al (2007) J Exp Bot, 58, 339-349

USE OF BIOPHYSICS PLANT MODELLING IN DETERMINATION OF COMPLEX PHENOTYPIC TRAITS

Jérémy Lecoœur
SUPAGRO, UMR 759 LEPSE, 2 place Viala, 34060 Montpellier cedex 01, France
No abstract

AUTOMATIC MEASUREMENT OF PLANT FEATURES USING IMAGEJ AND MRI CELL IMAGE ANALYZER

Volker Backer

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ImageJ is a public domain image analysis and processing program. A rapid image analysis application development framework called "MRI Cell Image Analyzer" has been developed on the basis of ImageJ at the microscopy and imaging facility Montpellier RIO Imaging. It adds a visual scripting interface to ImageJ's capabilities, which allows creating applications from existing operations by drag and drop. It provides support to create batch applications as well as interactive applications. This framework has been used to create different plant growth applications. Framework and applications are freely available under the GNU General Public License. One application is the automatic measurement of the total surface of the rosettes of Arabidopsis plants. The images, that are taken by an automate are of low resolution and the principal difficulty is the low contrast between the plant and the earth. In a second application high resolution images are taken manually. In this case the scale varies from image to image, since the distance of the camera can be different. This is taken into account by the semi-automatic solution. Other applications include the measurement of plant cells and, still under development, the measurement of root lengths and of local lesions on leaves. For the automatic measurement of root lengths, a direct exploratory tracing algorithm has been implemented in ImageJ.

QUANTITATIVE IMAGING OF LEAF AND ROOT GROWTH AT THE JÜLICH PLANT PHENOMICS CENTER

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At the Research Center Jülich, assemblies and procedures that will allow non-invasive characterisation of plant morphology, growth and dynamic response to the environment are currently being developed and refined. Most of these methods are based on image processing. Leaf growth of dicotyledonous plants and primary root growth have been monitored by image sequence analysis methods in our group extensively throughout the last years. It was shown in *Arabidopsis thaliana* by comparing growth of mutants and wild type how diel leaf growth cycles are affected by carbohydrate metabolism. Other patterns of diel leaf growth cycles have been analysed in the context of gene expression, shift of photosynthesis from C3 to CAM metabolism, adaptation to different ecological niches and response to alterations of ecofactors. For root growth, the reaction towards alterations of above- and below-ground abiotic factors and towards biotic stress applied to the shoot was monitored and interpreted in terms of shoot-root communication and trade-offs between investment in above- versus below-ground biomass production. More recently, an automated method was devised to screen a large number of plants for total (projected) leaf area and to calculate and interpret relative leaf growth rate of plant populations for a variety of research questions. Based on these and other non-invasive methods, the Jülich Plant Phenomics Center will become a facility enabling rapid selection of optimal germplasm for crop and bioenergy production under specific environmental conditions and that will serve as a research platform for fundamental questions of plant development.

SMARTROOT : INTERACTIVE ANALYSIS AND ANNOTATION OF ROOT MORPHOLOGY IN TIME SERIES OF DIFFICULT ROOT SYSTEM IMAGES.

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2D-imaging is currently the only technique that allows much of the morphological structure of root systems to be captured in a short period of time (seconds) and with a high spatial resolution (10-100 μ m). Unfortunately, false root branching resulting from unavoidable criss-cross between roots in the field of view of the camera often prevents the automated extraction of morphological features from 2D root images. In many cases, this issue can be solved by visually inspecting the image for interesting roots or root segments (i.e. those that are the focus of the study, or which lay in less complicated areas of the image), and proceeding with the morphological analysis of the selected objects. The SmartRoot software automatically vectorizes roots (selected by the user) using morphological constraints expressing a range of acceptable root behaviors (e.g. bending, change of diameter), which allows much of the false branchings to be detected. The resulting suite of vectors can be adjusted locally or manually redrawn (in the worst cases). Root pixels are separated from background pixel using an adaptive thresholding algorithm which improves the processing of images with non uniform background. When using time series of images, vectorized roots from different images can be viewed simultaneously (based on their user-supplied ID) even if the 2D images cannot be superposed. Annotations can be placed along roots and are shown on the other images of the series. Finally, the complete set of morphological data (length, diameter, angle, location) and annotations can be exported to any ODBC-compliant database system.

CHEMICAL IMAGING OF GROWTH OF ROOT USING FOURIER TRANSFORM INFRA-RED SPECTROSCOPY

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We have previously shown that FT-IR spectroscopy is a powerful tool to detect a wide range of cell wall modifications in various mutant backgrounds (Mouille et al, 2003). The purpose of the work presented here is to better define the cell wall modifications that occurred during cell elongation in the root. We acquired Infra-red spectra along the elongation axis of the root from the root cap to the first root hair. We then developed new statistical tools to identify zones in the root where specific changes of the spectra occurred. This approach allowed us to define specific wave-numbers diagnostic of the cellular differentiation stage in the root. A few growth conditions were tested and the various growth patterns deduced from the Infra-Red spectra will be discussed.

VISUALISING PLANT GROWTH IN FOUR DIMENSIONS USING OPTICAL PROJECTION TOMOGRAPHY.

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We have explored the use of Optical Projection Tomography (OPT) as a method for capturing 3D morphology and gene activity at a variety of developmental stages and scales from plant specimens, in collaboration with the Medical Research Council and Bioptonics. OPT can be conveniently applied to a wide variety of plant material including seedlings, leaves, flowers, roots, seeds, embryos and meristems. At the highest resolution large individual cells can be seen in the context of the surrounding plant structure. 3D domains of gene expression can be visualized using either marker genes such as β -glucuronidase, or more directly by whole-mount in situ hybridization. To interactively analyse and quantify 3D OPT data we are developing software using haptics to accurately place points on volumes in 3D space. These tools will enable us to create 3D statistical shape models to analyse phenotypic variation in leaves and flowers. For naturally semi-transparent structures, such as roots, live 3D imaging using OPT is possible. 3D gene expression patterns in living transgenic plants expressing fluorescent GFP markers can also be visualised by OPT. We are exploring the possibility of using heat shock induced GFP sectors to track leaf growth in 4D, by obtaining OPT time-course data for Arabidopsis plants growing in the OPT device. Computer vision techniques will be developed to analyse sequential time-course OPT datasets. The combination of 4D time-course data, 3D point-placing, sector tracking and modelling will allow us to understand mechanisms controlling growth and shape from earliest stages of growth to maturity.

CAPACITIES OF THE EAZYX S/W AND READER H/W FOR GROWTH ANALYSES

Kris Ver Donck

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POSTERS ABSTRACTS

Abstracts are classified in alphabetical order (1st author), **underlined names** are people present at the workshop

QTL ANALYSES OF GROWTH RELATED TRAITS UNDER POTASSIUM & PHOSPHATE STARVATION REGIMES

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The purpose of this study is to detect Quantitative Trait Loci - QTL involved in the responses of plant performance under nutrient regimes.

Growth-related traits (seed size, germination, rosette expansion rate, shoot and root weight, root length) were studied during vegetative phase. Natural variation of growth was analyzed using a set of 145 Recombinant Inbred Lines (RIL) of *Arabidopsis thaliana* derived from *Landsberg erecta* (Ler, Poland) and Kashmir (Kas-2, Kashmir) ecotypes. This RIL population was grown under 3 different regimes: Control ("C"), Phosphate starvation ("-P": -4.7 Fold) and Potassium starvation ("-K": - 3.4 Fold). In order to detect QTL, we used a mixed-model. This analysis took into account the variance between replicates (Blocks) and allowed us to analyze jointly the different traits and treatments. For each of the growth-related traits, common QTL were detected whatever the level of nutrients. These QTL have been considered as "constitutive" QTL for this trait. Another set of QTL were specific to phosphate and potassium starvation and have been considered as "starvations" QTL. Finally, QTL were detected specifically to a particular nutrient level and been considered as adaptive QTL. We detected also some QTL common whatever the analyzed trait and the starvation and these QTL have been considered as "master" QTL.

The results obtained describe the genetic architecture of growth in response to different nutrient levels. Molecular characterization of the major QTL obtained, will allow us to better understanding GxG interactions in plant performance.

NON DESTRUCTIVE NODULATED ROOT PHENOTYPING OF PEA RECOMBINANT INBRED LINES

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Root compartment is responsible for nitrogen acquisition, which is a combination of symbiotic fixation and mineral assimilation in legumes. A quantitative trait loci approach was initiated in order to identify major loci controlling nitrogen nutrition in pea. 117 different recombinant inbred lines were experimented in a controlled environment in pouches inoculated by *Rhizobium* and filled with a low mineral nitrate content solution. Twice a week from germination until the beginning of flowering, nodes, roots and nodules were counted. Roots were also drawn in different colors according to their order, and red indicated nodules. At the beginning of flowering, plants were taken out from their pouches, and both shoot and nodulated roots carefully spread out in two different transparent films and scanned. Shoot, root and nodule dry weights were also measured and shoot N content will be determined. There were significant differences in shoot, root and nodule biomasses and numbers among pea lines at the beginning of flowering, in agreement with a previous controlled pot experiment performed on the same lines. The total leaf area and the area of different green colors in leaves were calculated using image analyses of spread shoots and related to different shoot chlorophyll contents. The sequential development of roots and nodules will be also determined using WinRhizo analyses of both the nodulated root draws at the successive stages and the spread nodulated root images at the beginning of flowering. All these measurements will be used to identify relevant QTLs of nitrogen nutrition in pea.

IDENTIFICATION AND VALIDATION OF A QTL CONTROLLING ROOT – SHOOT PARTITIONING USING RECOMBINANT INBRED LINES, NEAR ISOGENIC LINES AND A SET OF MODEL PARAMETERS IN *ARABIDOPSIS THALIANA*

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Leaf and root growth are intimately related. Among others, an obvious trophic link exists between these two compartments with roots supplied with photosynthates produced by the leaves and leaves supplied with water and minerals by the roots. However, in genetic studies of plant growth, shoot and root are most often considered separately. This study was performed to identify common and specific genetic basis of shoot and root growth and to test the relevance of a set of model parameters related to biomass allocation between both sides of the plant.

A RIL population of *Arabidopsis thaliana* (Bay-0 x Shahdara, 162 individuals) was grown in hydroponics under standard conditions and harvested at two dates (20 and 24 days after sowing). Several size-related variables were measured (root, blade and petioles dry weight, blade area, primary and lateral root length, branching density...). Moreover, the two harvests were used to estimate relative growth rates of both shoot and root compartments. Finally, simple models of plant growth and biomass allocation were used to derive parameters related to root-to-shoot biomass partitioning. Most variables and parameters showed medium to high heritabilities (0.5 to 0.7). Genetic models could explain 20 to 50% of the total variance with model parameters having highest rp^2 values. All root-to-shoot parameters identified at least two consensus regions at chromosomes I and III governing biomass allocation between both sides of the plant. None of these regions contained QTL related to gross plant size. Near isogenic lines derived from RILs heterozygous at target markers successfully confirmed the presence of at least one of these QTLs. These data suggest that (i) genetic variability of shoot growth is likely to hide most of that of root growth; (ii) simple models can be used to identify QTLs of parameters related to the partitioning of biomass between both sides of the plant. Future studies will question the functional basis behind these root-to-shoot QTLs and their fate when plants are prone to a range of environmental stresses.

MODELLING N NUTRITION IMPACT ON ROOT ARCHITECTURE OF *ARABIDOPSIS THALIANA*.

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The aim of this work was to estimate in what extent homogeneous limiting N supply affects root architecture via the decrease in carbon availability, due to the reduction of leaf area. *A. thaliana* plants (WS) were grown in rhizotrons at two levels of N supply and on a combination of CO₂ or radiation levels.

An object oriented model was implemented for root system architecture and was designed to account for the effect of C availability. The model compares supply and demand for C within the root system described as a network of individual roots. "C supply" was defined from the flux of accumulated root dry weight, which was calculated from data as an input variable. The "C demand" was calculated for each root according to its diameter and summed for the whole root system. If C availability was higher than the sum of total demands, all apices grew at their potential. If not, each apex was supplied proportionally to the ratio diameter/(sum of all diameters). Its elongation was then calculated from its diameter, via a close relationship found between the both variables.

Only 12 parameters were needed to simulate contrasted responses of root system architecture under our culture conditions. Root length and branching were well predicted. Time of emergence of lateral roots showed the expected distribution. Even if our model does not simulate local effect of nitrogen availability and presents limited conditions of validity, this approach showed that the major effect of global N availability on root system architecture was mediated by modifications of C fluxes.

TRANSLATING SHORT-TERM ENVIRONMENTAL EFFECTS ON LEAF GROWTH RATE INTO DIFFERENCES IN WHOLE-PLANT PROFILES OF MAIZE LEAF AREA: IMPACTS OF TEMPERATURE, EVAPORATIVE DEMAND AND SOIL WATER STATUS

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Leaf growth is one of the first processes affected by changes in environmental conditions. Physiological studies often concentrate on short-term mechanisms, thus increasing the gap with whole-plant models designed to predict biomass accumulation, transpiration and yield at plant-cycle scale in field conditions. To bridge this gap, we have developed a model in maize, combining (i) an existing model predicting effects of QTLs on leaf expansion rate in short-term response to environmental conditions and (ii) a model coordinating the developments of the different leaves of a plant. The latter was based on three field experiments where all leaves were measured every second day from their initiation. The resulting whole-plant model was interfaced with the model APSIM for simulation at canopy level.

Twelve field situations in North and South France with contrasting temperatures, evaporative demands and soil water status were used to test the model. High evaporative demand reduced leaf area at a whole-plant level, consistent with the model. Short water deficits affected only leaves developing during the stress, either visible or still hidden in the whorl, independently of other leaves. The model adequately simulated whole-plant profiles of leaf area with a single set of parameters which applied to a genotype in all experiments. The model was also tested for predictions of biomass accumulation and yield in two field experiments in Australia.

This model extends to the real world physiological knowledge of the control of leaf elongation, and can help to determine how the genetic controls of these responses affect yield in a large range of climatic scenarios.

UNRAVELING THE INTERPLAY BETWEEN VASCULARIZATION AND LEAF GROWTH

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Formation of vascular tissue is crucial for leaf growth and morphology. The presence of veins stimulates local growth, because they deliver growth factors to nearby cells. This local growth drives leaf growth and morphology. On the other hand, there has to be some growth before vascular tissue can develop. This suggests a tight link between vascularization and growth, reflected by the growth disturbance of many vascular mutants. The leaf area of most mutants with an impaired vascular network is reduced and some mutants have altered leaf morphology. Is vascular tissue formed if there is enough space available? Is vascular tissue needed to induce further growth? Or, is there really a feedback loop between growth and vascularization? To answer these questions we use image analysis algorithms to measure vascular parameters and leaf area during leaf development of vascular mutants. Ultimately, changes in leaf growth and morphology will come down to changes in cell cycle activity. We selected several core cell cycle genes which show an expression pattern related to the vascular system. The cell cycle inhibitor KRP2 is one of them. Overexpression of KRP2 gives serrated leaves and shows an underdeveloped vascular network. This again confirms the link between vascularization and leaf growth. In the end we want to implement the flow of auxin, the formation of vascular tissue and its effect on cell division in a mathematical computer model. This will allow us to simulate hypotheses and to visualize the effect of cellular changes on tissue level and its influence on growth.

IDENTIFICATION OF MECHANISMS INVOLVED IN NITROGEN DEFICIENCY TOLERANCE OF WHEAT USING A SIMPLIFIED MODEL OF C/N RELATIONS AND ROOT ARCHITECTURE

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The breeding of wheat varieties adapted to low input management systems, and especially to low N supply, is a new goal for plant breeders. Therefore, we aimed at identifying plant functioning processes involved in plant adaptation to N deficiency. Our objective was to identify the key determinants involved in nitrogen use efficiency (NUE) which are related to root architecture, and finally to use this characterization as a screening tool for genetics studies. We conducted root profiles in the field under limiting and non-limiting nitrogen conditions for two wheat varieties, chosen for their contrasted NUE. On the same two varieties, we also characterized under controlled conditions (rhizotron) root and shoot biomasses, N absorption, total root length and root architecture. The results were analysed using plant functioning model(s), as a screening tool, to identify the parameters that differed between genotypes. We first used a simplified conceptual model, and we aimed at developing a numerical model. Once the key parameters were identified, we focussed on two points: 1) what is the genetic diversity available for studying those parameters, and 2) what are the genetic determinants of those parameters. For this, we used a set of 11 wheat varieties and a set of 120 doubled haploid lines obtained from the cross between the two contrasted varieties previously studied. The study of the 11 varieties allowed the validation of the model for a larger range of genetic diversity, and the identification of the polymorphism prone to help in further breeding programs. The doubled haploid lines allowed us to identify QTLs for the model parameters.

USING TOOLS FROM MOLECULAR BIOLOGY TO TEST A BIOPHYSICAL MODEL FOR LEAF GROWTH RESPONSES TO WATER DEFICIT

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The discovery of aquaporins as membrane channels facilitating water transfer and recent insights into their gating properties have opened new avenues for elucidating water transport mechanisms at a cellular level.

Here we show that the manipulation of plant water transport by aquaporin gating is also a powerful tool to understand how plants adapt to challenging environmental conditions. Plant response to atmospheric and soil water deficits in particular is characterised by rapid changes in stomatal conductance and leaf growth. These responses are mediated by a combination of hydraulic and chemical messages that can be uncoupled by manipulating the plant hydraulic balance. Using this approach we can characterise the hydraulic signalling cascade.

We show that manipulating aquaporin activity in maize roots completely modifies the whole plant hydraulic architecture inducing large impacts on integrated processes. We induced aquaporin gating by acid load, H₂O₂ and anoxia under various atmospheric water deficits. Under these conditions, aquaporin gating reduced both root and leaf water potential, cell turgor in the leaf elongation zone and leaf growth. However, these effects could only be observed when the plant transpired substantially revealing an interaction between water supply and evaporative demand. We demonstrate that cell turgor in the growing cells of the leaf is at the cross-talk of this interaction.

The integration of molecular biology with whole plant physiology reveals a hydraulic signalling pathway which largely mediates growth adaptation. We will show that this pathway validated under artificial manipulation of aquaporins is preserved under fluctuating environmental conditions

AN INFORMATION SYSTEM FOR THE PHENOTYPING PLATFORM PHENOPSIS.
Realization of a database for the PHENOPSIS system.

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An automated system, PHENOPSIS, was designed to grow more than 500 *Arabidopsis thaliana* plants in pots in highly homogenous and reproducible micro-meteorological conditions with automatic control of soil water content and automatic acquisition of individual plant pictures.

This system produces a large amount of data : more than 500 pictures per day, 5 meteorological variables recorded every 15 minutes, and a large collection of qualitative and quantitative variables describing plant growth. This high production of data, which will be largely increased by the installation of two new automaton at the end of 2007, needs a tool to control data and users.

Therefore we are carrying out an entire information system consisting in a database built on MySQL, and a Web interface linking users, data, and applications on database like statistics programs with R software.

The information system scheme, the logical data model for the database and variables collected in the database are presented here.

MODELLING THE ARCHITECTURAL GROWTH AND DEVELOPMENT OF ROSEBUSH USING L-SYSTEMS

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The study of the rosebush architecture is important for understanding the organs layout involved in the beauty of plants which is essential for their commercial value.

We developed a virtual plant model of *Rosa x hybrida* 'Knock out' that simulates in 3-dimensions plant growth and development in a non-stressed environment. The model is based on the Lindenmayer systems (L-systems) and the C language interfaced by the Lstudio software, it uses basic production rules deduced from both literature and plant observations. Morphometric measurements (e.g. diameter and length of the internodes), architectural sequences (e.g. foliar sequence) or physiological data (e.g. plastochron, growth rate) were analysed and integrated in the model according to thermal time (see poster Guéritaine et al.). Data analyses have pointed out inter-relationship parameters and functional rules. An allometric relationship between the final length and the relative growth rate was presented for the internodes and a mean normalised curve of the number of leaflets in function of the relative axis rank was introduced in the model to describe the foliar sequence along the axes.

The model will be used as a tool to test the effects of exogenous factors on plant architecture and to investigate the parameters which describe modifications of growth and development in relation with gene expression. This joint approach with ecophysiologists and geneticists will enable taking into account environmental and genomic effects on the plant architecture.

ROBUST PROBABILISTIC METHODS TO TRACE AND LABEL A GROWING ROOT

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We present a method to semi-automatically trace and label a root imaged on a digital camera. The user selects the start point, and the software traces the line of the root down to the tip. The algorithm is able to label sections of the root trace that differ from the normal growing angle, such as is caused by root gravitropism. The system uses a particle filter tracker, normally applied to tracking targets moving through time, here novelly implemented in a system for tracing a root in a single image. The algorithm builds a probability field estimating where the root lies, and then traverses the resulting graph using heuristics to produce a final description of the root trace. This combination of methods allows for a robust approach to root tracing, especially in the presence of lighting changes, image noise and distracting clutter.

The technique is significantly faster, easier and less laborious than comparable manual measurement methods. An objective measure of distance in image pixels is provided along the length of the trace. With camera calibration, this could be used as an accurate measure of root length in real-world units, and provide an estimate of the point of onset of gravitropism.

SPATIAL AND TEMPORAL ANALYSIS OF CELL DIVISION AND TISSUE ELONGATION IN SILKS OF MAIZE PLANTS SUBJECTED TO SOIL WATER DEFICIT

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Maize styles, or silks, have to considerably elongate to emerge from the surrounding husks to be exposed to pollination, and their growth rate is particularly sensitive to drought. A detailed spatial analysis of silk growth was carried out using the temperate line F252 subjected to different levels of soil water potential.

Although silks share common morphological features with roots and monocot leaves (longitudinal growth, cell files), the spatial and temporal distribution of their growth was close to that of dicot leaves. During a first phase, cell division and elongation occurred together all along the silk. Then, cell division progressively ceased from apex to base, while relative elongation rate remained constant at any spatial position. As silk emerged out of husks, elongation ceased in the emerged portion, while elongation rate progressively decreased in the part enclosed in the husks.

Decreasing soil water potential did not change this succession of events but modified growth rates and durations. The lower the water potential, the slower the relative rates of tissue elongation and cell division, resulting in delayed silk emergence. Nevertheless the duration of cell division was not affected by water stress. In all cases, the end of cell division in the silk coincided with anther dehiscence in the terminal inflorescence. As a consequence, the anthesis-silking interval, largely used by breeders to characterise the response of cultivars to stress, corresponded to a precise developmental phase of the silk : between end of cell division and arrest of cell growth in silk apex.

GROWTH AND ARCHITECTURAL DEVELOPMENT OF THE ROSEBUSH ACCORDING TO THERMAL TIME

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The architecture of ornamental plants, such as the rosebush (*Rosa x hybrida*) determines their shape and aesthetic quality, thus their commercial value.

The team is developing a 3D dynamic model that simulates the development of rosebush plant in a non-stressed environment. This work is organised in two complementary parts (1) experimental data acquisition to define plant growth and development at the organ scale according to thermal time (2) model implementation on the basis of the system of Lindenmayer (see poster Favre et al.).

Two greenhouse experiments, studying the growth and architectural development of plants, are in progress. Data are recorded using (a) plant photography and their analyses with MATLAB software, and (b) direct observations, collecting morphometric measurements (e.g. leaf length, internode diameter), architectural sequences (e.g. leaf pattern, gradient of bud break) and physiological stages (e.g. visible floral buds).

These data are used to establish growth curves of individual internodes and leaves lengths according to thermal time, to determine allometric relationships, to estimate the co-ordinations between vegetative and floral stages, and to monitor the evolution of architecture (e.g. the secondary axis layout) according to thermal time. Some of these relationships are presented. These analyses will enable to identify the parameters required to characterise the development of rosebush, parameters which will be incorporated into the 3D dynamic model.

TOWARDS SYSTEMS BIOLOGY IN ARABIDOPSIS – ATIDB AND ARABIDOPSIS REACTOME

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Capturing biological data in a computer readable format is the foundation for Systems Biology enabling analysis of high-throughput functional data, investigation of networks and pathways and generation of biological models. We currently develop two systems: the Arabidopsis Reactome (AtReactome) and the Arabidopsis thaliana Integration Database (ATIDB). The AtReactome is a curated, web-accessible knowledgebase designed to describe biological pathways ranging from the basic processes of metabolism to complex regulatory and signalling pathways. Within AtReactome, gene products are described in terms of the reactions in which they participate and these reactions are hierarchically grouped into pathways. To assist curation and enable utilization of the SkyPainter, we have automatically imported Arabidopsis pathways from AraCyc and KEGG, whilst we undergo the much slower and more precise process of manually curating pathway modules in collaboration with scientific experts. We have completed modules involved in carbohydrate metabolism and the cell cycle. We are currently developing modules relating to leaf growth and development and we would welcome input from scientific experts in this area. AtReactome data is available in a variety of standard formats, including BioPAX and SBML. In the future the pathways will be projected to other plants using orthology mapping with OrthoMCL and the data will be accessible using the MART web service. The proteins captured in AtReactome are linked to ATIDB which maps various datasets such as microarray probes and transposons onto the latest gene model sequence data. Both AtReactome and ATIDB are open source, open access projects.

ROOT GROWTH DYNAMICS OF *NICOTIANA ATTENUATA* SEEDLINGS ARE DIFFERENTLY AFFECTED BY MECHANICAL LEAF-WOUNDING OR ORAL SECRETIONS OF *MANDUCA SEXTA*

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Many studies demonstrate resource based trade-offs between growth and defence on a large time scale. Yet, the short-term dynamics of this growth reaction are still completely unclear, making it difficult to explain growth-defence trade-offs mechanistically. In this study, image-based non-destructive methods were used to quantify root growth reactions happening within hours following simulated herbivore attack. The induction of wound reactions in *Nicotiana attenuata* in the seedling stage led to transiently decreased root growth rates. Application of the oral secrete of the specialist herbivore *Manduca sexta* to the leaves led to a transient decrease in root growth that was more pronounced than if a mere mechanical wounding was imposed. Root growth reductions were more pronounced than leaf growth reactions. Root growth reduction occurred in the same intensity, when fatty acid-amino acid conjugates were applied to wounds. Timing of the transient growth reduction coincided with bursts of jasmonate (JA) and ethylene emissions reported in literature. Simulation of a wound response by applying methyl-jasmonate (MeJA) led to more prolonged negative effects on root growth. Increased nicotine concentrations, trichome lengths and densities were observed within 72 h in seedlings which were treated with MeJA or which were mechanically wounded. Overall, these reactions indicate that even in a very early developmental stage, the diversion of plant metabolism from primary (growth-sustaining) to secondary (defence-related) metabolism can cause profound alterations of plant growth performance.

TOWARD A MECHANISTIC MODEL OF LATERAL ROOT INITIATION IN *ARABIDOPSIS THALIANA*

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Lateral root formation is an important determinant of the root system shape and of its ability to explore and exploit the soil, a heterogeneous and changing environment. It takes place according to an acropetal gradient with the first step of lateral root development, lateral root initiation, occurring close to the root apex. Lateral root development is an auxin-dependent, two-step process, combining initiation and emergence. Little is known about the mechanisms regulating the succession of lateral root initiation, and unlike the regular arrangement of leaves in aerial parts, emerged lateral roots do not show strong macroscopic structure along the primary root.

Interestingly, recent recent data suggest a link between lateral root initiation and another process dependent on auxin fluxes in the root apex: gravitropism.

We found that gravistimulation and lateral root initiation are co-regulated in *Arabidopsis thaliana*, through the mediation of auxin fluxes. We used this property to alter and analyse the regulatory system that controls the patterning of lateral root initiation. From our experimental data, we built an auxin-based model of lateral root initiation during the primary root growth. We designed new

experiments to validate the model, and showed that the effects of gravistimulations on lateral root initiation density can accurately be predicted.

This modelling approach suggests that lateral root initiation is controlled by “inhibitory fields”, highlighting an unexpected analogy with the main models of phyllotaxis for the shoot apical meristem based on similar inhibitory fields assumption.

SHORT-TERM ADAPTATION OF LEAF ELONGATION IN WHEAT PLANTS IN RESPONSE TO WATER STRESS IMPOSED BY PEG.

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In order to investigate the mechanisms of the short-term responses of leaf elongation to water stress, wheat plants (2 weeks old) were exposed to water stress by adding PEG 6000 to the hydroponics solution .

The water deficit imposed resulted in a rapid cessation of elongation rate measured on the 3rd growing leaf , as a result of turgor decrease. For this period, the stomatal conductance was maintained temporarily before going down, whereas water potential tended to decline .

After approximately 30 min, the osmotic potential of the leaves started to decrease, while turgor recovered, and returned to a higher level than before the stress. At the same time, the leaf elongation rate partially recovered. The recovery of turgor was quite associated with the osmotic adjustment.

CONTRIBUTION OF A STRUCTURE-FUNCTION MODELLING APPROACH IN UNDERSTANDING BRANCH PHYSIOLOGICAL DIFFERENCES IN TWO APPLE CULTIVARS.

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The vegetative and reproductive growth of fruit trees depend on assimilate production which is controlled by tree canopy structure and leaf functions, both modulated by their environmental responses. This study aimed at unraveling the relative contribution of leaf spatial organization and functions on light interception, transpiration and photosynthetic capacities of branching systems through a 3D virtual plant approach.

Two apple (*Malus x domestica* Borkh.) cultivars with contrasted architectures, ‘Fuji’ and ‘Braeburn’ were compared. Three eight-year-old trees per cultivar were digitized to obtain 3D representations of foliage geometry and compare their structural organization. The branch physiological capacities were estimated and compared using a functional-structural plant model (FSPM). The RATP (Radiation Absorption, Transpiration and Photosynthesis) model was used to evaluate the impact of virtual switches of leaf organization and/or leaf functions on light interception, transpiration and photosynthetic capacities of branches in both cultivars.

‘Fuji’ trees presented a higher proportion of leaf area borne on long shoots, less leaves per shoot length unit, and a larger individual leaf area than ‘Braeburn’ trees. This resulted in a lower leaf area density (LAD) and consequently in higher light interception in ‘Fuji’ than in ‘Braeburn’ branches. Transpiration and photosynthesis were also higher in ‘Fuji’ branches than in ‘Braeburn’. The analysis of virtual scenarios revealed that leaf organization and functions reduced similarly the transpiration rates in Braeburn branches while leaf organization prevailed in lowering its photosynthetic rates compared to Fuji. This study demonstrated the efficiency of FSPM to disentangle physiological differences between cultivars through in silico scenarios.

IS IT POSSIBLE TO GROW PLANTS IN A REPRODUCIBLE WAY IN 10 DIFFERENT LABS WITH THE SAME PROTOCOL? THE AGRON-OMICS PILOT LEAF GROWTH PHENOTYPING EXPERIMENT

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The key objective of the AGRON-OMICS project is the understanding of the molecular pathways involved in the regulation of Arabidopsis leaf growth but leaf growth variables are under numerous environmental and genetic influences. To combine results obtained by the different partners of the AGRON-OMICS project, it was important, as a first step, to determine if the partners could obtain comparable leaf growth phenotypes when identical genotypes were grown under (as far as possible) the same environmental conditions. This was the aim of the Pilot Leaf Growth Phenotyping Experiment presented here which assessed the biological and inter-laboratory variabilities of leaf growth. Ten plants of 3 genotypes (Col, Ler and Ws) were grown in labs of 10 participating partners using a common protocol, common soil, common nutrient solution, common pots, common batch of seeds and identical micro-meteorological conditions. Rosette leaf growth was assessed by identical methods from whole rosette leaf area to epidermal cellular patterns in leaves of plants grown by the 10 participants. The results of this experiment will be developed on the poster.

THE ARABIDOPSIS TOR KINASE LINKS PLANT GROWTH, YIELD, STRESS RESISTANCE AND MRNA TRANSLATION

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The target of rapamycin (TOR) protein is an ancient and conserved kinase that has emerged as a central controller of mRNA translation, metabolism and cell growth in response to environmental cues. Plants, unlike animals, have a plastic and undetermined organ growth which is largely dependent on environmental informations. However, little is known so far on how these informations are perceived and transduced into coherent growth and developmental decisions.

The TOR kinase is probably present in all eukaryotic organisms and regulates a wealth of biological processes, including transcription of ribosomal components and translation of mRNAs, collectively contributing to cell growth. The Arabidopsis genome contains a single Tor gene (AtTor) but, in contrast to other organisms, plants appear to be resistant to rapamycin.

Plants that overexpress the AtTor mRNA accumulate more leaf and root biomass, produce more seeds and are more resistant to osmotic stress. Conversely, the down-regulation of AtTor by constitutive or inducible RNAi leads to a reduced organ growth, to an early senescence and to a post-germinative halt in development which is more severe in osmotic stress conditions. The size of leaves and epidermal

cells is directly correlated to the level of expression of AtTor. Moreover the number of leaves is also affected by changes in AtTor expression in certain growth conditions.

Thus, we propose that the AtTor kinase is one of the main contributor to the link between environmental cues and organ growth in plants by regulating several biological processes including mRNA translation.

THE ARABIDOPSIS NRT1.1 TRANSPORTER ACTS AS A NITRATE SENSOR AND IS CRUCIAL FOR NITRATE SIGNALLING GOVERNING ROOT COLONIZATION OF NITRATE-RICH PATCHES

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Nitrate is both the main nitrogen source for nutrition of higher plants, and a signal molecule regulating their metabolism and development. The roots sense the nitrate concentration in the soil solution, and trigger signalling pathways allowing plant adaptation to changes in the external availability of this key nutrient. Localized proliferation of lateral roots in NO₃--rich patches is a striking example of the nutrient-induced plasticity of root development. In Arabidopsis, NO₃- stimulation of lateral root elongation is apparently under the control of a NO₃- signaling pathway involving the ANR1 transcription factor that is thought to transduce the NO₃- signal internally, but the upstream NO₃-sensing system is unknown. We show that mutants of the NRT1.1 nitrate transporter display a strongly decreased root colonization of NO₃--rich patches, resulting from reduced lateral root elongation. This phenotype is not due to lower specific NO₃- uptake activity in the mutants and is not suppressed when the NO₃--rich patch is supplemented with an alternative N source. These results show that NRT1.1 promotes localized root proliferation independently of any nutritional effect, and indicate a role in the ANR1-dependent NO₃- signaling pathway. We concluded that NRT1.1, which is localized at the forefront of soil exploration by the roots, is a key component of the NO₃--sensing system that enables the plant to detect and exploit NO₃--rich soil patches. However, the sensing mechanism is unknown and several hypothesis will be discussed.

ANALYSING THE DYNAMICS OF ROOT GROWTH IN INCREASING LIGHT INTENSITY AT THE SHOOT LEVEL BY USING DIGITAL IMAGE SEQUENCE ANALYSIS

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Root growth is a complex process that is characterised by specific spatio-temporal patterns. To analyse short-term adaptations of roots to variations of light intensity, growth was analysed by using digital image sequence processing methods. Therefore images of root tips (growing in agar) were acquired with standard CCD cameras. Three moving stages which were controlled by an image based tracking algorithm allowed to maintain the root tip in the field of view. The acquired image sequences were used to calculate the velocity of the root tip and the distribution of relative elemental growth rate (REGR) along the root growth zone. This calculation was done automatically by a set of image sequence processing algorithms and allowed to detect modifications of the root growth parameters with a high spatial and temporal resolution.

In this study, the hypothesis was tested, whether a sudden increase in light intensity at the shoot level leads to an immediate increase of root growth. Seedlings of *Nicotiana tabacum* were subjected to light intensities of 60 and 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Seedling biomass, sucrose concentration as well as primary root growth increased significantly with light intensity. In transition experiments from low to high light intensities, root growth increased within four days, reaching the steady-state level measured in plants that were cultivated in high-light conditions (300 $\mu\text{mol m}^{-2} \text{s}^{-1}$). During the first three hours

after light increase, strong growth fluctuations were repeatedly observed and these patterns are discussed in the context of hydraulic and photosynthetic acclimation to the altered conditions.

ELONGATOR IN PLANTS: A NOVEL VIEW ON ORGAN GROWTH

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Growth, although well-defined as the interplay between cell division, cell expansion and differentiation, remains enigmatic since the exact mechanisms driving these processes are not yet fully elucidated. The focus of our research unit is to unravel genetic mechanisms underlying growth, using the *Arabidopsis* leaf as a model system. The presented work shows how this approach has led to the identification of the Elongator complex in plants.

Elongator is a histone acetyl transferase (HAT) complex, consisting of six subunits, that co-purifies with the elongating RNAP II in yeast and humans. Yeast Elongator mutants are retarded in growth and in humans, a mutation in one of the components is associated with familial dysautonomia. We demonstrated that point mutations in three *Arabidopsis thaliana* genes, encoding homologs of the yeast Elongator subunits ELP1, ELP3 (HAT), and ELP4, were responsible for the phenotypes of the *elongata2* (*elo2*), *elo3*, and *elo1* mutants, respectively. The *elo* mutants were characterized by narrow leaves and reduced root growth that resulted from a decreased cell division rate, showing that histone acetylation in plants is an important regulatory mechanism for organ growth.

Furthermore, the plant Elongator complex was genetically positioned in the process of RNAP II-mediated transcription downstream of Mediator. The purification of the *Arabidopsis* Elongator complex using the Tandem Affinity Purification (TAP) technology confirmed structural conservation of the complex in plants, positioned the complex in the process of transcription elongation and revealed putative novel regulatory proteins/pathways.

USING GREENLAB MODEL TO STUDY THE INFLUENCE OF INTRA-SHOOT TROPHIC COMPETITION ON SHOOT DEVELOPMENT OF TWO GRAPEVINE CULTIVARS (*VITIS VINIFERA* L.)

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The grapevine shoot displays a high phenotypic plasticity in development, especially when considering the branches. This plasticity was analysed by assuming that the local trophic competition between organs plays a major role on shoot organogenesis.

Experiments were carried out using two cultivars ('Grenache N.', 'Syrah') in well-watered conditions. Various levels of trophic competition were obtained by imposing a large range of cluster load levels per plant. The biomass flux between organs were estimated by using a functional-structural model describing the plant as a set of organs with topological connections (GREENLAB).

Shoot organogenesis displayed large variations according to the levels of fruit loads. These variations which were not uniform along the shoot were mainly due to decreases of duration of development, and of leaf appearance rate of the branches. Two spatial gradients were identified. The first one was related to the type of branches. The grapevine shoot was made of three types of branches (P0, P1, P2). The P1 and P2 branches were the most affected. The second gradient was related to the topological distance of the branches to the clusters, which were the main sinks inside the shoot. GREENLAB model was able

to capture the biomass flux over time. Sink-source ratios (Q/D), simulated with GREENLAB model showed a correlation between branch development and the levels of carbon supply.

These preliminary results indicated that the potential shoot development is related to the thermal time experienced by the plant and the achievement of this potential depends on the level of trophic competition mainly driven by the cluster demand.

WHICH ROLE FOR ABA IN THE CONTROL OF LEAF GROWTH ? ANALYSIS OF MAIZE LINES TRANSFORMED ON THE NCED GENE IN CONTRASTING EVAPORATIVE DEMAND AND SOIL WATER STATUS

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The controlling effect of the plant hormone abscisic acid (ABA) on the leaf growth of water stressed plants is controversial in the literature, with positive, negative or no effect depending on authors. The working hypothesis was that ABA has several opposite physiological effects in case of water deficit, so the overall whole-plant response to ABA varies with experimental conditions. We tested this hypothesis with a series of transformed maize lines affected on the NCED/VP14 gene which codes for a key enzyme of ABA synthesis. Three antisense and two sense lines were analysed with their respective WT on the phenodyn platform. They had contrasting ABA concentrations in the xylem sap, which translated into contrasting stomatal conductances. (i) During nights or days with low evaporative demand, sense lines that overproduced ABA had a lower leaf elongation rate (LER) and cell production rate than their WT, with an opposite behaviour in antisense lines. (ii) During days with high evaporative demand, sense lines had a higher LER than their WT, linked to a lower transpiration rate and a more favourable water status. Anti-sense lines had the opposite behaviour. (iii) During a rewatering or during evenings, leaf rehydration was faster in sense line and slower in antisense lines, with intermediate values in wild types, suggesting a positive effect of ABA on root hydraulic conductivity. These results suggest the existence of three effects of ABA on growth, an intrinsic negative effect observed during nights, and two positive effects observed during days, on plant water status and root hydraulic conductivity.

POSITIONAL CLONING OF GENES INVOLVED IN LEAF GROWTH

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Leaves are lateral determined organs whose primary function is photosynthesis and are also important for nutrient storage, defence and stress responses. The final shape and size of these organs result from a tight coordination between cell proliferation and cell expansion. Although an organ-size checkpoint controlling leaf size remains to be identified, several *Arabidopsis thaliana* mutants impaired in cell proliferation display compensated cell enlargement. To genetically dissect compensation and other mechanisms underlying plant organ growth, we have analyzed a large collection of leaf mutants. The exigua (exi) mutants display small and dark leaves, which contain mesophyll cells of a reduced size. We have initiated a positional approach for the identification of the EXI genes, which should be instrumental to understand how cell expansion is controlled during leaf growth. We are studying in addition several denticulata (den) and apiculata (api) mutants, which display pale, indented and small leaves. The phenotype of the den and api mutants is reminiscent to that caused by Kip-related protein (KRP) overexpression, which includes compensation. Our first attempts to identify the pathways in which these genes are involved will be presented.

**ARABIDOPSIS THALIANA RESPONSES TO WATER DEFICIT.
SOIL VS. HYDROPONICS**

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Hydroponics is a widely used method to grow plants in laboratories because it allows sampling of the root system for biochemical and molecular analysis and manipulations of the root environment. In principle, it should be suitable to study root growth changes in response to water deficit using PEG as an osmoticum. However, shoots and roots are intimately and functionally linked and it is essential to guaranty that the hydroponic system allows normal growth and development of both roots and shoots. It also essential to guaranty that PEG has no anoxic effect as it is reported to lower oxygen conductivity. Here we compared growth, development and water relations of either well watered or water stressed soil and hydroponically grown plants of *Arabidopsis thaliana*. Well watered plants from hydroponics were intistinguishable from soil grown plants in terms of phyllochron, leaf expansion, biomass accumulation and specific leaf area.

Plants were then exposed to moderate water deficit either by withholding water supply to soil or by using PEG to hydroponics solution. Pre-dawn water potentials of soil grown plants were always 0.1 to 0.2 MPa lower than osmotic potential of PEG-solution. Stress levels were however comparable by showing similar decreases of relative expansion rate. Both soil – and PEG generated water deficit resulted in similar decreases of stomatal conductance, relative water content and increases of osmotic potential. Moreover, PEG-generated water deficit strongly reduced shoot growth without altering root growth as classically found in soil. PEG-solution were copiously aerated which probably prevented hypoxia as revealed by the lack of induction of ADH 1, a hypoxia – responsive gene. Together, these data suggest that combining PEG and hydroponics is relevant in order to study root and shoot growth and functioning under water deficit.

SCREENING OF PLANT LEAF CANOPIES BY NOVEL, NON-INVASIVE APPROACHES.

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Plant crop yields are closely linked to leaf canopy growth which is strongly affected by abiotic stresses. The dynamics of the plant response are largely unknown due to a lack of adequate growth measurement methods.

The aim of this study is to establish non-invasive growth analysis procedures that quantify the effect of temperature and water availability on leaf growth in time scales of hours or days in some model plant species.

Methods involved include custom-made image analysis software, pictures taken with digital cameras and an automated image acquisition platform: Growscreen.

Six different species were grown in five experiments to investigate the effect of these abiotic stresses. These experiments (carried out both in research sites of Jülich and Montpellier) were able to account for relative growth rate (RGR) reduction during abiotic stress periods but also for growth recovery afterwards. Results of these experiments will soon enable plant canopy growth modelling approaches.

MODELLING N NUTRITION IMPACT ON PLANT FUNCTIONING IN VARIOUS GENOTYPES OF *ARABIDOPSIS THALIANA*.

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High-throughput phenotype analysis using simulation tools requires the use of models with few and easy measurable parameters. With the aim to characterize genetic variability of plant response to N availability and to identify the key parameters determining their efficiency toward low N nutrition using a modelling approach, we present a simple compartmental model of C and N absorption and partitioning for *A. thaliana* during vegetative growth. This model combines integrative variables (biomasses, N or nitrate accumulated), exchange surfaces for shoot but not for root, and efficiencies (radiation conversion to biomass and nitrogen specific uptake).

The model was used to quantitatively interpret the behaviour of five contrasted genotypes of *A. thaliana* grown in soil (rhizotrons) with 3 levels of nitrate supply. Parameters were estimated on one genotype and one N level and checked on the other genotype x nutrition conditions. We found that specific nitrogen absorption (snu) and specific carbon assimilation (sca) were the key variables that explain the responses of the five genotypes to nitrogen nutrition under steady state conditions.

In a last step, the determining role of nitrogen uptake efficiency in plant response to N supply was validated in *Arabidopsis*, using the mutant *AtNRT2.1a*, deleted on two genes involved in the High Affinity Transport System of Nitrate. We showed that the model simulated well the behaviour of this mutant, what pointed out the major role of the gene *AtNRT2.1* in plant response efficiency to N limited nutrition.

TESTING THREE DIFFERENT IMAGE ANALYSES SYSTEMS IN THE DETERMINATION OF THE CANOPY SURFACE IN *ARABIDOPSIS THALIANA*.

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Image analyses systems are today an accessible and accurate tool for obtaining objectives and repeatable measures of various parameters describing plants morphology. Many software packages, with a wide range of options and prices are available but it is difficult to determine which is the most suitable to our needs. In this work we have tested the precision of three different measuring methods to estimate the canopy surface in *Arabidopsis thaliana*: commercial package Optimass Bioscan 5 (OB), freeware ImageJ combined with commercial package Photoshop (P+IJ) and freeware ImageJ combined with the freeware “Landini’s Treshold Colour” (P+L) software. Thirty individual plants of different sizes were used. Two types of tests were executed: (i) comparison of image analyses and manually conducted measurements of canopy surface; (ii) comparison between canopy surface area measurements performed with OP, IJ+P and IJ+L. The results suggest that the three methods provide highly accurate measurements of canopy surface. There were small differences between manually and image analyses measurements except in the case of IJ+L which shows a slight tendency to overestimate the results. Comparisons of the programs generated almost equal surface estimates for OP versus IJ+P but showed overestimation when comparing IJ+L to any of the others two programs. Estimations were always well reproducible.

SIMULATION OF ARCHITECTURAL DEVELOPMENT OF APPLE TREES : A MIXED APPROACH INTEGRATING BIOMECHANICAL AND MARKOV CHAIN MODELS

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In this talk, we present a new model, MappleT, of plant architecture development which couples both statistical and mechanistic components that interact over several growth cycles. The statistical model controls the development of tree topology and is used to describe accurately the branching patterns according to field observations. The mechanistic model is used to derive a realistic geometry for the axes, taking into account their mechanical properties and organ growth. Both models were developed in previous studies, and we focus here on the emergence of tree form due to their interaction.

MappleT integration was carried out using L-Systems. Growth rules generate branching patterns according to hidden semi-Markov chains (HSMC), with parameters depending on the shoot type. The type of shoot developing from a terminal bud is determined as a function of tree age. Organ dimensions and mass, wood properties, branching angles, and the calendar of events during a growing season are based on experimental data. The radial growth of internodes is estimated by a pipe model. Biomechanical simulations make use of the concept of fast information transfer in L-systems. Bending moments are calculated in a basipetal information transfer phase from masses of the borne branches and organs, negative geotropism, non-elastic wood deformations, and resistance from the reaction wood. This information is used in an acropetal information transfer phase to determine the form of the whole tree.

Topological and geometric descriptors of the simulated trees (represented as MTGs) were calculated at different scales (whole tree, branches and shoots) and made it possible to compare the simulated trees with digitized trees.

CORRELATIONS BETWEEN LEAF GROWTH VARIABLES IN *ARABIDOPSIS THALIANA* : A QTL ANALYSIS

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Leaf growth can be studied through various variables at different organizational levels, from cell, to leaf to the whole plant. The functional links between variables which are determinant for leaf growth and its plasticity to environmental conditions are not clearly identified. The aim of this study was to elucidate the genetic bases of the relationships between variables involved in leaf development in *Arabidopsis thaliana*.

The approach consists in (1) growing a population of recombinant inbred lines (Ler*An-1) in optimal environmental conditions, (2) estimating a large data-sets of leaf growth variables, (3) identifying correlations between these variables, (4) and detecting associated QTLs.

Correlations between leaf growth variables were found both within and between the different organisational levels. QTLs involved in rosette leaf area were identified. By breaking down further the rosette area in its underlying components revealed groups with colocations of QTLs with different physiological functions. In addition, QTLs involved in the control of underlying variables without effects on rosette area were identified.

This work highlights the usefulness to analyse correlations between variables that define an integrated trait such as leaf area. Colocations of QTLs for variables which were correlated highlight a genetic basis and a functional interpretation for the phenotypic correlations observed.

APPARITION, LOCALIZATION AND GROWTH OF NODULES WITHIN THE ROOT SYSTEM OF PEA, AS AFFECTED BY ASSIMILATE AVAILABILITY

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In pea, symbiotic nitrogen fixation is highly related to nodule biomass. Despite many studies have focussed on nodule organogenesis at both the cellular and the organ scale, little information has been gained concerning the dynamic of nodule biomass during the plant growth cycle. Therefore, our aim was to characterize nodule establishment and growth at the plant level as a function of internal (carbon and nitrogen fluxes) and external factors (temperature, radiation and nitrate).

The effect of carbon nutrition on pea nodule growth was investigated in two experiments either by modifying carbon availability (using plant shading) or by modulating competition for carbon between nodules and other plant organs using contrasted nodulation conditions (produced by inoculating at week intervals from radicle emergence, which resulted in various shoot and root developments).

Plants grown hydroponically were harvested several times a week during the whole vegetative period. The taproot was divided in 2 cm segments for spatial observations (number and maximal length of lateral roots, number and biomass of nodules carried by the taproot segment and by its lateral roots).

The respective effects of C and N nutrition on nodule number, growth and localisation within the root system will be presented, as related with N uptake by symbiotic fixation. The acquired knowledge will be integrated in a model combining both trophic and architectural approaches. This will help us testing hypothesis concerning trophic and environmental factors that rule nodulated root establishment and functioning for N uptake. This model will become a powerful tool for analysing genetic variability associated to N nutrition of legumes.

ARE SOURCE AND SINKS STRENGTHS GENETICALLY LINKED IN MAIZE PLANTS SUBJECTED TO WATER DEFICIT ? A QTL STUDY OF THE RESPONSES OF LEAF GROWTH AND OF ANTHESIS-SILKING INTERVAL TO WATER DEFICIT

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Leaf growth and Anthesis-Silking Interval (ASI) are the main determinants of source and sink strengths of maize via their relations with light interception and yield, respectively. They depend on the abilities of leaves and silks to expand under fluctuating environmental conditions, so we tested the possibility that they may have a partly common genetic determinism in a mapping population which segregates for ASI. Maximum leaf elongation rate per unit thermal time and the slopes of its responses to evaporative demand and soil water status were measured in the Phenodyn platform (greenhouse and growth chamber), in two series of 120 recombinant inbred lines (RILs) studied in 2004 and 2005. ASI was measured in 3 and 5 fields under well-watered conditions and water deficit, respectively. For each RIL, the maximum elongation rate per unit thermal time was reproducible over several experiments in well-watered plants. It was accounted for by 5 QTLs, among which three colocalised with QTLs of ASI of well-watered plants. The alleles conferring high leaf elongation rate conferred a high silk elongation rate. The responses of leaf elongation rate to evaporative demand and to predawn leaf water

potential were also common to several experiments. The slopes of these responses had 3 QTLs in common with ASI of plants under water deficit. The alleles for leaf growth maintenance were those for maintained silk elongation rate. These results may have profound consequences for modelling the genotype x environment interaction and for designing drought tolerant ideotypes.

SPATIO-TEMPORAL LEAF GROWTH OF ARABIDOPSIS THALIANA AND EVIDENCE FOR SUGAR CONTROL OF DIEL LEAF GROWTH

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A digital image sequence processing (DISP) based non-invasive technique for visualizing and quantifying highly resolved spatio-temporal dynamics of leaf growth has been established for the model plant *Arabidopsis thaliana*. This has the potential to characterise temporal leaf growth in mutants and transgenic plants to analyse the molecular control underlying diel growth.

Arabidopsis thaliana leaves showed highest relative growth rates (RGR) at dawn and lowest RGR at the beginning of the night. Along the lamina, a clear basipetal gradient of growth rate distribution was found, similar to lots of other dicotyledonous species.

Analysis of growth of mutants in starch metabolism, known to be retarded in growth dependent on the day length, revealed changed temporal growth patterns. The starch-free mutant *stf1* shows an endogenous change in sugar availability *in vivo*. *Stf1* lacks any starch storage for the dark period and accumulates free sugars during the day. In correlation to the sugar availability, *stf1* showed reduced nocturnal growth rates and higher RGR in the afternoon compared to the wild type.

The sugar-sensing mutant *gin2-1* does not show any changes in spatio-temporal growth, indicating that the glucose-sensor hexokinase 1 (*AtHXK1*) does not control the wild type diel leaf growth under the chosen conditions.

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