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C5/P7 — LEAF FORM AND FUNCTION

C5/P7.1
10:30 Monday 29th June 2009
TBC

Neelimha Sinha (University of California Davis)

doi:10.1016/j.cbpa.2009.04.365

C5/P7.2
11:15 Monday 29th June 2009
Understanding developmental mechanisms underlying diversification of leaf form

Miltos Tsiantis (University of Oxford)

A key problem in biology is to understand how diversity in organismal form is generated. Diversification in the form of reproductively isolated species has been difficult to study because of the paucity of experimental systems where the developmental genetic changes underlying morphological variation can be accurately identified. To investigate this problem we study the genetic mechanisms underlying variation in the form of the predominant photosynthetic organ of plants, the leaf. Leaf form can be classified as simple, where the leaf blade is entire as in the model organism *Arabidopsis thaliana*, or dissected where the blade is divided into distinct units called leaflets. Mechanisms that determine specification of dissected versus entire leaf shape and regulate the number, position and timing of leaflet production are poorly understood. To obtain an in-depth and unbiased understanding of these mechanisms we established *Cardamine hirsuta* – dissected leaf relative of *A. thaliana* – as a versatile experimental system where both forward genetics, and stable genetic transformation can be deployed to study diversification of leaf form. Here we discuss how comparisons between *A. thaliana* and *C. hirsuta* have illuminated our understanding of processes underlying the evolution of leaf morphology.

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C5/P7.3
11:55 Monday 29th June 2009
How to dissect a leaf: A role for the *NAM/CUC3* genes and the microRNA *miR164*

Patrick Laufs (INRA (Versailles))

Leaves show a wide diversity of shapes which can be seen as resulting mostly from variations of their dissection patterns and levels. Two main leaf architectures can be distinguished: simple leaves formed by a single unit and dissected/compound leaves grouping several leaflets. The margins of each of these structures can show different patterns of incisions, ranging from entire to serrated or lobed.

We have analysed the role of the *NAM/CUC3* genes in the dissection of leaves with different architectures. The *NAM/CUC3* genes have been identified initially for their function in the definition of the boundary domain surrounding organ primordia in the apices of the aerial plant parts. In Arabidopsis the three *NAM/CUC3* genes *CUC1*, *2* and *3* are expressed in this boundary domain and their inactivation leads to the fusion of adjacent organs and perturbed meristem function.

We will show that *CUC2*, and in a more minor way *CUC3*, have a central role in the elaboration of dissected leaf margins in *Arabidopsis*. The analysis of a selection of compound-leafed species reveals that the *NAM/CUC3* genes have an evolutionary conserved role in all types of leaf margin dissection. Our observations suggest that the boundary domain defined by the *NAM/CUC3* genes has a dual role promoting leaflet separation locally and leaflet formation at distance.

The *NAM* genes are regulated by a microRNA, *miR164*. We will provide evidence for the role of miR164 regulation during leaf development and that the *MIR164* and *CUC* genes are part of a complex regulatory network.

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C5/P7.4

13:30 Monday 29th June 2009

The role of (stem) cell division in leaf growth

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The growth of an organ is the result of the spatio-temporal regulation of cell division and cell expansion. We have developed kinematic analysis to quantify the role of these processes in response to perturbation of key regulatory genes. Here we report two surprising findings: the analysis of DELLA mutants shows that GA signalling affects cell division and the stem cell regulators SHR and SCR play a role in leaf development. For integrating experimental observations into a full understanding of the leaf developmental system, modelling is crucial. Our first modelling efforts show the potential of this approach, but also the challenges ahead.

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C5/P7.5

14:30 Monday 29th June 2009

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

Christine Granier (INRA-LEPSE), Sébastien Tisné (INRA-LEPSE), Catherine Massonnet (INRA-LEPSE), Juliette Fabre (INRA-LEPSE), Nathalie Wuyts (INRA-LEPSE), Denis Vile (INRA-LEPSE), Myriam Dauzat (INRA-LEPSE)

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

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C5/P7.6

15:40 Monday 29th June 2009

Deciphering the rules relating cell division and leaf form

Andrew J. Fleming (University of Sheffield)

It is self evident that organs consist of cells, yet the relationship between observed patterns of cell division and the form that an organ takes is complex and frequently non-intuitive.

Using the *Arabidopsis* leaf as an experimental organ, we have performed an analysis of cell division pattern during the earliest stages of leaf development and used a variety of computer-based tools to quantify the changes of leaf shape that occur in parallel. We have then applied a number of molecular tools to alter the pattern of cell division during specific phases of leaf development. A spatio-temporal quantitative comparison of the changes in cell division pattern and leaf shape allows us to define how the relationship of cell division and morphogenesis alters during leaf development.

These data indicate that the rules relating cell division and leaf form are under developmental regulation. Manipulation of cell division can be used to alter form, but only in a specific developmental context. Moreover, the outcome of altered cell division pattern on differentiation can have physiological consequences that feedback onto growth and, thus, form.

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C5/P7.7

16:20 Monday 29th June 2009

Cell division in the *Arabidopsis* leaf epidermis

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The *Arabidopsis* leaf epidermis is a tissue consisting of a single layer of cells of various sizes, shapes and functions, arranged in an intricate two-dimensional pattern. We aim to understand this pattern using a combination of time-lapse imaging and computational modeling techniques.

We have developed the ability to image *Arabidopsis* seedlings continuously for up to seven days using time-lapse confocal microscopy. The resulting movies capture the dynamic nature of leaf development. From these movies we extract the information about cell growth, timing of cell divisions and placement of division walls, which is used to produce an initial descriptive model. We then gradually replace direct data with hypothetical deterministic rules of cell division within a growing leaf epidermis, and verify the results by comparing the output to the data.

The model has already made it possible to test existing theories of how and when cells divide, and resulted in the falsification, in the case of *Arabidopsis* leaves, of several rules previously reported in the literature. We have also verified rules that predict the position of dividing walls in non-differentiated cells. The model is currently being extended to include rules for the timing of cell division and the differentiation of stomata. The model thus provides a framework for understanding how complex patterns of epidermis cells develop and accommodate differentiated cells, and suggests that the observed complexity may by an

emergent property of a small set of simple, possibly deterministic rules.

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C5/P7.8

16:40 Monday 29th June 2009

The volumetric component of individual leaf expansion: Taking into account sub-epidermal tissues in the description of leaf expansion over time

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Most leaf development studies at the cell and organ levels have been limited to the leaf surface, with data referring to the leaf surface area and to the number and surface area of epidermal cells. However, leaf sub-epidermal tissues, the palisade and spongy mesophyll, contain the main actors in photosynthesis. The number and thickness of palisade cell layers and the volume occupied by spongy mesophyll (cells and intercellular spaces) affect the accumulation of photosynthates and, as such, whole plant growth. Studies into the leaf phenotype of growth-affected *Arabidopsis thaliana* mutants have revealed a higher variability in leaf thickness than in leaf surface area. In general, there is no correlation between these two variables, which means that to describe a leaf phenotype, leaf volume has to be taken into account. A method has been developed for high-resolution imaging of leaves in three dimensions using multiphoton laser scanning microscopy, and for the analysis of images, providing data on volumes and volumetric proportions of cells and tissues and cell density. The method has been used in the study of *A. thaliana* leaf expansion from emergence to the onset of senescence for leaves located at different nodal positions in the rosette, completing our knowledge of individual leaf development processes with their volumetric component. The method will further be applied in the study of leaf plasticity in response to the environment for both *A. thaliana* and apple tree, a model and an agronomic species, respectively.

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C5/P7.9

09:00 Tuesday 30th June 2009

Asymmetry and pattern in the leaf epidermis

Dominique Bergmann (Stanford University), Juan Dong (Stanford University), Greg Lampard (Stanford University), Cora MacAlister (Stanford University), Charles Hachez (Stanford University), Matthew Rowe (Stanford University), Carrie Metzinger (Stanford University)

Stomata are epidermal pores found on the surfaces of the aerial portions of most land plants. They function to regulate gas exchange between the plant and the atmosphere. We use the development of stomata as a model for the generation of asymmetries during plant development. Stomatal guard cells are created via a stereotyped set of asymmetric cell divisions whose number and orientation are dictated by local cell–cell interactions and longer range signals from other tissues or from the outside environment. We are interested in the nature of the positive and negative inputs into this system and how they are integrated. Our focus is on three major elements: (1) a set of related bHLH transcription factors that regulate the cell divisions associated with establishing, maintaining and terminating the stomatal lineage, (2) a negative regulatory circuit previously defined by receptor-like proteins and a Mitogen Activated Protein Kinase (MAPK) cascade, and (3) novel proteins that carry out the asymmetric division process. We have established direct regulatory relationships between the MAPK kinases and one of the bHLHs and will discuss how stomatal development provides a test system for deciphering complex regulatory networks. We will also introduce the novel and asymmetrically localized protein BASL and a model for its activity in differentiation and self-renewing cell divisions.

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C5/P7.10

10:30 Tuesday 30th June 2009

Control of differentiation and cell cycling in leaf development

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Leaf development is characterised by a period of mitotic cycles which control the cell number in the developing organ followed by cell expansion and an associated switch to endocycles. D-type cyclin (CYCD) activity plays an important role in determining the timing of this switch during leaf development. Leaf development also requires the down-regulation of meristem markers such as SHOOTMERISTEMLESS (STM), whose continued expression promotes the maintenance of an undifferentiated state and continued mitotic cycles. Cytokinin appears to be closely involved in determining the mitotic state acting both downstream of STM and upstream of CYCD activity and the interplay of these factors with cell cycle control and the transition from mitotic cycles to endocycles will be discussed.

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C5/P7.11

11:10 Tuesday 30th June 2009

Elucidation of the molecular basis of auxin regulation of vascular development in woody plants

Rishi Bhalerao (UMEA)

Phytohormone indole-acetic acid (auxin) is a key regulator of vascular development. In vascular tissue of woody plants, a concentration gradient of auxin overlaps the developmental gradient of secondary xylem. This overlap between auxin gradient and

developing secondary xylem has lead to the suggestion that auxin gradient could act like a morphogen in regulating secondary xylem development. In addition auxin also plays a key role in environmental control of vascular cell division activity in wood plants. I will discuss our results based on analysis of auxin responsive transcriptome in woody tissues and the effect of modulation of auxin responsiveness on secondary xylem development in model plant hybrid aspen to explain the role of auxin in regulation of vascular development in woody plants. We have also analysed the mechanism underlying day length regulation of vascular meristem activity and I will discuss our results that show that modulation of auxin signaling by short day signal is important for seasonal control of the vascular meristem activity.

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C5/P7.12

11:50 Tuesday 30th June 2009

'Microfilament Analyzer' as a tool to study the cytoskeletal (re) organisation during pavement cell development in the *Arabidopsis thaliana* leaf

Eveline Jacques (University of Antwerp), David D'Haese (University of Antwerp), Dirk Adriaensen (University of Antwerp), Jean-Pierre Verbelen (University of Antwerp), Kris Vissenberg (University of Antwerp), Jan Buytaert (University of Antwerp)

Epidermal pavement cells of the *Arabidopsis* leaf develop in a very specific way from simple rod-shaped to complex puzzle-like structures. This morphogenesis is accompanied by a reorganisation of the cytoskeletal components. Both microtubules and actin are visualised using GFP transgenic lines, being respectively the double mutant glabrous1:α-Tubulin6 (g1:TUA6)-GFP and Fimbrin Actin Binding Domain 2 (FABD2)-GFP.

To make an accurate and objective analysis of the cytoskeletal organisation in the obtained pictures, a software programme is developed and named 'Microfilament Analyzer'. Besides the more standardised interpretation of images it also facilitates the analysis of higher amounts of data. The programme consists of a three-step-process, starting with the detection of the (micro)filaments on the digital images. Next, the detected filaments are assigned to individual cells and finally the orientation of the filaments is determined.

With this tool, a detailed study of cytoskeletal (re)organisation in developing epidermal cells of the fourth leaf (cotyledons not included) was conducted.

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C5/P7.13

12:10 Tuesday 30th June 2009

Body plan transitions in the evolution of leaves

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Life on land is dependent on leaves for the production of oxygen and food, yet we currently have minimal understanding of how they evolved. The main hypothesis suggests that flowering plant leaves arose from simple dichotomising shoot systems in which the fate of some branches was modified such that they became reduced and flattened at the side of a main shoot. This hypothesis does not account for major early changes in plant body plan that led to the formation of leaf like organs in the haploid generation in basal plant groups such as mosses and liverworts. Such changes in body plan are mimicked within the life cycle of extant mosses such as *Physcomitrella*. Their developmental basis, and similarities between *Physcomitrella* and *Arabidopsis* leaf development will be explored.

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C5/P7.14

13:30 Tuesday 30th June 2009

Photosystem II the water splitting complex of photosynthesis: Structural analyses

James W. Murray (Imperial College), Jim Barber FRS (Imperial College)

About 2.5 billion years ago, an enzyme, photosystem II (PSII), emerged with the ability to use light to oxidize water to oxygen and equivalents of hydrogen. The hydrogen equivalents are used to reduce carbon dioxide to organic molecules, and the oxygen is released into the atmosphere. PSII is a large, multisubunit membrane protein–pigment complex, a 700 kDa dimer.

The water-oxidation reaction within PSII is catalysed at an unusual Mn_4Ca metal cluster, ligated by a highly conserved protein environment. Understanding the structure and mechanism of PSII has been greatly enhanced by recent crystallographic structure information. In addition to the original refined structure (Science 303 (2004)1831–1838), we have used anomalous X-ray scattering techniques to confirm the location of calcium in the metal cluster and identify two chloride binding sites. Both are essential for water splitting activity. To investigate channels leading to and from the metal cluster to the bulk solvent we have employed both theoretical and experimental approaches.

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C5/P7.15

14:30 Tuesday 30th June 2009

The evolution of C4 photosynthesis

Julian M. Hibberd (University of Cambridge)

The C4 pathway has evolved independently at least 45 times. These multiple evolutionary origins have arisen despite the fact that most C4 leaves have altered biochemistry, cell biology and morphology compared to those of C3 species. We are interested in the role of photosynthesis in veinal cells of C3 plants before they become highly specialised in the C4 pathway as well as the alterations in gene expression responsible for the generation and maintenance of a C4 leaf.

We have combined the use of GAL4-VP16 enhancer trap lines with RNAi to remove photosynthesis from veins of the C3 plant *Arabidopsis thaliana*. Our analysis shows that photosynthesis in veinal and nearby cells of C3 plants receives little CO_2 that enters the leaf via stomata, but that photosynthesis around veins is important in supplying carbon skeletons to the shikimate pathway, controlling leaf senescence, and that it increases fitness. Photo-

synthesis in mid-veins from *A. thaliana* is able to use CO_2 derived from organic acids present in the transpiration stream because of significant activities of four-carbon decarboxylases in the mid-vein. To analyse how the regulation of genes encoding these decarboxylases has altered during the evolution of C4 photosynthesis, we assessed the abundance of transcripts derived from each gene encoding the decarboxylases in mid-veins, and cloned orthologues from *Cleome gynandra*, the most closely-related C4 plant to *A. thaliana*. Comparative analysis allows us to identify mechanisms underlying the alterations in gene expression required for a functional C4 leaf.

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TBC

Uli Schurr (University of Juelich)

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C5/P7.17
16:20 Tuesday 30th June 2009
CP12 mediated redox regulation of chloroplast metabolism is essential for leaf development

Christine A. Raines (University of Essex), Thomas P. Howard (John Innes Centre), Alisdair Fernie (MPI Potsdam), Julie C. Lloyd (University of Essex)

Redox regulation of leaf metabolism mediated by thioredoxin proteins in the chloroplast has been known for some time. More recently the small redox sensitive chloroplast protein, CP12, has also been shown to be subject to regulation via thiredoxin f. Unexpectedly, CP12 antisense plants have a severe growth phenotype with abnormal leaf shape and altered mesophyll cell size. These changes in development were unexpected given that the only known role of the CP12 protein was in the regulation of NADP–GAPDH and PRKase in the photosynthetic carbon reduction (Calvin) in responses to changes in light intensity. However, photosynthesis in the CP12 antisense plants was reduced by a maximum of 25–30% and PRKase and GAPDH activities are similar to that in wild type plants. It is highly unlikely that these changes could account for the dramatic differences in morphology in these plants. Interestingly the activities of two additional thioredoxin regulated chloroplast enzymes, malate dehydrogenase and glucose-6-phosphate dehydrogenase, are altered as was carbon allocation with both starch and sucrose being reduced by 50%. Levels of oxoglutarate levels and aromatic amino acids were also reduced significantly but interestingly levels of polyamines increased which could be related to the abnormal leaf morphology. Our data have revealed that CP12 plays a central role in mediating thioredoxin modulation of chloroplast enzymes and that CP12 regulation of metabolism is essential for leaf development.

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C5/P7.18
16:40 Tuesday 30th June 2009
Stomatal conductance based on energy balance

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Evaporation of water inside a leaf into the humid air of the intercellular spaces is driven, in part, by absorbed radiation. A light beam interacting with a leaf penetrates the epidermis with little interaction and the largest part of the energy is absorbed by mesophyll cells. This asymmetric absorption of energy leads to the mesophyll becoming warmer than the epidermis and causes a temperature gradient which may drive evaporation and condensation within the leaf, carrying heat with it. We present data showing that variation in stomatal conductance at constant humidity and CO_2 is closely proportional to changes in fluxes of energy ($W\,m^{-2}$) absorbed by the leaf. We developed a model of energy exchange between the mesophyll and the atmosphere with different regimes of heat and water exchange operating on the inner and outer sides of the epidermis. The rate at which water is delivered to the inner side of the epidermis is determined by the radiation load on the leaf while the rate of water loss to the atmosphere is controlled by stomata. We posit that energy driven changes in water vapor delivery to the epidermis affect the water potential of the epidermis influencing the water potential gradient between mesophyll and epidermis. Water and solutes are transported within the apoplast along this gradient and stomatal conductance adjusts such that the epidermis is neither gaining nor losing water in steady-state. The model realistically simulates stomatal response to changes in radiation, temperature and, water vapor, and has the potential for upscaling water fluxes in ecosystems.

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C5/P7.19
Poster Session — Tuesday 30th June 2009
Wheat model rigid pubescence carriers: Cluster analysis of leaf quantitative traits

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Leaf rigid pubescence is considered to be one of crucial characters enabling resistance to a number of abiotic and biotic stressors. Three conditioned types of leaf quantitative traits have been highlighted using Two Way ANOVA-administrated cluster analysis based on a Euclidian similarity matrix applied to different morphometric properties of wheat. Since initial stages of ontogenesis, the area of the nucleus is ascribed to *the major quantitative trait*. This primary trait has been revealed to unify model wheat lines possessing rigid pubescence beginning from the very stage of seedlings. Length of rigid trichomes is attributed to “backward" (flexible) quantitative traits that occur less significant at early ontogenetic stages to become critical by the stage of anthesis through waxy ripeness. Trichome density is referred as the *secondary quantitative trait* forming the united cluster of rigid pubescence carriers exclusively at final stages starting from waxy ripeness. Under these conditions, belated formation of such a united cluster of the rigid-trichome carriers has been detected both for adaxial, and abaxial blade surfaces. Thus, unique features of rigidly

pubescent model wheat lines are determined rather early, at the stage of seedlings, by the area, as optionally a functional state of trichomal nucleus. So, various developmental plots of rigid trichome functioning under the stress may be "turned on" particularly at this stage.

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C5/P7.20

Poster Session — Tuesday 30th June 2009

Mitotic inhibitor WEE1 kinase, a part of DNA damage checkpoint in green alga *Chlamydomonas reinhardtii*

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WEE1 kinase is a major regulator of mitosis. During S and G2 phase it phosphorylates and inactivates cyclin dependent kinase (CDK), the key activator of cell cycle progression. In mammals and higher plants is WEE1 a part of DNA damage checkpoint that ensures maintenance of cell viability in cells which have suffered genomic damage. *Chlamydomonas reinhardtii* is a green alga dividing by multiple fissions into 2, 4, 8 or 16 daughter cells of distinct cell size. Its genome encodes a single homolog of WEE1 kinase. We cloned a GFP tagged CrWEE1 cDNA under Ni inducible Cyc6 promoter. Recovered transformants showed a clear phenotype in the presence of $NiCl_2$. The over-expression of GFP-WEE1 slowed down the growth so the cells divided later compared to controls. Interestingly, although they reached the same mother cell size they divided into less daughter cells of bigger size implying a role for WEE1 kinase in the cell sizer. Also, the cells in the presence of $NiCl_2$ showed significant increase in the survival rate upon exposure to zeocin as DNA damaging agents indicating a role for CrWEE1 in the DNA damage response. We crossed GFP-WEE1 over-expressing lines to uvs11, a known DNA checkpoint mutant of *C. reinhardtii*. *C. reinhardtii*' WEE1 plays, in contrast to *Arabidopsis* WEE1, dual role in regulating mitotic progression and coordinating DNA damage response.

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C5/P7.21

Poster Session — Tuesday 30th June 2009

Stomatal function in the microphyllous plant family *Selaginellaceae*

Elizabeth M. Ruszala (The University of Bristol), Simon J. Hiscock (The University of Bristol), Alistair M. Hetherington (The University of Bristol)

Stomata are pores made up of two specialised cells called guard cells. They are present in epidermal tissues and are responsible for controlling the exchange of water vapour and CO_2 between the interior of the leaf and the atmosphere. Stomata first appear in the fossil record approximately 410 MYA in primitively vascularised sporophytes (Kenrick and Crane, 1997). Interestingly, the guard cells of these fossil species show remarkably similar anatomy to modern forms of higher megaphyllous plants (Edwards et al., 1998). Little is known about how lower plant stomata function, but this physical similarity suggests that they may behave in a manner akin to higher plant stomata. In higher plants, both pore aperture and the number of stomata that form are controlled by endogenous plant hormones, such as abscisic acid (ABA), and environmental signals including photon irradiance, atmospheric CO_2 concentration and atmospheric relative humidity (RH) (Hetherington and Woodward, 2003). Using epidermal peels of lycophyte plants (specifically *Selaginellas*) I have confirmed that microphyll stomata alter in aperture in responses to many stimuli known to regulate the aperture of megaphyll stomata including light/dark transitions and ABA. Current work is centred on investigating whether *Selaginella* guard cells use similar signalling networks to those employed in higher plants. A major interest is in calcium-based signalling pathways. To investigate whether calcium is involved in *Selaginella* guard cell signalling, I am using a biolistic approach (Bothwell et al., 2006) to introduce the calcium indicator dye FURA2 into guard cells of this species.

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C5/P7.22

Poster Session — Tuesday 30th June 2009

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

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

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

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

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

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C5/P7.23

Poster Session — Tuesday 30th June 2009

'Microfilament Analyzer' as a tool to study the cytoskeletal (re) organisation during pavement cell development in the *Arabidopsis thaliana* leaf

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Epidermal pavement cells of the *Arabidopsis* leaf develop in a very specific way from simple rod-shaped to complex puzzle-like structures. This morphogenesis is accompanied by a reorganisation of the cytoskeletal components. Both microtubules and actin are visualised using GFP transgenic lines, being respectively the double mutant glabrous1:α-Tubulin6 (g1:TUA6)-GFP and Fimbrin Actin Binding Domain 2 (FABD2)-GFP.

To make an accurate and objective analysis of the cytoskeletal organisation in the obtained pictures, a software programme is developed and named 'Microfilament Analyzer'. Besides the more standardised interpretation of images it also facilitates the analysis of higher amounts of data. The programme consists of a three-step-process, starting with the detection of the (micro)filaments on the digital images. Next, the detected filaments are assigned to individual cells and finally the orientation of the filaments is determined.

With this tool, a detailed study of cytoskeletal (re)organisation in developing epidermal cells of the fourth leaf (cotyledons not included) was conducted.

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Poster Session — Tuesday 30th June 2009

Cell cycle arrest in response to DNA damage in *Chlamydomonas reinhardtii*

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Although cellular genome is relatively stable, it can be altered both spontaneously and after exposition to various DNA damaging agents. Cells usually respond to the genome damage by activating a DNA damage response pathway. We studied the effects of zeocin, a bleomycin family antibiotics causing double stranded breaks, and caffeine, methylxantine synergistic with many DNA damaging agents, on the growth rate, cell survival and cell cycle progression of the alga *Chlamydomonas reinhardtii*. *C. reinhardtii* is a model unicelullar green alga dividing by multiple fissions. Its cell cycle consists of long G1 phase followed by *n* alternating rounds of S phase and mitosis, terminated by cell division into 2^n daughter cells. Increasing concentrations of zeocin severely affected cell survival and blocked nuclear and cell divisions. Moreover, *Chlamydomonas*' cells were hypersensitive to DNA damage if combined with the presence of caffeine. In the presence of caffeine only, the cells divided faster comparing to untreated control, probably due to degradation of inhibitor of mitosis, Wee1. Here, we show that application of caffeine can override a cell cycle arrest following to DNA damage. It implies DNA damage pathway is conserved from algae to higher eukaryotes.

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Poster Session — Tuesday 30th June 2009

DNA damage checkpoint targets both WEE1 and major mitotic kinase CDKB1 in *Chlamydomonas reinhardtii*

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Genomes of all organisms are exposed to various stress factors. Therefore it is vital for the cell to continuously check the integrity of genetic material and repair it immediately when damaged. DNA damage response pathway is protein network responsible for sensing, reacting to and repairing of damaged DNA. Two related kinases are involved in this pathway: ATM, which is activated by double-strand breaks, and ATR primarily responding to single-strand breaks or stalled replication forks. In animals activated ATM/ATR trigger pathway leading to cell cycle arrest by regulation of CDC25 and WEE1. Plants lack a functional homolog of CDC25 and DNA damage checkpoint is controlled by WEE1 kinase operating in ATM/ATR dependent manner. In the model organism, green alga *Chlamydomonas reinhardtii* there is one homolog of each CDKB1 and WEE1 kinases. The roles of both proteins in the regulation of *C. reinhardtii* cell cycle are unknown. We analyzed expression and activity of CDKB1 and WEE1 in the response to DNA damage. Both of the genes are transcriptionally induced upon DNA damage and post-transcriptionally regulated in ATM/ATR dependent manner leading to a cell cycle arrest prior to S/M phase with high tyrosine phosphorylated inactive CDKB. We conclude that CDKB1 is a major mitotic kinase in *C. reinhardtii* and it is also a major target of WEE1 kinase. Both proteins are required for the response to DNA damage.

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Poster Session — Tuesday 30th June 2009

The role of the trehalose-6-phosphate sugar signal in coordinating leaf development to plant sugar status in *Arabidopsis thaliana*

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Trehalose-6-phosphate (Tre6P) is emerging as a major signal metabolite responding strongly and specifically to sucrose availability in the plant and regulating starch metabolism downstream. Previous work has shown that constitutive modulation of Tre6P levels in plants leads to strong developmental and growth phenotypes affecting both reproductive as well as vegetative development, as well as being one of the only examples of genetic modification of leaf photosynthetic capacity. This has often been interpreted as a pleiotropic effect to phenotypes related to starch metabolism. In this study we demonstrate that modulation of Tre6P levels in *Arabidopsis thaliana* through over-expression of bacterial enzymes synthesising or degrading Tre6P affects

cell division and cell expansion processes in leaves proportionally to the levels of Tre6P in the plant, resulting in large changes in leaf size and growth rate as well as affecting leaf shape, photosynthetic capacity and specific leaf area. We compare these changes to the developmental responses of leaves to high and low light, a manipulation of plant sucrose status. In addition, we employ the starchless *pgm* mutant to demonstrate that these effects are independent of starch, as previously thought. These results suggest that Tre6P may be an important signal transducing plant sucrose availability into leaf developmental responses and could be involved in adaptive growth responses resulting in optimisation of leaf functional properties in response to environmental conditions.

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Poster Session — Tuesday 30th June 2009

Tapping into the mechanical link of expansins in leaf morphogenesis

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Expansins are cell wall-loosening proteins implicated in many aspects of plant development. Expansin's role in leaf morphogenesis is indicated by the aberrant primodium initiation and modified leaf shape through local induced expression. However, mechanistic function of expansins remains elusive.

In order to verify the direct role of expansins in modulating cell wall extensibility during leaf growth, tissue-level atomic force microscopy (AFM) has been adopted for in vivo quantification. Preliminary results provide the first direct evidence of a distinct difference of cell wall properties between the adaxial and abaxial side of leaf primodium, showing abaxial side to be much more extensible and a drastic shift at the leaf margin. Further investigations will be carried out on development across different plastochron stages in conjunction to mapping the expression pattern of leaf-specific expansins.

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Poster Session — Tuesday 30th June 2009

Manipulation of *Arabidopsis* leaf shape by chemically inducible *AtCYCD3.1* expression

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Plants are sessile organisms and during the course of evolution they have had to develop several mechanisms to optimise survival in a changing environment. As the major *photosynthetically* active organ, the leaf plays an essential role. Leaf size and shape differs between plant species and frequently reflects habitat, but the mechanism underlying this variation in form remains unclear. The emergence of the final leaf form is clearly based on the genetically determined developmental plan, but intercellular biophysical interactions and external environmental factors also have a strong influence on the final leaf shape.

Chemical inducible gene expression systems are a valuable tool for studying the complex mechanism of leaf shape regulation since they allow the staged manipulation of specific parameters during development. Here we present the outcome of altered cellular dynamics on leaf form following the staged induction of CYCLIND3.1, a key factor in the plant cell cycle. Using a combination of molecular, imaging and computational approaches we show that dynamic manipulation of CYCD3.1 gene expression leads to various cell division patterns and changes in leaf shape. This allows for a deeper understanding of the spatial and temporal correlation between shape determining cell divisions and further leaf growth.

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Poster Session — Tuesday 30th June 2009

What is the phenotype of a genotype? An inter-laboratory comparison of leaf growth and profiling signature in three *Arabidopsis thaliana* accessions

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Several projects aim at identifying, understanding and modelling the molecular processes involved in the phenotypic control in *Arabidopsis thaliana*. One approach used in these interdisciplinary projects is to grow different genotypes at different locations and analyse them by different molecular profiling platforms. The integration of different data types would be simplified if similar phenotypes could be observed at different locations. We present here the results of a first public European inter-laboratory experiment (ten laboratories from six different countries) addressing the feasibility of obtaining identical leaf growth phenotype and profiling signature for three contrasting genotypes using a standardized protocol. In a first preliminary experiment, we determined the appropriate environmental conditions and a minimum set of leaf growth variables to reveal the contrasts among these three genotypes. Based on this experiment, a detailed protocol was shared among all participating laboratories to test the reproducibility of the leaf growth phenotype at different sites. Statistical analyses of phenotypic data revealed significant differences both between the laboratories and within a same laboratory. Not only the absolute values of leaf growth variables differed among the different groups, but also, the ranking of the genotypes according to their leaf growth variables. Transcriptomic and metabolomic signatures were analysed to determine to what extent profiling data were related to this phenotypic variability. All together these findings highlight the difficulty of assigning a phenotype to a given genotype.

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Poster Session — Tuesday 30th June 2009

Analysis of the impact of cellular processes on leaf growth of *Arabidopsis thaliana* by a high-throughput mutant approach

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Leaf growth is a dynamic process in 3D that results from a combination of two key cellular processes: cell division and cell expansion. The mechanisms underlying the control and coordination of these two processes are not well identified. In this study, we propose to use a high-throughput mutant approach to try to elucidate

the role played by these cell processes in leaf expansion both in area and thickness. One hundred mutants affected either in cell cycle regulation, cell wall properties, organisation of the cytoskeleton or endoreduplication processes were grown under similar controlled environmental conditions in the PHENOPSIS phenotyping platform. Leaf growth was analysed in area and thickness at different organizational levels, such as cells, the individual leaf and the whole plant.

Among the 100 mutants, 76 did not modify significantly their leaf growth compared to the wild-type neither in area nor in thickness. The remaining 24 mutants were affected either in their leaf area, or leaf thickness or both. There was no relationship between leaf area and leaf thickness within the 100 mutants. Leaf thickness depended more on the proportion of lacuna parenchyma tissue which was highly negatively correlated to the proportion of palisade parenchyma tissue. Our analysis shows an independent control of leaf area and leaf thickness during leaf growth and revealed the necessity to consider these two variables independently in the perspective of a dynamic 3D leaf growth model.

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Poster Session — Tuesday 30th June 2009
Influences of Nitrogen on leaves in winter wheat

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Studies have revealed the influence of seed size on the appearance rate and elongation rate of leaves together with an effect on the final number of spikelets and tillers in winter wheat. In the present study the focus was to identify if the availability of Nitrogen had any influence on these parameters. This was achieved by making regular emerging leaf measurements on the mainstem of wheat plants, tagged at emergence and growing in various plots in a rotational section of the Broadbalk Classical Experiment at Rothamsted. The manurial treatments of the plots in this study range from 35 t ha^{-1} FYM applied annually since 1852 to P K and Mg only since 1843 and also included plots that had inorganic Nitrogen applications made to them reflecting commercial usage. Results indicate that there were influences in the final number of leaves etc. and that differential growth rates when leaf extension was expressed as mm °C day^{-1} could be identified between treatments during the season and between seasons. However the higher rates from the Nitrogen treatments did not appear until partway through the season. Leaf tip to indent measurements also revealed differences between treatments and clearly reflected an increase in length following Nitrogen application, the length of the indent to ligule portion of the leaf increased one leaf earlier.

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Poster Session — Tuesday 30th June 2009
Impact of transient chilling temperatures on individual leaf extension and plant leaf area dynamics in maize

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In northern areas, temperatures below 15 °C frequently occur and reduce maize yield through negative impacts on growth and photosynthesis. Yield reductions associated with early cold events are mainly due to an alteration of the growth of leaves not yet visible during the stress. However, little is known about the relationships between chilling response of growth at the cell, leaf and whole plant levels. To address this question, we investigated the effects of a transient chilling stress in the course of vegetative growth on cell dimensions, kinematic of leaf extension and final leaf area of the whole leaf profile. Plants were grown in a temperate greenhouse (24/20 °C, control treatments T0) and moved into a cold compartment (14/10 °C) for a week either before (T1) or after (T2) tassel initiation. Severe reductions of leaf length were observed for the 3–4 leaves emerging after transfer. These effects were maintained until the topmost leaves in T2 but not in T1, resulting in strong differences of total plant area. Using temperature compensated time unit, kinematic analysis of leaf elongation indicated that the duration of the exponential phase and the elongation rate during the linear phase were reduced for all the leaves emerging after transfer, both in T1 and T2. These results, consistently with measured cell numbers and sizes, suggest that change in leaf lengths is under control of two driving forces affecting the functioning of the leaf elongation zone: direct short-term effects of cold and long-term feedbacks of whorl architecture. The timing of chill affected differentially both processes.

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Poster Session — Tuesday 30th June 2009
Alkaline and acidic α-galactosidase activities are essential during leaf senescence and AtAkαGal3 is a chloroplast alkaline α-galactosidase in *Arabidopsis*

Ruey-Hua Lee (National Cheng-Kung University), Hao-Jen Huang (National Cheng-Kung University)

Leaf senescence involves a complex regulatory networking in response to developmental and environmental signals. We have previously identified a rice leaf senescence-associated gene, *OsAkaGal*, encodes a chloroplast alkaline a-galactosidase in rice. Our previous studies showed that OsAkaGal is involved in the degradation of digalactosyl diacylglycerol on the thylakoid membranes during rice leaf senescence. We extended this finding, and investigated all the related genes in *Arabidopsis* genome. Alkaline and acidic a-galactosidases have same catalytic mechanisms. Both enzyme families capable of hydrolyze a variety of glycosides possessing non-reducing terminal and side-chain a-galactosyl residues. In this study, the expression profiles of transcripts and the relative amount of these two groups of enzymes were examined during different stages of leaf senescence in *Arabidopsis*. We also studied the ultrastructural changes during chloroplast degradation. We also examined whether the encoded proteins for all the putative alkaline a-galactosidase genes in *Arabidopsis* indeed possess alkaline a-galactosidase enzyme activities. The subcellular localization of gene products was also examined. T-DNA insertion mutants were also characterized. From these studies, we suggested that one of *Arabidopsis* alkaline a-galactosidase (AtAkaGal3) is the function ortholog of OsAkaGal. We also proposed that alkaline a-galactosidases though only occupied small portion of total a-galactosidase activities in the leaf tissues, but they could have decisive roles in the speed and progression of leaf senescence process.

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Poster Session — Tuesday 30th June 2009

Controlling leaf growth parameters by altering cell wall characteristics

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The control of organ size is a fundamental problem in biology. Previous investigations in plant biology have revealed that final leaf size is correlated with the maximum rate of leaf expansion, leading to the hypothesis that this represents a causal relationship. I set out to test this theory by manipulating the biophysical extensibility of the plant cell wall, since this parameter is thought to play a key role in restricting plant cell growth.

Using a tetracycline-inducible promoter system in *Nicotiana tabaccum* (tobacco), I have up-regulated the expression of a cell wall protein (expansin) previously shown to have cell wall loosening properties. By applying the inducer at targeted phases of development, before, during and after the maximum rate of leaf expansion, I could investigate the consequences on leaf growth.

My data indicate that the efficacy of expansin action depends on the presence of other factors which are present in a developmentally controlled fashion, so that increased expansin gene expression is only effective in promoting leaf growth during the phase of development corresponding with maximum leaf expansion. This experiment has provided a new insight into potential means of controlling leaf growth.

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