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P6–DEVELOPMENTS IN PLANT BIOLOGY

P6.1

The physiological role of *Arabidopsis* CP12 gene family

P. Singh, C. Raines, (University of Essex)

CP12 is a small nuclear encoded chloroplast protein with a molecular mass of 8 kDa for the mature protein. CP12 has been found in all photosynthetic organism including cyanobacteria, algae and higher plants. In vitro studies have indicated that CP12 forms a multiprotein complex together with PRK and GAPDH and has a role in the regulation of the activity of PRKase and GAPDH. In *Arabidopsis* there are three CP12 gene sequences, one on chromosome 1, CP12-3, on chromosome 2, CP12-1 and on chromosome 3, CP12-2. Sequence alignment analysis showed that CP12-1 and CP12-2 amino acid sequence are almost highly similar (86%) over the entire protein including transit peptide while the mature protein showed 98% similarity. CP12-3 is distinct and has close homology to CP12 from cyanobacteria. Preliminary expression analysis of the CP12-1 and CP12-2 gene of *Arabidopsis* has shown that they are differentially expressed. To extend this work further we studied the expression analysis of all the three CP12 genes of *Arabidopsis* dependent on tissue type, light and under different stress conditions. These studies has found that the CP12-1 showed highest expression in flower buds and expressed in dark while the CP12-2 expressed more in leaves, shoots and light grown tissue, While CP12-3 expressed more in dark grown tissue and under hypoxic condition. To further investigate this we have cloned the 5' upstream sequences for each of the *Arabidopsis* CP12 genes and have made transcriptional fusion constructs with the GUS reporter gene and studied the promoter:GUS expression for all the three CP12 genes of *Arabidopsis*.

To address the in vivo role of the individual members of the CP12 gene family, transgenic tobacco and *Arabidopsis* plants were produced using an antisense construct. *Arabidopsis* antisense plants showed complex phenotype and have some similarity to that of tobacco antisense plants, comparison of the CP12-1,-2 and -3 antisense lines revealed differences in the phenotype, dependent on which CP12 gene was being targeted

for down-regulation. The hypothesis from our work would indicate wider role of CP12 proteins than to regulate the PRK and GAPDH in the Calvin cycle.

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P6.2

Towards in vivo characterization of selected *Arabidopsis* formins

F. Cvrckova, M. Grunt, (Charles University – Prague); D. Pickova, (Academy of Sciences of the Czech Republic); V. Zarsky, (Charles University – Prague and Academy of Sciences of the Czech Republic)

Formins (FH2 proteins) are evolutionarily old actin-organizing proteins. Studying their function in plants is complicated by large gene family size – *Arabidopsis* has 22 formin-encoding loci in two distinct subfamilies (for bibliography see <http://kfrserver.natur.cuni.cz/genes/formins/>). A number of single or double T-DNA insertion mutants lack readily observable phenotypes, indicating functional redundancy.

Unrelated mutations that challenge the actin cytoskeleton might be used to bring about a phenotype in at least some formin mutants. This is documented by segregation ratio distortion in a cross between a mutant lacking the “outlier Class I” formin AtFH12 and a transgenic line expressing the heterologous GFP–talin fusion frequently used to visualize actin. This construct is somewhat toxic even in wild-type background (Ketelaar et al., 2004), and apparently more deleterious in the absence of functional AtFH12. Expression of dominant mutant alleles and/or tagged proteins provides another useful tool, as shown previously e.g. for AtFH8. We observed that both N- and C-terminally GFP-tagged ClassII formin AtFH16 could associate with actin cables when transiently expressed in tobacco leaf epidermis. We have now constructed transgenic *Arabidopsis* lines expressing the same fusion proteins, opening a way

towards comparison of the behaviour of AtFH16 in the heterologous expression system and in the more realistic *Arabidopsis* environment.

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Ketelaar et al., 2004. Plant Physiol. 136, 3990.

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P6.3

Unravelling the roles of two senescence-enhanced MYB transcription factors

N. Warner, E. Breeze, E. Harrison, V. Buchanan-Wollaston, (Warwick HRI)

Senescence is an active and highly regulated process of deterioration occurring in later stages of development that is visible during autumn when leaves change colour and are shed. Efficient senescence enhances the survival of the plant by recycling its nutrients and transferring them to growing regions. It is an adaptive process regulated by a complex network of signals combining internal and external factors. These include nutrient, stress, development, pathogen response and hormonal signals, which require the up and down regulation of specific sets of genes, de novo gene expression and protein synthesis.

Two senescence-enhanced MYB transcription factors have been identified in *Arabidopsis* by microarray analysis and further confirmed by real time PCR. These are MYB90 and MYB75, which have been previously implicated in the flavonoid pathway. The first aim of the projects is to analyse their role in the regulation of anthocyanin biosynthesis during senescence. Changes in the expression of MYB and other flavonoid pathway genes during development and stress, and the downstream effects of a knockout mutation and RNAi interference on gene expression and the production of secondary metabolites are examined. The second aim is to identify upstream factors that regulate their expression by the characterisation of the *cis*-acting elements of the promoter regions required for senescence-specific expression.

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P6.4

Cellular metabolomic chemical imaging for fingerprinting and targeted analysis: The application of focal plane array Fourier-transform infrared microscopy to study plant development

S. Thain, (Environmental Research Institute/UHI); P. Robson, (IGER); L. Mur, (University of Wales Aberystwyth); J. Wilson, (Varian UK)

Fourier-transform infrared (FT-IR) microscopy imaging was used to acquire chemical data from specific tissues at a cellular and sub-cellular resolution. Data gathered from a number of plant developmental processes including embryogenesis and pathogen infections will be presented. Using a mid-infrared sensitive focal plane array detector camera containing a grid of 64×64 detector elements, spectrographic information from 4000 to 900 cm⁻¹ on chemical composition was recorded to yield spatially resolved “chemical images” at a maximum spatial resolution of 5.5 μm from tissue preparations. The results show that FT-IR imaging data is suitable for multivariate data analysis methods to yield both fingerprinting and targeted metabolomic information.

The metabolic processes that occur continually within cells form a complex network of chemical reactions. Metabolomic data is currently obtained mostly at very low levels of spatial discrimination. For instance root and aerial green tissue may be separated for bulk biochemical analysis. Technical issues such as visualisation, manipulation and equipment sensitivity are often limiting factors. Thus even within single organs information from a range of tissues or cell types will be summed during analysis to yield the averaged metabolome. This potentially results in a masking of important chemical patterns that were present in specific tissues or perhaps even just a few cells. Without the ability to resolve this level of spatial discrimination in metabolomic analysis, with quantitatively, reproducibly and with sufficient replication, our understanding of metabolism will be incomplete. FT-IR can help contribute to this important level of information acquisition in many different study systems.

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P6.5

Comparison of xylem flow velocities determined by MRI and a non-invasive heat pulse technique in Golden Alder and Silver Birch

C. Helfter, D. Hand, (Heriot-Watt University); C. Windt, H. Van As, (Wageningen University); M. Mencuccini, (The University of Edinburgh)

Research is currently under way at Heriot-Watt University (Edinburgh, UK) to develop a robust, portable, non-invasive system for field measurements of xylem and phloem sap flow in trees. The system uses a near-infrared laser source ($\lambda=812$ nm) for local application of heat pulses to the stem. The dimensions of the heated area are typically 1 mm high×5 mm wide; the optical power at the sample is ca. 500 mW and causes a localised temperature rise of 5–10 °C. An infrared camera monitors the temporal evolution of the temperature field around the point of heating; thermometric profiles are used for the calculation of flow velocities.

Detection of xylem and phloem by our technique has been demonstrated (Helfter et al.). The performance of the laser-based approach was compared with xylem velocities determined by MRI flow imaging (Windt, etl al.) at the Wageningen NMR Centre (NL). The custom-built MRI system consists of a 0.72 T electromagnet of planar geometry allowing for lateral insertion of the plant and simultaneous MRI and heat pulse measurements. A near 1:1 agreement between MRI and heat pulse xylem flow velocities was found in both Golden Alder and Silver Birch saplings over several night and day cycles.

References:

Helfter, C., Shephard, J.D., Martinez-Vilalta, J., Mencuccini, M., Hand, P.D. *Tree Physiology*. 27, 169–179.

Windt, C., Vergeldt, F.J., de Jager, P.A., Van As, H. *PCE*. 29, 1715–1729.

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P6.6

Early signals in the wound response of *Arabidopsis thaliana*

K. Morker, (Lancaster University)

Various signalling pathways are involved in the response to wounding in plants. However, the mechanisms by which plants initially perceive wounding are largely unknown. We have investigated two early responses to wounding that may represent primary mechanisms for sensing leaf damage. The first of these is the generation of reactive oxygen species (ROS), which have previously been suggested as signals in the wound response. Using histochemical assays and luminescence imaging, we have established that superoxide, hydrogen peroxide and singlet oxygen are all generated in leaves within minutes of wounding. Furthermore, we have identified the primary site of superoxide and hydrogen peroxide generation as the chloroplast, as a direct result of perturbation of photosynthetic electron transport. The major burst of ROS produced in response to wounding is, therefore, light-dependent. In order to investigate more fully the interaction between light and wound stress, we have used microarray analysis to explore wound-induced changes in the *Arabidopsis* transcriptome in the light and in the dark. Whilst both wounding and light/dark shifts have a major impact on the transcriptome, we have identified only a few significant interactions between the two conditions. Secondly, we have identified both molecular and physiological responses to changes in leaf water relations that occur rapidly in wounded leaves. Current experiments are focussed on determining the signals involved in mediating changes in stomatal conductance, transpiration and assimilation by gas exchange measurements in wild type and mutant plants.

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P6.7

Functional characterization of a recombinant *Selaginella* xyloglucan endotransglucosylase/hydrolase and its effect on cell wall mechanics

K. Vissenberg, V. Van Sandt, D. Souslov, J. Verbelen, (University of Antwerp)

The plant cell wall is a solid, yet dynamic structure with a fibrous skeleton composed of cellulose microfibrils that are coated and tethered by xyloglucans. During cell elongation the cooperation of a set of enzyme families enables the wall to grow without losing its strength. Xyloglucan endotransglucosylase/hydrolases (XTHs) are believed to be involved in this ‘wall loosening’. They break and rejoin xyloglucan chains, allowing the cellulose microfibrils to move apart driven by protoplasmic pressure.

With diverse PCR techniques based on conserved domains known in higher plant XTH-genes, a *Selaginella*-specific XTH-sequence has been isolated. *In silico* analysis revealed several features that are characteristic for XTH proteins, identifying the sequence as *SkXTH1*. The protein was heterologously expressed in *Pichia pastoris* to allow its functional analysis. From results obtained by isoelectric focusing of *Selaginella* protein extracts combined with the fluorescent XET assay, SkXTH1 seems to be a member of a protein family in *Selaginella* of at least 4 XTH proteins displaying the endotransglucosylase (XET) activity.

We have studied the effect of SkXTH1 on the mechanical properties of the epidermis of growing onion bulb scales in constant-load experiments. Addition of SkXTH1 to heat-inactivated onion epidermal cell walls restored on average more than 70% of the protein-dependent creep transverse to the cellulose microfibrils that was lost during boiling. Parallel to the net cellulose orientation, no changes in extensibility were measured. These results suggest that XTH can act as a cell wall-loosening enzyme.

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P6.8

Reactive oxygen species produced by NADPH oxidase are involved in pollen tube growth

M. Potocký, V. Žárský, (Academy of Sciences of the Czech Republic); M. Jones, N. Smirnov, (University of Exeter); R. Bezvoda, (Charles University)

NADPH oxidase (NOX) activity is required for plant development, notably for the tip growth of *Arabidopsis* root hairs. However, it is unclear whether this requirement for NOX-derived reactive oxygen species (ROS) is a general requirement for the control of polarized plant cell growth. To investigate this possibility, we examined the role of ROS in pollen tube growth. Tip-localised ROS were detected in growing pollen tubes by chloromethyl dichlorodihydrofluorescein diacetate oxidation, while tip-localised extracellular superoxide production was detected by nitroblue tetrazolium reduction. We have cloned a fragment of pollen-specific tobacco NOX closely related to a

pollen-specific NOX from *Arabidopsis*. Transfection of tobacco pollen tubes with NOX-specific antisense oligodeoxynucleotides (ODNs) resulted in decreased level of NtNOX mRNA and lower NOX activity, inhibited ROS formation and pollen tube growth. ROS scavengers and the NOX inhibitor diphenyleneiodonium chloride inhibited growth and ROS formation in tobacco pollen tube cultures. Exogenous hydrogen peroxide (H₂O₂) rescued the growth inhibition caused by NOX antisense ODNs, suggesting that H₂O₂ mediates the effect of NOX activity on pollen tube growth. Exogenous CaCl₂ increased NBT reduction at the pollen tube tip, suggesting that Ca²⁺ increases the activity of pollen NOX *in vivo*. Our results show that pollen tube growth requires tip-localised ROS produced by a NOX enzyme, and suggest that this is likely to be a general mechanism in the control of tip growth of polarized plant cells.

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P6.9

The targeted early differentiation of functional cells is required for pattern resolution in the *Arabidopsis* epidermis

M. Pullen, K. Lindsey, (University of Durham)

Pattern definition in the shoot epidermis involves the resolution of specific functional cells (stomata and trichomes) from an initial 'field' of undifferentiated cells. In *Arabidopsis*, components of an evolutionarily conserved transcription factor cascade involving MYB and MYC/bHLH proteins, define trichome cells early in leaf development which exit the cell cycle into an endoreduplication phase, and promote guard mother cell fate and terminal differentiation with a final division to form the guard cell pair. Other known stomatal patterning genes encode signal perception and transduction proteins.

The hydra mutants of *Arabidopsis*, deficient in key sterol biosynthetic enzymes, have a seedling lethal pleiotropic phenotype. Mutant trichomes differentiate either as adjacent cells, or appear to result from an incomplete division. Their variably sized stomatal clusters have characteristics of mutants from both the MYB/MYC signal cascade and stomatal signal perception and transduction.

Transcriptional reporters of HYD1 indicate gene activity only at the point of cell cycle exit in root epidermal cells, trichomes and stomata. Therefore, HYDRA metabolic products affect multiple stages of pattern definition within the epidermal cell layer in a cell non-autonomous manner. This implies a role for sterols in pattern resolution both between adjacent cells and at the tissue level. Mutant hydra tissues have elevated cell cycling (pCYC1At::CDB::GUS) activity, indicating a delayed progression to differentiation. The limitation of HYDRA1 activity to non-cycling functional cells in the developing leaf epidermis implies that the timed cell cycle exit of targeted cells within the epidermal field is crucial for pattern resolution in these tissues.

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P6.10

Arabidopsis APP1 is a rate-limiting component of auxin signalling required for activation of DR5-like auxin response elements

A. Murphy, S. Makam, W. Peer, (Purdue University)

Arabidopsis APP1 is a membrane-associated soluble aminopeptidase with activity against N-terminal Arg >> Tyr > X-Pro > Leu residues. APP1 is a single copy gene and APP1 was originally isolated by its weak affinity for the auxin transport inhibitor 1-naphthylphthalic acid (NPA). Enzymatic activity of APP1 is partially inhibited by NPA and the similar auxin transport inhibitor DCPFA. Mutations in APP1 result in drastically reduced growth. Overexpression results in increased growth, enhanced lateral root formation, and decreased seed set. Treatment of app1 with auxin does not restore growth. Treatment of APP1 overexpressors restores wild-type growth. APP1 expression is induced by auxin, wounding, and jasmonic acid. However, IAA treatment in excess of 3 uM results in decreased APP1 protein levels, indicating enhanced turnover. APP1 exhibits affinity for VPP peptide motifs and binds diprolyl motifs in AUX-IAA proteins (IAA1 and 9) with relatively high affinity *in vivo* and *in vitro*. APP1 binding to AUX-IAAs is auxin insensitive. Binding decreases when *iaa1* and *iaa7* mutant peptides are substituted in the pull downs. DR5:GUS activity and transcription are barely detectable in the app1 mutant background, even after auxin treatment. Overexpression of APP1 results in ectopic DR5:GUS expression. However, app1 mutants exhibit no detectable alterations in auxin accumulation or transport. These results suggest that activation of DR5-like auxin response elements by auxin response factors requires APP1-dependent degradation of Aux-IAA repressor proteins. These results suggest that APP1 functions as an activator of AUX-IAA-dependent signalling and auxin-dependent AUX-IAA degradation, either by enhancing IAA-TIR1/AFB binding or enhancing SCF-TIR access to AUX-IAA proteins.

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P6.11

The role of ARR22 and two-component systems during *Arabidopsis* pod development

E. Naomab, Z. Gonzalez-Carranza, J. Roberts, (University of Nottingham); S. Gattolin, (The University of Birmingham); M. Alandete-Saez, (University of California); K. Elliott, (Institute of Food Research, Norwich)

Response regulators are part of a multi-component phosphorylation system and are implicated in playing pivotal roles in modulating plant responses to critical environmental signals such as cytokinin and ethylene.

ARR22 (*At3g04280*) is an atypical response regulator gene, whose function in *Arabidopsis thaliana* is unknown. Time course RT-PCR and Northern blot hybridization analysis reveal transcripts of *ARR22* pre-dominantly in reproductive organs (Gattolin et al., 2006). Furthermore, β -glucuronidase (GUS) reporter gene expression driven by *ARR22* promoter (*ARR22::GUS*) is specifically localized at the seed: funiculus junction. However, GUS activity is primarily visible when pods or seeds are mechanically wounded. Promoter analyses have shown no indication that the 183 bp 5'UTR intron regulates the wound inducibility of *ARR22*. Over-expression of *ARR22* using the constitutive 35SCaMV promoter resulted in extreme dwarf transgenic phenotypes. *ARR22* knock-out (KO) transgenic lines are phenotypically indistinguishable from wild-type individuals under the conditions studied. Double knock-out lines of *ARR22* and *ARR24* are also similar to wild-type plants and whilst these two genes exhibit 66% amino acid similarity there is no evidence that they are functional homologous. Microarray approach has been followed to identify *ARR22* co-regulated genes during pod development. *ARRE* is another gene having 75% amino-acid sequence similarity to *ARR22* but which is structurally highly divergent, having two possible open reading frames. The two open reading frames were over-expressed separately using the constitutive 35SCaMV promoter. Homozygous *35S::ARRE* transgenic lines have similar phenotypic characteristics to wild-type individuals.

Gattolin, S., Alandete-Saez, M., Elliott, K., Gonzalez-Carranza, Z., Naomab, E., Powell, C., Roberts, J.A., 2006. Spatial and temporal expression of the response regulators *ARR22* and *ARR24* in *Arabidopsis thaliana*. *J. Exp. Bot.*, 57 (15) 4225–4233.

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P6.12

Acyl-activating enzyme 2: Part of a developmental fail-safe mechanism?

M. Hooks, J. Turner, B. Grail, (University of Wales Bangor); T. Larson, I. Graham, (University of York)

From a survey of publicly available microarray data, AAE2 displayed expression properties suggesting possible metabolic involvement in seed germination. AAE2 is expressed highly in early developing seedlings, but is also appears highly induced upon seed imbibition. In order to determine possible germination phenotypes, physiological analyses were conducted on two independent homozygous T-DNA insertional mutants in the *AAE2* gene. Both *aae2* alleles germinated and established more rapidly than the corresponding wild-type lines under optimal growth conditions. However, when seedlings were germinated and grown in the dark for six or more days, only a small proportion of the mutants showed normal cotyledon greening

and expansion. Upon infection with powdery mildew, both mutants were able to mature and set seed.

However, seed from these plants exhibited marked reduction in both germination potential and subsequent establishment compared to wild-type, which suggests that metabolic programs mediating recovery from pathogen infection are disrupted in *aae2* mutants. The nature of the phenotypes suggests that AAE2 functions in pathways required for normal development, at least under situations of stress.

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P6.13

Investigating plant-aphid interactions in *Arabidopsis* – The use of functional genomics to investigate the role of plant amino acid transporters

H. Kemp, J. Pritchard, J. Newbury, J. Bale, (The University of Birmingham)

Extensive use of pesticides has led to widespread development of resistance in aphids, consequently posing a problem for the future success of chemical control. The development of the understanding of interactions between aphids and their host plants may help to inform new control strategies. It is hypothesized that aphid performance is limited by the amount of nitrogen in their diet.

Previous work has suggested that altering sieve element (phloem) composition can affect an aphid's acceptance or rejection of the phloem and have effects on growth and reproductive performance. This was investigated using the peach potato aphid, *Myzus persicae*, the mealy cabbage aphid, *Brevicoryne brassicae*, and the model plant *Arabidopsis thaliana*. The effect of an insertional (knock out) mutation in the amino acid permease gene, *AAP1* was determined by measuring whole plant phenotype, aphid feeding behaviour and aphid performance.

Aphid probing behaviour, measured using Electronic Penetration Graphs (EPGs), was significantly altered in *aap1* knockout plants. The mean time taken to reach sustained sieve element ingestion (E2), along with the mean time spent in pathway was significantly greater for aphids feeding on *aap1* plants. This suggests that the disruption to *AAP1* altered the cues used by the aphid to locate the sieve element. Ongoing work regarding the effect of *AAP1* on aphid performance is currently being undertaken.

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P6.14

Functional analysis of candidate wind stress genes using gene silencing by RNA interference (RNAi)

R. Bennett, A. Shirsat, (University of Wales Bangor)

Mechanical stress in plants may be created in nature by wind, rain, and through contact with animals. Wind stressed plants are

typically physiologically dwarfed; characteristics include shorter (and often thicker) stems, reduced leaf surface area and changes in leaf angle. With the exception of vibration, the growth responses to mechanical stress are similar. We have previously found that subjecting *Arabidopsis* plants to wind stress (fans), thigmic stress (touch) and seismic stress (shaking) results in the expected dwarfing of plants with respect to an unstressed control. In contrast, subjecting *Arabidopsis* seedlings or plants to vibration stress promotes growth.

RT–PCR was used to look at gene expression in issues sampled from plants subjected to wind and other mechanical stresses. Candidate wind/mechanical stress genes; genes encoding plant cell wall proteins, genes involved in lignin biosynthesis and those encoding cell signalling pathway intermediates were found to be differentially expressed in plants subjected to these stresses. We are currently using the technique of gene silencing of selected candidate wind/mechanical stress genes by RNA interference (RNAi) to analyse the role of these genes in the response and adaptation of plants to wind stress. RNAi constructs targeting Expansin 3, Cellulose synthase 3, Touch gene 4, Cinnamyl CoA reductase 2, and the Cinnamyl CoA reductase family of *Arabidopsis* have been generated.

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P6.15
Immunolocalization of trehalose-6-phosphate synthase in leaf segments of genetically engineered tobacco plants expressing the *AtTPS1* gene

A. Almeida, D. Santos, S. Araújo, P. Fevereiro, (ITQB); M. Santos, E. Villalobos, J. Torné, (IBMB)

Following the establishment of a transgenic line of tobacco (B5H) expressing the trehalose-6-phosphate synthase (TPS) gene from *Arabidopsis thaliana*, a preliminary immunolocalization study was conducted using leaves of adequately watered B5H and wild-type plants (WT). Immunocytochemical staining followed by electron microscopy showed that the enzyme was detected in both B5H and WT plants at two different levels. Signal quantification showed to be two to three times higher in transgenic plants than in wild-type. This enzyme was markedly present in the vacuoles and the cell wall, and to a lesser extent in the cytosol. Moreover, high profusion of gold particles was detected in adjacent cells and in the sieve elements. Occasional spots were also detected in chloroplasts and nucleus, especially in transgenic B5H line. No labeling signal was detected in mitochondria. Protein localization seems to underline the important role of TPS in sugar metabolism and transport through the plant that can explain their role in plant stress tolerance. Finally, it can be expected that TPS from tobacco to have a relatively high similitude to the TPS of *Arabidopsis thaliana*.

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P6.16
Glycogenins in plants? Starch granule and polymer initiation

A. Kolasa, (John Innes Centre)

The synthesis of starch polymers, amylose and amylopectin, is a well understood process. In contrast, the question of how starch granule or polymer synthesis is initiated still remains open. In animals and yeasts, the storage form of carbon is glycogen, a molecule structurally similar to amylopectin. Glycogen synthesis requires the initiating protein glycogenin that is able to self-glucosylate using UDP-glucose as a substrate. It is possible that glycogenin-like proteins might be responsible for the initiation of amylopectin molecules in plants. Two different approaches have been undertaken to explore this hypothesis. First, sequence similarity searches identified several plant genes similar to glycogenins. To reveal their role, if any, in starch synthesis single, double and triple *Arabidopsis* knock-out mutants for five carefully selected genes were isolated and analysed. Second, we identified a self-glucosylating activity within starch granules. Two starch–granule-bound proteins are able to self-glucosylate in the presence of ADP-glucose. ADP-glucose is also the substrate for glucan chain synthesis. These self-glucosylating proteins are unlikely to be any of the well-characterised starch synthases. For example, mutants lacking major granule-bound isoforms of starch synthase retain both glucosylating activities. Our major goal now is to purify these self-glucosylating granule-bound proteins and obtain protein sequence information. This will enable identification of the genes and further studies of their function.

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P6.17
The *in vivo* activities of phosphoribulokinase and glyceraldehyde phosphate dehydrogenase can be rapidly altered through protein–protein interactions

T. Howard, J. Lloyd, C. Raines, (University of Essex)

There is a requirement during photosynthesis that the consumption of energy (ATP) and reducing power (NADPH) by the Calvin cycle is closely coordinated with their supply from electron transport; this is in order to optimise their use and to prevent their depletion. It has long been established that thioredoxin is capable of the rapid activation of key Calvin cycle enzymes in response to increased supply of energy and reductive power following illumination. Whilst phosphoribulokinase (PRK) and glyceraldehyde phosphate dehydrogenase (GAPDH) are thioredoxin activated, the mechanism(s) for the deactivation of these enzymes, and for the fine-regulation of these processes during light-shade transitions is less well understood. Previously, a regulatory multi-enzyme protein complex has been reported that forms between PRK, GAPDH

and an 8.5 kDa protein, known as CP12. Here we report that the dissociation and re-association of the complex is extremely rapid in response to changes in illumination, that the amount of the protein complex is determined by light intensity, that activity of the electron transport chain is required for complex dissociation and that the *in vivo* activities of PRK and GAPDH correspond closely with the presence or absence of the protein complex. We hypothesise that this protein complex allows the rapid deactivation of these enzymes in response to decreased light intensity, and further to this, that it is in part responsible for the rapid activation of PRK when light becomes available.

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P6.18

The *POLARIS* gene is essential for correct ethylene signalling in *Arabidopsis*

S. Mehdi, K. Lindsey, (University of Durham)

POLARIS (PLS) encodes a 36-amino acid peptide required for correct root growth and vascular development in *Arabidopsis thaliana*. Mutation of *pls* results in an enhanced ethylene-response phenotype and defective auxin transport and homeostasis. These defects can be rescued by inhibition of ethylene signalling but not of ethylene biosynthesis, and *pls* does not over-produce ethylene, indicating a role for the peptide in ethylene signalling. Ethylene as an important regulator of root development and our data suggests that *PLS* negatively regulates ethylene responses to modulate cell division and expansion via downstream effects on auxin signalling thereby influencing root growth and lateral root formation. This mechanism is predicted to involve a regulatory loop of auxin–ethylene interaction. We are investigating the molecular mode of action of *PLS*, and hypothesize that *PLS* may directly interact with the components of ethylene signalling pathway. We are using Yeast Two Hybrid and proteomics analysis to address this possibility, and, in addition, transcriptional analysis will be used to identify the downstream targets regulated by *PLS*.

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P6.19

Hot plants: The physiology and behaviour of thermoregulatory flowers

R. Seymour, (University of Adelaide)

The flowers, inflorescences and cones of several groups of early seed plants produce heat during blooming. Some species are able to regulate heat production and maintain relatively constant floral temperatures in widely variable environmental tempera-

tures. This presentation explores temperature regulation in plants from its initial discovery to current experiments. We have examined the phenomenon at different levels of organization, from the molecular control of heating to its ecological significance. Respiration rates can be extraordinarily high, and floral temperature can reach 35 °C above the air. The control mechanism is still unknown, but investigations on gene expression, selective inhibitors and isotope fractionation reveal involvement of the alternative oxidase (AOX) and uncoupling protein (UCP), depending on the respiratory substrate (carbohydrate—AOX; lipid—UCP). Oxygen is supplied by diffusion, and the morphology of the pathway is described in *Philodendron selloum*. Thermogenesis is associated with scent production in most species, but temperature regulation continues past the attractive phase and is associated with insects (chiefly endothermic beetles) that reside for a day in a floral chamber, where they eat, digest and mate. Measurements of the energy costs of insect activity show that as little as 4 °C elevation in floral chamber temperature in *Philodendron solimoesense* can reduce the costs of activity 5-fold. Floral temperatures (ca. 32 °C) in the Amazon water lily *Victoria amazonica* approximate preferred activity temperature of its scarab beetle pollinator (*Cyclocephala* sp.). Thus thermogenic flowers can provide a direct energy reward to insect visitors, and temperature regulation may be associated with it.

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P6.20

Endophyte assisted phytoremediation of trichloroethylene (TCE)—An environmental contaminant

Z. Khan, (University of Washington)

Trichloroethanol (TCE) is the most common environmental pollutant at many of the hazardous waste sites. TCE pollution became prevalent primarily through the use as an industrial degreasing agent. It is persistent in the environment and has toxic effects to kidney, liver and the central nervous system. Due to its widespread contamination finding innovative ways to clean this pollutant has become a priority in the remediation field. Phytoremediation, the use of plants for the restoration of environments contaminated with pollutants is a relatively new technology that is more benign than current engineering solutions to treat contaminated sites. Endophytes are microbes that live within plants. The benefits of combining endophytic bacteria with plants for increased remediation of pollutants has been successfully tried for toxic metal removal from contaminated soils. In our studies we screened several varieties of sweet potato (a hardy plant) for TCE uptake and the results show good removal rates from hydroponics and production of the early metabolite, trichloroethanol (TCEOH) by the sweet potato plants. The percentage removal was higher than wild poplar plants which makes it a potential phytoremediation plant. We are in a process of identifying the endophytic bacteria of

sweet potato and determine if they help the plants either in metabolizing the pollutant or in contributing to general plant health. It is hoped that not only will plants be able to efficiently remove environmental contaminants they will also provide a source of income after the remediation project is complete, thereby making the process cost effective.

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P6.21

Response of wild C₄ crop progenitors to subambient CO₂ highlights a possible role in the origin of agriculture

J. Cunniff, C. Osborne, M. Charles, G. Jones, (University of Sheffield); B. Ripley, (Rhodes University);

The synchronous origin of agriculture in at least four independent climatic regions at the end of the glacial period points to a global limiting factor for crop domestication. One hypothesis proposes that a rapid CO₂ increase from 180 ppm to 280 ppm during deglaciation caused a significant increase in growth rates of wild crop progenitors, thereby removing a productivity barrier to their successful domestication. However, early C₄ crops present a challenge to this hypothesis, because they were among the first domesticates, but have a carbon-concentrating mechanism that theoretically makes photosynthesis insensitive to CO₂. We investigated the CO₂-limitation hypothesis using a set of five C₄ and one C₃ crop progenitors, all 'founder crops' from distinct centres across the globe. Plants were grown in controlled environment chambers at glacial (180 ppm), postglacial (280 ppm) and current ambient (380 ppm) CO₂ levels. Growth rates and phenology were tracked, and photosynthetic and transpiration rates measured, before the plants were harvested at set developmental stages for biomass measurements. An increase in CO₂ from glacial to post glacial levels caused a significant gain in biomass of up to 50% in C₄ crop progenitors. Investigation into the underlying mechanisms showed C₄ photosynthesis to be limited by low CO₂ levels. The same increase in CO₂ caused a significant reduction in transpiration rate through a ~30% decrease in stomatal conductance, showing that CO₂ may have a further role in water relations. Our data provide experimental support for the CO₂-limitation hypothesis, and highlight a possible role for global atmospheric change in the origin of agriculture.

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P6.22

Control of seed dormancy and germination by environmental signals

S. Penfield, I. Graham, (University of York); K. Halliday, (University of Edinburgh)

Seeds of most temperate plant species exhibit dormancy and germinate only after specific cues from the environment such as temperature, light and nutrient availability. These signals control the levels of gibberellin (GA) and abscisic acid (ABA) in the seed, and these hormones in turn regulate the transition from dormancy to germination. How the signals of these two hormones are integrated in the seed and used to break dormancy has remained unknown for many years.

Here we show that in *Arabidopsis* the DELLA protein regulators of the GA response are required for seed dormancy and propose a model for the way these signals regulate germination. We show that dormancy correlates with cotyledon expansion in seedlings in 15 DELLA mutant combinations, and that known seed dormancy mutants all have previously unrecognised cotyledon expansion phenotypes in seedlings. Our data further shows that environmental signals, GA and ABA regulate cotyledon expansion inside the seed coat. We propose a model in which environmentally controlled cotyledon expansion breaks dormancy by breaking the seed coat, and that this initiates events subsequently leading to seed germination.

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P6.23

Biochemical regulation of tomato fruit growth

S. Sladjana, B. Vucelic-Radovic, R. Stikic, (University of Belgrade)

Tomato is a crop of worldwide economic importance, and the factors controlling its fruit growth attracted considerable research interest. Recent results suggested importance of cell wall peroxidase activity in regulation of fruit growth. The aim of this paper was to investigate the effects of new irrigation techniques (partial root-zone drying [PRD] and deficit irrigation [DI]) on the peroxidase activity in the exocarp of tomato fruit and to compare these results with the results obtained for fully irrigated [FI] plants. This could be beneficial for understanding the effects of these irrigation techniques on tomato fruit growth and yield. Tomato plants (*Lycopersicon esculentum* L., cv. Sunpak) were raised from seed in compost- (Potground H, Klasmann-Deilmann, Germany) filled seed trays in a growth chamber (photoperiod was 14 h; light intensity at plant level 300 μmol m⁻² s⁻¹, temperature 25/18 °C and relative humidity 70%). The enzyme activity was determined by a guaiacol test, detailed by Chance and Machly (1955). Obtained results showed strong correlation between peroxidase activity and growth termination. Generally, the peroxidase activity was significantly higher in DI and PRD treatments compared to those of FI, pointed out on the significant role of this enzyme in tomato fruit growth.

Keywords: Tomato; Peroxidase activity; Partial root drying (PRD); Deficit irrigation (DI); Dull irrigation (FI)

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P6.24**Use of RAPD in assessing genetic variability in *Tilia cordata* to facilitate appropriate reestablishment of native trees**

A. Mylett, S. Brown, C. Casey, (University of Lincoln)

Lincolnshire contains some of the most important examples of small-leaved lime woodland in Britain. The trees have been managed since the 11th century by coppicing and many of the trees studied are several hundred years old.

The genetic diversity of small leaved lime (*Tilia cordata*) is being investigated using RAPD markers to estimate the genetic relationships between trees. The RAPD technique provides a convenient method of assessing the differences in the genetic composition of related individuals when there is no DNA sequence information available. Initial results of RAPD analysis indicate that the trees from two separate woodland areas although largely similar show two separate clusters. This suggests that RAPDs will be a suitable tool in this study and will show how the genetic variation of the trees is affected by their location not only in their own wood but also within nearby ancient woodlands.

Tissue culture techniques are also being developed to enable the successful micro-propagation of *T. cordata*. Factors such as sterilization procedures, plant growth regulator concentrations, temperatures and day length are being manipulated to produce optimum conditions for the production of explants. Using the plant growth regulators 6-benzylaminopurine (BAP) and naphthaleneacetic acid (NAA) it has been possible to induce both root and leaf material. Using tissue culture techniques to produce new seedlings would enable the woods to be managed in a way that maintains the inherent genetic integrity of the *Tilia cordata*.

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P6.25**Bioinformatics: A tool to explain gene diversity**

R. Gaur, (Mody Institute of Technology and Sciences); R.K. Gaur, K.P. Sharma, V. Mishra, (Department of Biotechnology, Faculty of Arts, Science and Commerce, MITS, Lakshman-garh-332311, Sikar, Rajasthan, India)

Sequencing technology has now reached such a level of sophistication that it is quite common for a large stretch of DNA to be sequenced and for that sequence to be manipulated/stored in a computer database. It is possible once a nucleotide/protein sequence has been deduced to search an existing database for a similar, homologous, sequence and for generic gene or protein coding region. It is more relatively straightforward to use sequence analysis software to search a new sequence for identity within a chosen database.

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P6.26**Genetic modification of plant stature by manipulation of gibberellin metabolism: An alternative to chemical growth regulators**

A. Bhattacharya, M. Davey, B. Power, (University of Nottingham); D. Ward, A. Phillips, P. Hedden, (Rothamsted Research)

Gibberellins (GAs) are endogenous plant hormones that control key aspects of growth and development. Chemical growth retardants that inhibit GA biosynthesis are used extensively in horticulture to modify plant stature, increasing production costs, manpower and associated environmental risks. An alternative strategy is genetic manipulation of GA metabolism in plants to induce similar phenotypic changes. Two species, *Solanum nigrum* and *Nicotiana glauca*, have been employed as targets for *Agrobacterium*-mediated gene delivery. The constructs used in this study contained the CaMV 35S promoter driving GA-biosynthetic genes including *MmGA3ox1* and *MmGA3ox2* from *Marah macrocarpus*, *AtGA20ox1* from *Arabidopsis thaliana* and *des* from the fungus *Gibberella fujikuroi*, which may increase bioactive GAs and promote plant growth. The *PcGA2ox1* gene from *Phaseolus coccineus* may decrease the concentrations of bioactive GAs by deactivation, so decreasing stature. Double transformations of both species with 35S::*MmGA3ox1*+35S::*MmGA3ox2*, 35S::*MmGA3ox1*+35S::*AtGA20ox1* and 35S::*PcGA2ox1*+35S::*des* genes are also being carried out in order to evaluate the combined effect of both genes in modifying GA metabolism. Both species have been transformed with *MmGA3ox1*, *MmGA3ox2*, *PcGA2ox1*, *AtGA20ox1* and *MmGA3ox1*+*MmGA3ox2* genes; gene presence and expression are being confirmed by PCR and RT-PCR, respectively. GC-MS analyses are being undertaken on transgenic plant tissues to determine the changes in the contents of precursor, bioactive and deactivated GAs. Increase in stature has been observed in *S. nigrum* transformed with 35S::*MmGA3ox1*, 35S::*MmGA3ox2* and 35S::*MmGA3ox1*+35S::*MmGA3ox2* genes. Modification of plant stature by such a transgenic approach may have application in commercial horticulture.

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P6.27**Sugar beet plant–water uptake and plant–water relationships under saline growth conditions**

A. Dadkhah, H. Moghtader, (Ferdowsi University)

Growth and water uptake both decreases when sugar beet plants are irrigated with saline water. To determine the relative condition of physiological traits to these decreases plant fresh and dry weight, plant leaf area, number of leaf per plant, leaf

water (ψ_w) and osmotic (ψ_p) potentials, gas exchange parameters, leaf chlorophyll and Na^+ content were investigated in the sugar beet (*Beta vulgaris* L.) plants, cvs Madison and 7233-P₂₉. Plants were grown in greenhouse condition, in sand culture, and irrigated with a complete nutrient solution supplied with 0 (control), 50, 150, 250 and 350 mM $\text{NaCl} + \text{CaCl}_2$ (in 5:1 ratio), over a period of 2 months. Salinity reduced plant dry weight, height and (reduction of plant leaf area and stomatal density) and physiological changes [reduction of stomatal conductance, transpiration and net CO_2 assimilation (A_{CO_2})]. Leaves appeared healthy and chlorophyll content per unit leaf area increased with increasing salinity. Although reduction of net A_{CO_2} at low levels of salinity can be attributed to stomatal conductance and stomatal density, at high levels of salinity non-stomatal factors cause reduction in net A_{CO_2} . Photosynthetic ability was inversely related to the concentration of either Na^+ or Cl^- in the leaf laminae sampled at the end of experimental period.

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P6.28 **Optimisation of micropropagation media for Malaysian banana (*Musa* spp.)**

P. Sipeen, P. Anthony, M.R. Davey, J.B. Power, (University of Nottingham)

The current study evaluates the *in vitro* responses of an economically important Malaysian banana cv. Pisang Nangka AAA to different combinations and concentrations of 6-benzylaminopurine (BAP) and indole-3-acetic acid (IAA) in Murashige and Skoog (MS)-based media. Currently, there are no reports on the successful micropropagation of this cv. and which focus specifically on the synergistic effects of plant growth regulators (PGRs). Data on shoot-tip explants survival, and shoots, leaves and roots formation were taken at 28 days after culture (DAC). Mean survival rates for cultured explants of $87.5 \pm 12.5\%$ to 100% were observed for all treatments. The highest mean number of shoots generated per explant was 5.46 ± 0.22 (20 mg l^{-1} BAP and 0.175 mg l^{-1} IAA). The highest mean number of leaves and roots per explant was 5.15 ± 0.16 and 12.40 ± 0.64 , respectively in control treatment (medium without PGRs). The shoots, leaves and roots size decreased with increasing concentration of BAP and with a fixed concentration number of leaves. Leaf ψ_w and ψ_p decreased with 0.175 mg l^{-1} IAA. The roots formation was totally salinity but leaf turgor pressures were significantly higher in salinised than control plants which suggests that bulk tissue turgor did not limit growth under salinity. Increasing salinity in the irrigation solution led to both morphological changes inhibited at 10–30 mg l^{-1} BAP and 0.175 mg l^{-1} IAA. MS medium with 20 mg l^{-1} BAP and 0.175 mg l^{-1} IAA will be used for shoot multiplication of this banana cv. Rooting of shoots (100%) was induced on a PGRs-free medium at 28 DAC. This micro-

propagation protocol will be a foundation for future studies on cryopreservation of banana germplasm.

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P6.29 **Galactose from the legume root cap: Structure, signal, toxin, trigger?**

M. Hawes, R. Celoy, I. Price, F. Wen, J. Ebolo, (University of Arizona)

An acidic beta-galactosidase activity was found to be localized in border cells of pea, bean, alfalfa, barrel medic, sorghum, and maize, and to be secreted into the extracellular environment. No activity was detected in radish and *Arabidopsis*, which do not produce viable border cells. A heterologous probe from a tomato galactosidase was used to identify a full length cDNA clone (BRDgal) from a pea root cap library. Whole mount *in situ* localization (WISH) was used to document that BRDgal mRNA expression is localized in peripheral cells of the root cap as they undergo border cell separation, and in detached border cells; no expression occurs within the body of the root. Multiple efforts to develop viable hairy root clones expressing BRDgal antisense mRNA were unsuccessful, suggesting that inhibiting expression of this enzyme is lethal to development. Galactose, which comprises up to 40% of the root cap mucilage where BRDgal is found and can act as a species-specific chemoattractant and nutrient for microorganisms, was reported by Knudson (1919) to be toxic to plant roots. We confirmed that galactose inhibits root growth, and its effects on cell viability, development, and growth were systematically compared with those of other primary and secondary metabolites encountered by root tips during penetration of the soil environment. Galactose is among the most potent metabolites in its effects, and may constitute a key signal for root cap control of growth and development. Future studies to establish whether cell death occurs by toxicity or by the induction of programmed cell death are warranted.

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P6.30 **Production, purification and characterization of 10 *Arabidopsis* xyloglucan endotransglycosylases/hydrolases (XTHs)**

K. Vissenberg, A. Maris, J. Verbelen, (University of Antwerp)

Xyloglucan endotransglycosylase/hydrolases (XTHs) are a class of enzymes that have the ability to cleave and rejoin xyloglucan chains and are considered to play a key role in both the construction and disassembly of the cell wall architecture.

The *Arabidopsis* genome encodes an XTH-family of 33 highly similar members. They share a conserved motif that is predicted to be necessary for their activity, as well as N-linked glycosylation sites and a putative signal sequence for translocation to the cell wall. The high degree of sequence similarity between these 33 *XTH* genes indicates that their proteins are likely to have analogous biochemical properties. However, the evolution of a large collection of related enzymes suggests the potential for diverse physiological functions.

Analysis has revealed that many members of the *XTH* gene family exhibit distinct organ- or tissue-specific expression profiles, but their exact functions remain largely unclear. To gain insight into their functional diversity we heterologously produced 10 isozymes in *Pichia pastoris* that are known to be predominantly expressed in roots.

The pPICZa-expression vectors are constructed in a way so that the yeast cells secrete the XTH proteins into the medium. Media are analyzed by means of SDS-PAGE, western blotting and a specific fluorescent xyloglucan endotransglycosylase (XET) activity assay. When the protein mix contains the active heterologous XTH protein, the sample is subjected to ion exchange chromatography, followed by a size exclusion column for final purification. Once a proper purification level is reached, the proteins are tested on their enzymatic characteristics.

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P6.31

Cytoskeleton and cell wall changes during the biphasic growth of dark-grown *Arabidopsis thaliana* Col-0 hypocotyls

K. Vissenberg, J. Van Orden, J. Verbelen, (University of Antwerp); S. Pelletier, T. Desprez, S. Vernhettes, H. Höfte, (INRA Versailles)

Dark-grown hypocotyls of *Arabidopsis thaliana* (Col-0) elongate in two phases. At 24 h after imbibition, cell elongation was initiated in all cells of the hypocotyls. Until 48 h all cells showed a comparable slow growth rate and reached a similar length of about 38 μm at 48 h. In the second phase (48–50 h), the growth of basal cells accelerated, while the cells at the top maintained at the slow elongation rate. During the next hours the growth acceleration propagated, following an acropetal gradient.

Changes in the cytoskeleton and in the cell wall were studied using MAP4-GFP and fimbrin actin-binding domain (FABD2)-GFP plants, and Field Emission Scanning Electron Microscopy (FESEM) and CESA3-GFP and CESA6-GFP plants respectively. At the beginning of the slow elongation phase, microtubules (CMTs) and microfibrils (CMFs) showed a random orientation. During the slow elongation phase and the transition to the fast elongation phase the CMTs and CMFs remained predominantly perpendicular to the elongation axis.

When cell elongation ceased, a reorganization occurred to an oblique and eventually to a longitudinal orientation.

The actin cytoskeleton changed in three steps: 1) at the start of the slow elongation, thick actin cables were found, especially around the nucleus; 2) just before the start of the fast elongation, there was a fine network of actin cables throughout the whole cytoplasm; 3) at the beginning of and during fast elongation, thick longitudinal cables were detected together with a fine network of actin cables throughout the cell.

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P6.32

QTL mapping for partial resistance to root knot nematode in *Oryza sativa*

R. Shrestha, M. Wilson, A. Price, (University of Aberdeen)

Rice (*Oryza sativa*) is the oldest grown cereal and the most important crop for half the world's population, predominantly in Asia, where 90% of the world's rice is grown. *Meloidogyne graminicola*, rice root knot nematode is a major pest of rice in Asia and West Africa for which resistant varieties are not currently available. Two sub species of *O. sativa* (*japonica*=Azucena and *indica*=Bala) have been used to create a mapping population at the University of Aberdeen and the two parent strains were found to differ quantitatively in their susceptibility to *M. graminicola*. *M. graminicola* also had an effect on yield of these two rice varieties. The total seed weights and root weight of Azucena were significantly ($P < 0.05$) reduced in the presence of nematodes. Gall numbers were estimated for 144–156 RILs 2 and 4 weeks after inoculation. A total of five and two significant or putative QTLs (LOD score =3.2 or =2.4, respectively) for nematode resistance were detected in the two experiments. The QTL on chromosome 6 was detected in both experiments. For two of the QTLs detected, *Azucena* was the donor of the resistant alleles, suggesting it will be possible to breed plants with greater resistance than the more resistant parent. Partial resistance genes are thought to be non-specific and effective against all races of pathogens and therefore are valuable for development of durable resistant varieties.

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P6.33

Quantitative analysis of amino acids composition in the sap of individual sieve elements of wheat (*Triticum aestivum* L.)

S. Gattolin, J. Newbury, J. Bale, J. Pritchard, (University of Birmingham); D. Barrett, (University of Nottingham)

The phloem of a plant consists principally of specialised cells called sieve elements, organised in sieve tubes that allow the

redistribution of organic nutrients, mainly sucrose and amino acids, from source tissues to sink tissues. A sieve tube is currently thought to function as a semi-impermeable pipe within which the mass flow of solutes is driven by a pressure gradient from regions of high solute concentration to regions of low concentration. While various studies focused on comparing phloem sap composition between plant species, or between different individuals at different stages of growth or under different environmental conditions, little is known about within-plant variation in the nutrient composition of phloem sap. By combining aphid stylectomy and Light induced fluorescence capillary electrophoresis (CE-LIF) we analysed the amino acid composition of individual sieve elements in wheat plants. Our results showed that whilst the relative abundance of individual amino acids was comparable in all samples analysed, a variation of up to 10-fold in total amino acids concentration could be measured from an individual sieve tube to another. Sap amino acid composition from individual sieve tubes was monitored over a period of 5 h, and preferential increase of some amino acids but not others was observed over this period of time, suggesting a time of the day-dependent selective loading of amino acids at the source tissue.

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P6.34

Pressurisation and gas flow within the sacred lotus, *Nelumbo nucifera*

P. Matthews, (Adelaide University)

Like many emergent aquatic macrophytes, the sacred lotus (*Nelumbo nucifera*) has overcome the problems associated with growing in flooded anoxic sediments by facilitating the movement of oxygen to its submerged organs through an interconnected system of gas canals. The lotus enhances gas movement by using pressure generated within its leaves to drive convective flow through its gas canals, down a pressure gradient. The mechanism of pressurisation was examined on plants growing in the Botanic Gardens of Adelaide. The environmental conditions of relative humidity, temperature, leaf temperature and solar radiation were recorded and regressed against leaf pressure measured within the petiolar gas canals. The difference between ambient and leaf water vapour pressure was demonstrated to be the most important factor in generating leaf pressure, with leaf temperature contributing to pressure by further increasing the water vapour pressure within the leaf. Patterns of convective flow generated by the leaves were measured using nitrogen injected into the petiolar gas canals and detected upstream or downstream with implantable oxygen probes. This information was related to stomatal aperture and gas canal anatomy to produce a complete picture of gas transport within the sacred lotus.

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P6.35

How do variable temperatures affect alpha-amylase in developing wheat grains?

A. Farrell, P. Kettlewell, (Harper Adams University College)

Environmental conditions during grain filling can have dramatic effects on grain development. Adverse conditions can result in the accumulation of alpha-amylase long before germination is initiated. Temperature fluctuations in the later stages of grain filling have been proposed as a key factor in stimulating the production of premature alpha-amylase, but the mechanisms involved have yet to be established.

A central question is, whether temperature acts directly on alpha-amylase production or indirectly by altering aspects of grain morphology. We examined the direct effects of cold and heat-shock on alpha-amylase through a series of controlled environment experiments. A comparison was made with plants grown under stable temperature conditions but with grain development altered by increasing assimilate supply using partial degrading. We used a recently developed ELISA assay to monitor alpha-AMY-1 (high pI alpha-amylase) during grain development and assess the impact of the two treatment regimes. Differences in grain morphology were assessed using image analysis (ImagePro Plus).

The results show a clear effect of fluctuating temperature on alpha-amylase levels with significant genotypic variation in the response. Degraining produced larger grains in many genotypes but did not result in higher alpha amylase levels. The results do not suggest a link between grain morphology and alpha amylase.

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P6.36

A genetic approach towards modulating pod-shattering in oilseed rape

T. Wood, (John Innes Centre)

Unsynchronized pod-shattering in oilseed rape (*Brassica napus*) is a serious agricultural and economic problem, with lost revenues of an estimated £42 million per annum for the U.K. alone. Therefore efforts to reduce this loss will provide benefits both for the society and the environment. A Random Impact Test (RIT) has been devised to measure the difference between levels of pod-shatter resistance (PSR) in *Brassica*. Material from a *B. napus* mapping population has been found to segregate for PSR and to contain a wide range of shatter susceptible, intermediate and resistant lines. Compared to the common cultivar 'Apex' (one of the parents for this population), some lines exhibited increased PSR of 6–8 times. PSR is perceived to be associated with development of the dehiscence zone (DZ) and potentially with an increase in the amount of vasculature in the pods. Continued efforts to

develop a molecular map, for this population will facilitate the isolation of genes/QTL associated with PSR and provide a valuable tool for marker assisted selection (MAS). A candidate gene approach using genes with known function in *Arabidopsis* fruit development is being adopted to investigate the genetic regulation of DZ development in *B. napus*. Previously, two QTL potentially linked to increased PSR in *B. napus* have been identified using a relatively small data set. Further analysis using data obtained during this study will be used to validate these and also to try to identify new QTL.

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P6.37

Towards understanding the physiological basis of root penetration QTLs in rice

L. Clark, R. Whalley, (Rothamsted Research); A. Price, (University of Aberdeen)

Strong soil due to soil compaction or drying can be a major constraint to root growth. Rice is an ideal model system for the study of root responses to mechanical impedance as there is evidence for cultivar differences in root penetration. Several quantitative trait loci (QTL) controlling root penetration have been identified in a Azucena × Bala mapping population (Price et al., 2000). Here, we present evidence from pairs of near-isogenic lines that differences in root penetration of wax layers are due to differences in bending stiffness. Root bending stiffness is strongly dependent on root diameter, with an approximately fourth power relationship. Studies of this kind have the potential to help elucidate the mechanism of action of root penetration QTLs.

Reference:

Price et al., 2000. Theor. Appl. Genet. 100, 49–56.

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P6.38

Brassinosteroids protect plants against heat stress

A. Confraria, (University of Porto and University of the West of England); R. Desikan, (Imperial College London); I. Santos, (University of Porto); S. Neill, (University of the West of England)

Brassinosteroids (BRs) are plant steroid hormones that control a wide range of processes such as cell division and expansion, xylem differentiation, seed germination, vegetative growth and apical dominance (Sasse, 2003). BRs have also been reported to increase plant resistance to biotic and abiotic stresses. However, the mechanisms underlying such protective properties of BRs remain largely unexplored. We have observed that 24-

epibrassinolide (EBR) could protect in vitro grown potato plants from heat stress. It is also apparent that EBR effectiveness in protection is affected by the concentration of ethanol – the solvent for EBR – in the growth medium. Protective effects of EBR against heat stress have also been detected in *Arabidopsis thaliana*, in keeping with a recent report by Kagale et al. (2007). In order to search for potential explanations for these effects of EBR, the effects of EBR on enzymatic antioxidants were determined; no significant effects were observed. Subsequently, a proteomics approach was taken. After 14 days of growth in modified MS medium supplemented with 20 nM EBR, potato plants were heat-stressed and leaf samples were harvested for protein extraction. Proteins extracts were then separated by 2D gel electrophoresis and differentially regulated proteins were selected for identification by mass spectrometry. Data on specific proteins will be presented and discussed.

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Kagale, S., et al., 2007. Planta. 225, 353–364.

Sasse, J.M., 2003. J. Plant Growth Regul. 22, 276–288.

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P6.39

Metabolism of fructans during the maturation of wheat kernels

A. Paradiso, E. Greco, L. De Gara, (Dep. Plant Biology and Pathology University of Bari, Italy); M. D'Egidio, C. Cecchini, (CRA Rome, Italy); C. Corradini, (Dep. of Chemistry University of Parma, Italy)

Fructans are polysaccharides occurring in 15% of flowering species; their structure is specie-specific, varying in glycosidic linkage, branching and degree of polymerization. In plants, fructans are known to be involved in the responses against hydric and termic stresses. Moreover, they recently received much attention in food science as prebiotics.

Fructans metabolism was investigated in wheat kernels. It has previously been shown that kernels are particularly rich in fructans during the first period of maturation (9–17 days form anthesis); whereas, their content decrease as the development proceeded (De Gara et al. 2003). In this study the changes in the levels and polymerization degree of the fructans stored in different kernel tissues has been further investigated. The variations in the activity/expression of the enzymes responsible for fructan synthesis and hydrolysis have also been analysed at different phases of kernel maturation.

Our results suggest that wheat are able to synthesise fructans in the kernels and that the enzymes involved in the biosynthesis or hydrolysis change their expression and/or activity during kernel development, according to the variations of fructan contents observed in kernel tissues.

De Gara, L., de Pinto, M.C., Moliterni, V.M. D'Egidio, M. G., 2003. Redox regulation and storage processes during maturation in kernels of *Triticum durum*. *J. Exp. Bot.* 54, 249–258.

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P6.40

Cellular metabolomic chemical imaging for fingerprinting and targeted analysis: The application of focal plane array Fourier-transform infrared microscopy to study plant development

S. Thain, (Environmental Research Institute/UHI); L. Mur, (University of Wales Aberystwyth); P. Robson, (IGER); J. Wilson, (Varian UK)

Fourier-transform infrared (FT-IR) microscopy imaging was used to acquire chemical data from specific tissues at a cellular and sub-cellular resolution. Data gathered from a number of plant developmental processes including embryogenesis and pathogen infections will be presented. Using a mid-infrared sensitive focal plane array detector camera containing a grid of 64×64 detector elements, spectrographic information from 4000 to 900 cm^{-1} on chemical composition was recorded to yield spatially resolved “chemical images” at a maximum special resolution of $5.5 \mu\text{m}$ from tissue preparations. The results show that FT-IR imaging data is suitable for multivariate data analysis methods to yield both fingerprinting and targeted metabolomic information.

The metabolic processes that occur continually within cells form a complex network of chemical reactions. Metabolomic data is currently obtained mostly at very low levels of spatial discrimination. For instance root and aerial green tissue may be separated for bulk biochemical analysis. Technical issues such as visualisation; manipulation and equipment sensitivity are often limiting factors. Thus even within single organs information from a range of tissues or cell types will be summed during analysis to yield the averaged metabolome. This potentially results in a masking of important chemical patterns that were present in specific tissues or perhaps even just a few cells. Without the ability to resolve this level of spatial discrimination in metabolomic analysis, with quantitatively, reproducibly and

with sufficient replication, our understanding of metabolism will be incomplete. FT-IR can help contribute to this important level of information acquisition in many different study systems.

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P6.41

Adhesion system of *Enteromorpha* spp.: Preventing zoospores from attaching

K. Mühlenbruch, A. Kesel, (University of Applied Sciences Bremen)

Fouling (benthic organisms like algae, barnacles, bacteria and mussels grow on any wet surface) is a huge problem in naval industries, that causes billions of extra costs every year. To prevent organisms from growing on, for example, ship hulls, anti-fouling substances, such as the extremely toxic and at least in EU forbidden tributyltin (TBT), are applied. To find nonpolluting substances the fouling organisms has to be known like the green macroalgae *Enteromorpha* spp which is one of the most harmful fouling organisms in coastal waters and prefers surface topographies close to its own size as zoospore which is around $5 \mu\text{m}$ (Callow et al., 2002). Experiments in summer 2006 with a possible nonpolluting anti-fouling substance showed contrary to this, that on any of the test panels with surface topographies of 0, 1, 3, 6, 9, 76 and $200 \mu\text{m}$ deep randomising structure and $256 \mu\text{m}$ deep linear structure (soft silicone shore $A=26$ and a low surface energy $<25 \text{ mN m}^{-1}$) *Enteromorpha* grew after 5, 10, 15 and 23 weeks in a North Sea harbour. Next to this the test panels showed no fouling except for some barnacles. The used material seems to have an ideal combination of microstructure, elasticity and surface energy to prevent fouling of any organism. In addition, especially in the case of *Enteromorpha* spp., there is no special microstructure needed at all. So this is a big step towards nonpolluting anti-fouling substances.

Callow et al., 2002. Biofouling.

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