



ELSEVIER

Abstracts / Comparative Biochemistry and Physiology Part A 137 (2004) S199–S220

www.elsevier.com/locate/cbpa

CBP

Society for Experimental Biology Annual Main Meeting
29th March–2nd April 2004, Heriot-Watt University, Edinburgh, UK

P5–GENERAL PLANT BIOLOGY

Organised by Richard Napier for the Plant Section

**P5.01 Genetic analysis of seed vigour in
Brassica oleracea L**

W.E. Finch-Savage, G.J. King and J.R. Lynn

Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

The ability of seeds to germinate and establish seedlings under a range of conditions (seed vigour) has a direct contribution to the economic success of commercial crops. There have been many studies on the influence of the environment on seed vigour during production and subsequent storage, but little is known of the genetic basis of differences in seed vigour. We are studying natural variation in the vigour of *Brassica oleracea* seeds to investigate the genetic basis of a number of germination and pre-emergence seedling growth traits that are associated with seed vigour. We have screened two segregating populations of doubled haploid *B. oleracea* lines for which there is an aligned molecular map. Heritability of the various traits was typically in the 10–15% range, with up to 25% in some traits. Quantitative trait loci (QTL) analyses were carried out on all measurements and revealed significant QTL on several linkage groups. Significant QTL for germination rate on linkage groups O1 and O3 have been fine mapped using backcross substitution lines. The germination behaviour of a substitution line with a single small introgressed region on linkage group O1, which accounts for much of the variation in germination rate, has been characterised. Reciprocal backcrosses have shown the effect on germination rate is embryo rather than seedcoat based. Apparent collinearity between this region of the *B. oleracea* genome and *Arabidopsis thaliana* chromosome 3 is being utilised to identify candidate genes for their potential contribution to future seed vigour testing.

**P5.2 ROCO, a novel component of the
photoperiodic flowering pathway**

J. Holmes^{1,2}, K. Morris¹, L. Codrai¹, A. Huttly³, I. Carré² and S.D. Jackson¹

1. Horticulture Research International, Wellesbourne
2. University of Warwick, Department of Biological Sciences, Coventry
3. Rothamptstead Research, Harpenden

Flowering time has been studied for many years but recent studies suggest that plants perceive day length through the coincidence of *CONSTANS* (*CO*) expression with light.

Regulator of CONSTANS (*roco*) is a novel flowering time mutant of *Arabidopsis* that has been positioned on the long day (LD) photoperiodic promotion pathway. The *roco* mutant flowers late in LD but at the same time as WT in short days (SD). The response to photoperiod is determined by a complex set of interactions between specific photoreceptors and the circadian clock which in turn control the rhythmic expression of *CO*. Coincidence of *CO* expression with light in LD promotes the expression of *FLOWERING LOCUS T* (*FT*) which in turn promotes flowering. Real-time quantitative PCR has shown that expression of both *CO* and *FT* is down regulated in the *roco* mutant. Sequence analysis has revealed that *ROCO* encodes a novel RING-finger protein. RING-finger proteins act as ubiquitin ligases, which are components of the ubiquitin proteasome pathway that targets specific proteins for degradation via the 26S proteasome. *Roco* flowers at the same time as WT plants in SD but flowers late in LD which suggests that it is defective in a photoperiodic inductive signal normally seen in LD. It is hypothesized that *ROCO* targets a repressor of flowering (a repressor of *CO* transcription) for degradation in inducing LD conditions. To test the hypothesis that *ROCO* is a ubiquitin ligase a GST-*ROCO* fusion protein will be used in an *in vitro* ubiquitination assay. To identify the putative repressor of flowering epitope tagged *ROCO* fusion proteins will be used to perform pull down experiments *in planta* followed by mass spectrometry to identify purified proteins.

P5.3 Characterisation of early and late flushing markers (osmoprotectant, compatible solutes, oxidative products) in common ash (*Fraxinus excelsior*) for breeding programs

Laurent Jouve and Jean-François Hausman (CRP-GL, CREBS Research Unit, Luxembourg)

One of the major problems connected to growing ash is frost induced damages in late spring, when young shoots are highly susceptible. This involves bud necroses and further timber forking leading to an economical depreciation of the wood. The importance of selecting for late flushing in breeding strategies for ash has not to be overstated but potentially may be achievable, as flushing is a highly inheritable trait.

Different experiments have been performed in order to characterise the physiology of ash bud-break and flushing during spring. Analyses of osmoprotectants and compatible solutes as proline, polyamines or carbohydrates and oxidative products as malondialdehyde or lipid peroxides have been performed. A first assay, using forced winter buds, allowed us to characterise phenotypically ash bud break in four stages related to their growth: winter bud, swollen bud, young stem and adult leaf. Moreover, sampling during forcing has been done and allowed us to study putative biochemical markers for early and late flushing. A field bud harvesting, during year 2003, was used to characterise and find interesting markers for early and late flushing trees. For instance, decrease in endogenous level of malondialdehyde, and accumulation of putrescine from March to May were strongly related to late flushing plants. Further studies and analyses will allow us to propose interesting markers for selection of plus-trees and to provide efficient tools for breeding programs.

P5.4A Cytogenetic and morphometric study of gamma irradiated cotton cultivars and their crossing progenies

Masoud Sheidai, Biology Department, Shahid Beheshti University, Evin, Tehran, Iran

A cytogenetic and morphometric study was performed of about 40 M4 gamma irradiated tetraploid cotton cultivars (*Gossypium hirsutum* L.) and their crossing progenies. The chromosome pairing and chiasma frequency as well as meiotic abnormalities were compared among the genotypes studied. Meiotic abnormalities including cytotoxicity, formation of the laggard chromosomes, stick-

iness as well as disorganised chromosomes. Were observed which may be responsible for the reduction in pollen fertility and abnormal pollen grain formation in cotton cultivars studied. Elimination of the micronuclei from periphery of the cell the along with some amount of cytoplasm was noticed in some of the cultivars. B-chromosomes were observed in some of the cultivars. The cultivars studied differed significantly in their cytogenetic and morphometric/agronomic characteristics indicating their genomic differences which may be used in cotton breeding. Cluster and ordination based on principal components analysis grouped those cultivars showing cytogenetic and morphometric similarities. The most variable meiotic characteristics were identified among the genotypes by using factor analysis.

P5.4B Elucidating QTL for leaf development in lettuce and their linkage to candidate genes

F.Z. Zhang, G.J.J. Clarkson (University of Southampton), R. Michelmore (University of California), M.S. Dixon, G. Taylor (University of Southampton)

Leaf development was studied using 60 F₂ recombinant inbreed lines derived from a cross between cultivated lettuce (*Lactuca sativa* cv. Salinas) and wild lettuce (*L. serriola* acc. UC96US23). This mapping population was tested in two separate field trials in Portugal and the UK. Leaf development traits, including leaf area, weight, specific leaf area, relative water content, chlorophyll content, stomatal density and index, epidermal cell area, epidermal cell number and cell sap osmolality were analysed. Additionally, the lettuce leaves were harvested for shelf life assessment. Significant correlations were found between shelf life with leaf area, weight, chlorophyll content, stomatal index and cell number, indicating that some leaf development traits are important to post-harvest processability of leaves.

Quantitative trait loci (QTL) analysis is underway using composite interval mapping and latest findings will be presented. The gene and environmental interaction effects of the QTLs relevant to leaf development were tested in the two different environment field trials. The genomic resource with more than 66 000 lettuce ESTs, providing a unigene set of 19 500, is available for identifying candidate ESTs involved in leaf development and processability. Candidate genes for leaf developmental traits and shelf life will be mapped using the same population. The identification of QTLs and candidate genes for leaf development and leaf processability will lead to

a better understanding of processability at a genetic and cellular level, and allow us to improve salad leaf quality through marker assisted breeding.

P5.5 Quantification of the mechanisms of wheat anchorage failure using Image analysis

A.R. Tams^{1*}, S.J. Mooney¹, P.M. Berry²

¹ Department of Environmental Science, School of Agriculture and Environmental Science, University of Nottingham, University Park, Nottingham, UK, NG7 2RD
² ADAS High Mowthorpe, Duggleby, Malton, N. Yorks, UK, YO17 8BP

The permanent displacement of plant stems from the vertical, known as lodging, affects all cereal species and is a major limiting factor on grain production worldwide. Two forms of lodging are recognised: stem lodging (when the stem base buckles) and root lodging (when the root-soil system fails). However, there is conjecture about the mechanism of how the root-soil system fails. This is mainly due to the difficulties associated with observing the process within its natural environment. To enable an investigation of the mechanisms of root failure *in situ*, a method for collecting undisturbed soil cores containing the root system cereal plants has been developed. This involves impregnating the soil surrounding the roots with varnish to solidify the top 100 mm of the root-soil complex. This has been carried out with winter wheat (*Triticum aestivum* L.) grown on three different soil types: clay at ADAS Boxworth, Cambridgeshire, silty loam at ADAS Rosemaund, Herefordshire and sandy loam at the University of Nottingham, Leicestershire at two planting densities (100 & 400 seeds/m²). Once collected, the root-soil samples were re-impregnated with crysolic resin in the laboratory and were either analysed in 3D using an x-ray Computed Tomography scanner or the samples were sectioned and each slice photographed. The resulting images were then analysed using image analysis software to quantify differences in the size, shape and distribution of soil pores and particles, together with the orientation and position of the below ground part of the cereal stem and its crown roots. This study has given the first insights about how the root-soil system of cereals fails using a method that does not disturb the region of failure.

P5.6 Long-term responses of stomata following five years of forest exposure to elevated CO₂ in the POPFACE experiment

P.J. Tricker¹, M.J. Tallis¹, G.J.J. Clarkson¹, H. Trewin¹, E. Kuzminsky², M. Sabatti² and G. Taylor¹

¹School of Biological Sciences, University of Southampton

²Dept. of Forest Environment and Resources, University of Viterbo

Stomatal development was measured over five years in three species of poplar exposed to elevated CO₂ at 550 ppm using a free-air enrichment system. In an open canopy, stomatal index was significantly influenced so that the numbers of stomata decreased in elevated CO₂. As the canopy closed, however, elevated CO₂ no longer influenced stomatal numbers. In contrast, stomatal conductance continued to be sensitive to CO₂ concentration, decreasing overall although this was dependent on species and soil N status. Leaf level water use efficiency increased significantly in all three species, in a closed canopy and after five years of exposure, and this increase was related to reduced stomatal aperture. These results describe the progression of stomatal sensitivity to elevated CO₂ over several years and demonstrate that stomatal conductance rather than development drives improvements in leaf level water use efficiency of these three poplar species in response to changing atmospheric CO₂ concentration.

These findings from field-grown trees are in contrast to recent reports following short-term exposure to elevated CO₂ and explanations for these differences are considered.

P5.7 Quantifying internal gas diffusion limitations in photosynthesising leaves using chlorophyll fluorescence imaging

E. Gallouët^{1,2}, T. Lawson¹, J.I.L. Morison¹, G. Cornic², & N.R. Baker¹

¹Department of Biological Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ UK

² Laboratoire Ecologie Systématique et Evolution, Bat 332, Université Paris-Sud, 91405 Orsay

The exchange of gases within leaves and their surrounding environment takes place predominately through the stomata. In heterobaric leaves, where extension of the bundle sheath to the upper and lower epidermis prevent lateral gaseous CO₂ diffusion ‘patchy’ stomatal behaviour results in heterogeneous assimilation rates across the leaf surface. However, it has been suggested that in homobaric leaves where there is no compartmentalisa-

tion of the leaf to prevent lateral CO₂ movements this non-uniform pattern of assimilation does not occur. Using chlorophyll fluorescence imaging simultaneously with gas exchange we are able to image large areas of the leaf lamina (10 cm²) and calculate patterns of assimilation rates and internal CO₂ concentration (C_i) over the leaf lamina. We have simulated patchy stomatal behaviour by applying 0.1 cm² areas of grease to the upper, lower or both leaf surface(s) of *Phaseolus vulgaris* (heterobaric) and *Commelina communis* (homobaric) and monitored the resulting patterns of internal CO₂ concentration, under different oxygen and carbon dioxide conditions. Analysis of transects across the patches reveal sharp decreases in CO₂ concentration from the patch boundary towards the centre. These data suggest that lateral gaseous resistance is high in both homobaric and heterobaric leaves. The contribution of the lateral resistance to calculated mesophyll resistance is discussed.

P5.8 Investigating the potential of thermal imaging in monitoring stress in crops and ecosystems

Olga M. Grant¹, Lukasz Tronina¹ & M. Manuela Chaves^{1,2}

1. Instituto de Tecnologia Química e Biológica, Oeiras, Portugal

2. Instituto Superior de Agronomia, Lisboa, Portugal

Stomatal closure is considered a sensitive response to soil water deficit, and hence has potential as an indicator of plant stress, of value in irrigation scheduling or in monitoring responses to environmental change in natural ecosystems. However, the use of porometers to monitor stomatal conductance is time-consuming, labour-intensive, and gives only point measurements. As stomata close, energy dissipation is decreased and so leaf temperatures tend to rise. Therefore it has been suggested that leaf or canopy temperature can be used as an indicator of plant stress. The use of thermal imaging systems allows us to rapidly and non-invasively obtain more integrated data for individual leaves or areas of canopies. While recent papers have emphasised the advantages of thermal imaging, experiments designed to test whether the technique is in fact as useful as more traditional physiological methods in distinguishing between stress treatments are lacking. We have recently conducted such experiments, and our results highlight a number of issues that could prevent thermal imaging from accurately distinguishing between stressed and non-stressed plants or canopies. We are now concentrating on improving the technique for detection of stress in crops or natural ecosystems.

P5.9 Molecular dissection of the ozone stress response

E.F. Short, M. McAinsh and A. Shirras

The gas ozone protects against the harmful effects of ultra-violet radiation in the stratosphere. However, in the troposphere ozone is toxic to plants and causes significant reductions in crop yields. Ozone is a reactive oxygen species (ROS) and can cause oxidative damage directly by entering stomata and interacting with cell wall and membrane components. Ozone can also form other ROS such as hydrogen peroxide and hydroxyl radicals that can cross the plasma membrane and cause further damage, leading to reduced transpiration, accelerated senescence and decreased photosynthesis. In addition, the plant itself can produce ROS, which are thought to be a component of the signalling pathway. Plants react to oxidative stress by increasing their antioxidant defences in an attempt to neutralise harmful ROS. The individual roles of several antioxidants have been extensively studied, however their regulation and interaction *in planta* have yet to be fully elucidated. The specificity of antioxidants and other stress-related molecules to each unique stress is also poorly understood. In this work a DNA microarray has been utilised to detect novel genes in *Arabidopsis thaliana*, that are regulated by ozone. Twenty genes, which are significantly up-regulated and one gene that is down-regulated by ozone treatment have been identified. Five of which have been chosen for further analysis. The expression of these genes in response to a range of ozone concentrations over time has been investigated using Real Time RT-PCR. These genes have been shown to be induced specifically in response to acute ozone stress and initial studies suggest that their expression is calcium-dependent.

P5.10 Rubisco turnover rate and its implications for field grown barley genotypes

Louis J. Irving¹, Kazuhiro Imai², Amane Makino², Tadahiko Mae², Roger Ellis³, William T.B. Thomas³ and David Robinson¹

¹ School of Biological Sciences, University of Aberdeen, Aberdeen, UK

² Department of Applied Plant Science, Tohoku University, Sendai, Japan

³ Scottish Crop Research Institute, Dundee, UK

Cereal crop NUE stands at approximately 33% worldwide, although N recovery in biomass is significantly

higher than this. However, it cannot be ignored that significant quantities of N based fertiliser are lost to the environment every year, with both economic and environmental impacts. Therefore, more N efficient plants are desirable to reduce the applications necessary for successful cultivation of cereals, especially in delicate environments. Whilst N uptake and assimilation are presently well understood (albeit incompletely), the mechanisms of N storage within plants are presently poorly known.

Here we present experimental data showing genotypic variation in Rubisco synthesis and degradation rates (the CO₂ fixing enzyme in photosynthesis and a major N store in cereal plants). We explore the mechanisms by which Rubisco quantity seems to be regulated, discussing this in the context of recent work on the subject. We will discuss the implications this has for both photosynthetic capacity and the nitrogen economy of the plant. Furthermore, we will report data from field trials to compare the N storage capacities of different barley genotypes. Finally, we will consider the evidence for correlations between Rubisco dynamics and plant morphological / developmental characters.

P5.11 Cold acclimation of plant respiration: are there changes in the capacity, number and/or size of individual mitochondria?

A.F. Armstrong, O.K. Atkin (University of York) and D. Logan (University of St Andrews)

Thermal acclimation of respiration results in cold-grown plants exhibiting higher rates of respiration than their warm-grown counterparts when measured at a moderate set temperature. The objective of this work was to elucidate some of the mechanistic changes underlying leaf respiratory acclimation to the cold. Work was conducted on mature warm-grown (WG), cold-treated (CT) and cold-developed (CD) leaves of *Arabidopsis thaliana*. The temperature response characteristics of respiration were measured using oxygen electrodes. Although both the CT and the CD leaves exhibited significantly higher rates of respiration across the temperature range than the WG leaves, the rates were substantially higher in the CD leaves, which achieved full acclimation (respiratory homeostasis). At moderate to high temperatures respiration tends to be limited by substrate supply and adenylate control, at lower temperatures, however, respiration tends to become increasingly limited by respiratory capacity. Respiration was increased not only at the moderate to high temperatures, but also at the lower temperatures, indicating that an increase in capacity could be involved. An increase in capacity could result from

an increase in rate per unit mitochondrial protein, an increase in the amount of mitochondrial protein per unit mass, or a combination of the two. In order to test this, the capacity of the cytochrome and alternative pathways of electron transport were measured per mg mitochondrial protein and per gram fresh mass in mitochondria extracted from WG, CT and CD leaves. In conjunction with this, the mitochondrial density of WG, CT and CD leaves was measured in *Arabidopsis* plants with GFP labelled mitochondria.

P5.12 Alternative Pathway of Protein Import in Plastids

Xianxi Yan^a, Sultan Khan^a, Stephen High^a, Michael J Emes^b, Toshiharu Hase^c and Caroline G Bowsher*

^a School of Biological Sciences, University of Manchester, 3.614 Stopford Building, Oxford Road, Manchester, M13 9PT, UK

^c Department of Botany, University of Guelph, Guelph, ON, N1G 2W1, Canada

^d Division of Enzymology, Institute for Protein Research, Osaka 565, Japan

* Corresponding Author

Plastids are organelles unique to plant cells and have a range of the metabolic functions^[1]. Many plastidic proteins are nuclear encoded and synthesized in the cytosol as precursors. The precursors are then targeted into the plastid. Protein import has been extensively studied in chloroplasts^[2]. This paper aims to characterise the mechanism of protein import into plastids from different tissue sources. A comparison of import of precursor proteins into chloroplasts and root plastids has been investigated. Photosynthetic proteins, RbcS and ferredoxin I, were imported into isolated chloroplasts but not into root plastids. In contrast, the non-photosynthetic ferredoxin III was imported into both isolated chloroplasts and root plastids. Chimeric ferredoxin I/III (transit peptide of FdI with mature region of Fd III) was imported only into chloroplasts. Ferredoxin III/I (transit peptide of FdIII with mature region of FdI) was imported into both chloroplasts and root plastids. These results suggest the transit peptide has a major role in the differential uptake of specific proteins into different plastids. The pathway for the import of nuclear encoded proteins between root plastids and chloroplasts will be discussed. References

Bowsher C G and Tobin A K (2001). *Journal of Experimental Botany*. 52, 513–527.

Chen X and Schnell D J (1999). *Trends in Cell Biol.* 9, 222–227.

P5.13 Characterisation of starch phosphorylase from developing wheat endosperm amyloplasts

P. Tickle, University of Manchester; I. Tetlow, University of Guelph, Canada; M. Emes, University of Guelph, Canada; S. Coates, Advanced Technologies, Cambridge; M. Burrell, University of Sheffield; C. Bowsher, University of Manchester.

Starch phosphorylase catalyses the reversible transfer of glucosyl units from glucose-1-phosphate to the non-reducing end of alpha-1,4-linked glucan chains, thus, has the potential to both synthesise and degrade starch. In higher plants two major isozymes of starch phosphorylase exist (Pho1 and Pho2) that differ in subunit size, kinetic properties and intracellular localisation. The larger of the two isozymes, Pho1, has a low affinity for branched polyglucans such as glycogen and is exclusively plastidial. Pho2, on the other hand, has a high affinity for branched polyglucans and is located solely in the cytosol. During endosperm development the number of amyloplasts per cell increases, as does the volume of the cell occupied by starch and during this phase the activity of starch synthetic enzymes increases. To test the hypothesis that Pho1, which is localised exclusively within the amyloplast in the developing wheat endosperm, is involved in the starch biosynthetic pathway the regulation of Pho1 gene transcription, protein levels and activity has been investigated during development. Results indicate that Pho1 may play some role in starch synthesis. Future work will include the purification and kinetic analysis of Pho1. This will allow putative *in vivo* substrates to be identified and hence give a greater understanding of the role of this enzyme in starch metabolism in the developing wheat endosperm.

P5.14 Pyruvate orthophosphate dikinase and its role in C3 plants

K. Parsley and J.M. Hibberd

The majority of plants are known as C₃ plants because the first product of photosynthesis, phosphoglyceric acid, contains three carbons. However, some plants are known as C₄ plants, because they use an alternative pathway in which the first product of photosynthesis is a four carbon compound. C₄ plants have evolved independently many times, but for C₄ photosynthesis to operate, alterations in anatomy, ultrastructure and biochemistry are needed.

One of the key enzymes used during C₄ photosynthesis is pyruvate orthophosphate dikinase (PPDK), which regenerates phosphoenolpyruvate (the primary acceptor of CO₂ in C₄ plants) from pyruvate in the chloroplasts of mesophyll cells; its role in C₃ plants is however unknown.

We have investigated *ppdk* in the C₃ plant, *Arabidopsis thaliana*, and shown that the single gene produces two transcripts. The longer transcript is formed because a second promoter element controls the production of an additional exon, and the shorter transcript is controlled by a promoter located in the first intron. We have shown that the first exon of *Arabidopsis ppdk* codes for a targeting peptide that delivers the longer form of the protein to chloroplasts.

Expression analysis using semi-quantitative RT-PCR and RNA blots shows the two forms are differentially expressed and suggests that the longer *ppdk* transcript is more abundant in photosynthetic tissue in agreement with its proposed localisation to the chloroplast. The shorter *ppdk* transcript is of lower abundance except in roots. The role of the respective proteins is being investigated using mutants.

P5.15 Mechanism of mushroom flavour biogenesis: evolution of 8-carbon volatiles from lipids and functional analysis of heterologously expressed lipid dioxygenase genes

E. Combet^{1,2}, K.S. Burton¹, G. Griffiths¹, D.C. Eastwood¹ and J. Henderson²

¹ Horticulture Research International, Wellesbourne, Warwick, CV35 9EF

² Coventry University, School of Science and the Environment, Priory Street, Coventry CV1 5FB

Mushroom flavour biogenesis is associated with production of 8-carbon compounds derived from the polyunsaturated fatty acid linoleic acid. Studying this pathway presents interests at different levels. Firstly, flavour is a key component to the crop quality. Also, a number of compounds (oxylipins) involved in the pathway have been postulated to play a role in wound response. Furthermore, new knowledge will be valuable for manipulation of lipid molecules for medicinal and industrial use.

Higher fungi have a unique mechanism of lipid metabolism for volatile production. The biochemistry involves the oxygenation and cleavage of linoleic acid to 1-octen-3-ol 'the mushroom alcohol' and a 10-oxodecanoic acid. The intermediate product is a 10-hydroperoxide, which has, unlike plant hydroperoxides, non-conjugated double bonds. The double bond position (at C8 and C10) is not a subtle difference, as it radically changes the predicted enzymology of the reaction. Consequently, the first enzyme involved, performing the dioxygenation reaction, is thought to be a heme-dioxygenase, as opposed to the lipoxygenase involved in the plant system. However, both types of enzymes are considered as potential candidates to elucidate this biochemical pathway.

There is little genomic information on higher fungi and only one fungal dioxygenase has been identified, presenting different specificities. To overcome this shortage of information, a variety of approaches were used (chemical analysis, GC-MS profiling of volatiles, lipid analysis, complex PCRs, mass gene screening, heterologous expression of candidate genes) in order to characterise the pathway involved in mushroom flavour biogenesis and lipid metabolism.

P5.16 Abstract not supplied

P5.17 The abscisic acid (ABA) relations of the parasitic association *Rhinanthus minor*/ *Hordeum vulgare*

Jiang Fan, W.Dieter Jeschke, Wolfram Hartung

Using the facultative root hemiparasite *Rhinanthus minor* and *Hordeum vulgare* as a host, the flows, depositions and metabolism of abscisic acid (ABA) within the host, the parasite and between host and parasite have been studied. Estimated flows of ABA on a per plant basis in barley were slightly increased by 13% in the xylem after infection. There were weak or no effects on biosynthesis and on ABA degradation in barley. Deposition was significantly affected in the leaf laminae (3 fold) and in leaf sheath (2.4 fold), but not in roots. Dramatic changes in ABA flows, metabolism and deposition on a per plant basis, however, have been observed in *Rhinanthus*. Biosynthesis in the roots was 12-fold higher after attachment resulting in 14-fold higher ABA flows in the xylem. A large portion of this ABA was metabolised, a small portion was deposited. Phloem flows of ABA were increased 13-fold after attachment.

The concentrations of ABA in tissues and transport fluids were higher in *Rhinanthus* by an order of magnitude than it in the host tissues and saps. The possible function of ABA in the parasite is discussed.

P5.18 LILIPUT, a dwarf mutant with an enhanced response to ABA

Bown, L.C.,¹ Kusaba, S.,¹ Codrai, L.,¹ Jackson, A.,¹ Guimond, S.,² Turnbull, J.E.,² Goubert, F.,³ Dupree, P.,³ Reed, J.,⁴ Jackson, S.¹

¹ Horticulture Research International, Wellesbourne, CV35 9EF, UK.

² University of Liverpool, School of Biological Sciences, Liverpool, L69 7ZB, UK

³ University of Cambridge, Department of Biochemistry, Cambridge, CB2 1QW, UK

⁴ University of North Carolina, Department of Biology, Chapel Hill, NC 27599-3280

Disruption of the *LILIPUT* (*LPT*) gene by T-DNA insertion results in a dwarf mutant with an enhanced response

to the phytohormone ABA. Successful complementation of the mutant by re-introducing the WT gene eliminates the possibility that another mutational event is responsible for the observed phenotype. In addition to an enhanced response to exogenously applied ABA, the *Lpt* mutant also produces an elevated level of the endogenous hormone compared to wildtype plants. It is possible, therefore, that the gene is involved, directly or indirectly, in the regulation of ABA biosynthesis although this remains to be clarified. Interestingly, the *Lpt* mutant also demonstrates a particular root phenotype typified by short, irregular root hairs of which a small percentage appear to have aborted prior to elongation. The reasons for this are, as yet, unclear but suggest a potential disruption to root hair positioning and cell elongation.

Sequence analysis of the *LPT* gene reveals 33% identity to the mammalian exostosin (*EXT*) genes, which are involved in the production of heparan sulfate (HS). In mammals HS functions as a cell surface co-receptor mediating cell responses to various growth factors. At present, there is no supportive evidence that plants produce HS, however, part of this work aims to address the possibility that the *LPT* gene may be involved in the synthesis of plant HS. As a preliminary approach to this we have investigated the action of HS-specific enzymes (heparases) on extracts from wildtype and *Lpt* mutant plants. Interestingly, although the analysis of wildtype tissue suggests a potential recognition of HS, no such result has been observed for the *Lpt* mutant. Current work aims to verify this observation.

P5.19 The effect of light quality on food reserve utilization and seedling development of *Pisum sativum*

C. Masterson and C. Wood, Biology, University of Newcastle upon Tyne

Pea is a starchy seed also containing about 1.4% oil. The embryo utilizes these reserves during germination and early seedling development and we have previously reported² that there is a switch in which food reserve is utilized at different stages of development. During initial imbibition and emergence stages, starch reserves are utilized whilst lipid levels remain constant. Following emergence of the plumule, development becomes light-requiring, involving greening and leaf development, resulting in an autonomous, photosynthetic plant. During this stage, lipid stores are utilized whilst starch levels remain constant. This switch to lipid utilization coincides with a peak of mitochondrial beta-oxidation activity in the cotyledon¹. This current report describes the ability of light of different wavelengths to trigger this switch.

Chlorophyll synthesis was optimal in white light whereas dark- and far-red-grown seedlings remained yellow. Red- and blue-light-grown seedlings attained chlorophyll levels of approximately 50% of the white-light controls. Starch utilization was linear throughout the growth period in dark and far-red light conditions, but starch utilization ceased with the onset of chlorophyll synthesis in white- and blue-light-grown seedlings. Parallel to this, lipid utilization was negligible in dark- and far-red-grown seedlings, whereas utilization of stored lipid was observed with the onset of greening in seedlings grown in white and blue light. It appears that the switch from starch to lipid utilization is blue-light controlled and this may be the light trigger for mitochondrial beta-oxidation.

References:

1. Masterson & Wood 2001 *Proc.R.Soc.Lond.B* 268, 1949–1953.
2. Masterson et al. 2003 *Comp.Biochem.Phys.A* 134 (Issue 3, Supplement 1) S155.

P5.20 Fractal patterns in species distributions of some British scarce plants

G.H. Chekuimo Tagne¹, W.E. Kunin², M. Pocock², R. Aston²
(azpa01@yahoo.com)

The spatial distribution and fractal structure of two British scarce plants, *Lobelia urens* (heath lobelia) and *Phyteuma orbiculare* (round-headed rampion), have been examined at several different scales.

The two species have similar degrees of local patchiness at scale coarser than 50 km and have contrasting coarse-scale between 50 km and 1 km scales, but differed consistently in the slopes of their scale-occupancy curves distributions at scale finer than 1 km. The slope of the log-log plot of *L. urens* is not constant, but varies systematically with spatial scale, and from habitat to habitat at the same spatial scale.

Abundance estimates suggest that the species *P. orbiculare* is found to be clumped at all scales, whereas *L. urens* is dispersed at intermediate scale. Fractal dimension analysis suggests that this changes through scale. The distribution varied in their pattern from highly clumped to randomly dispersed. Fairly predictions of *L. urens* can be made from 50 m and 200 m.

Some issues affecting management of species abundance, as well as underlying mechanisms and conservation schemes have been highlighted.

P5.21 Role of peroxidase and amine oxidases in heat susceptible and tolerant cultivars of developing wheat grains

Bavita Asthir¹, A P S Mann¹ and William Spoor²

¹Department of Biochemistry & Chemistry, Punjab Agricultural University, Ludhiana, India. ²Crop Science & Technology Department, Scottish Agricultural College, West Mains Road, Edinburgh, EH9 3JG, Scotland, UK

Histochemical localization and activity pattern of soluble peroxidase, diamine oxidase (DAO), polyamine oxidase (PAO) together with the characterization of peroxidase have been studied from the developing grains of heat-susceptible (WH 542) and heat-tolerant (PBW 343) cultivars of wheat (*Triticum aestivum*, L). Peroxidase, DAO and PAO were mainly localized in the chalazal cells, nucellar projection, seed coat, aleurone, vascular tissue and crease regions of both the cultivars. However, the intensity of the color varied with the cultivar and their stage of development. Activities of peroxidase and amine oxidases were significantly high at mid-milky stage of grain development. PAGE of peroxidase, DAO and PAO revealed some additional bands in WH 542 that appeared only at specific stage of grain development. Peroxidase purified from the mid-milky stage grains of heat-susceptible and heat-tolerant genotypes showed marked differences in pH optima, Km and Vmax values. Inhibitory effect of sulphhydryl group modifiers and various metal ions was much more pronounced in the susceptible genotype than in the tolerant genotype. The possible involvement of these enzymes during lignin/suberin deposition for modulating assimilate supply to the developing grain is discussed.

P5.22 Differential response of peroxidase in healthy and infected red rot susceptible varieties of sugarcane

Kanwal Preet, Bavita Asthir and A P S Mann

Dept. of Biochemistry and Chemistry, Punjab Agricultural University, Ludhiana, India

Involvement of peroxidase during plant defense response mechanism have been studied in sugarcane (*Saccharum officinarum* L.) against red rot fungus *Colletotrichum falcatum*. The area covered by the fungal mycelium increased gradually and was maximum on 15th day of infection. A positive correlation was found between peroxidase activity with the degree of infection. Histochemical localization studies further confirmed the presence of peroxidase in the internodal cells of sugarcane. Peroxidase isolated from cane juice at the stage of maximum infection was fractionated by 0–80% ammonium sulphate precipitation followed by DEAE-cellulose

column chromatography from CoJ64 (Healthy) and CoJ64 (Infected) varieties. Isolated peroxidase retained sufficient activity for a period of one month stored at 4°C. The optimum pH of peroxidase for CoJ64 (Healthy) was 6.0 while that for CoJ64 (Infected) was 6.5. Peroxidase exhibited an optimum temperature between 40–45°C. Complete inhibition of the enzyme activity was found in the presence of Mn^{2+} , Tris and Hg^+ followed by Ca^{2+} and EDTA. PAGE of purified enzyme indicated well defined bands of enzyme preparation. The appearance of new bands following infection may be correlated with the induction of catalytic activity of more isozymes leading to an overall increase in peroxidase activity.

P5.23 Variation in Carbon Isotope Discrimination and Other Traits Related to Salt Tolerance in Sugar Beet Cultivars

Ali. R. Dadkhah (Mashad University) and H.Griffiths (Cambridge University)

The effect of cultivar and salt stress on the physiological traits, such as carbon isotope discrimination (Δ), photosynthesis (A), transpiration (E), stomatal conductance (g_s), predawn leaf-water potential (Ψ_1) and leaf water content (LWC) were studied on sugar beet cultivars (Madison, 7233-P₁₂, 7233-P₂₁ and 7233P₂₉). The morphological traits, such as live leaf area and specific leaf weight (SLW) were also evaluated. Positive associations between Δ and A , E and g_s were observed under increasing salt condition. The A/g_s ratio, was negatively associated with Δ , and a negative correlation was observed between SLW and Δ . An apparent genotypic variation in Δ was observed in plants after treatment with saline water. A remarkable correlation between LWC and gas exchange traits was found, whereas SLW was negatively correlated with A , and LWC. Carbon isotope discrimination might be a useful tool for selecting salt-tolerant sugar beet genotypes but more studies are required to define more precisely the sampling conditions and the influence of factors affecting Δ .

P5.24 Expression of UDP-glucose pyrophosphorylase gene in *Arabidopsis* leaves as affected by interaction of phosphate, hormones and sugar

I. Cierieszko, University of Bialystok, Poland, and L.A. Kleczkowski, Umeå University, Sweden

The effects of inorganic phosphate (Pi) deficiency, sugar and ABA/ethylene status on expression of UDP-glucose pyrophosphorylase (UGPase) gene (*Ugp*), involved in

sucrose/polysaccharides metabolism, were investigated. Both wild-type (wt), *aba* and *abi* mutants (ABA-deficient and -insensitive), *etr*, *ein* and *eto* (ethylene resistant and overproducing) grown on phosphate-deficient (-P) and complete nutrient solutions, as well as *pho1* (P-deficient) mutants of *Arabidopsis thaliana* were used for experiments.

Generally, both P-deficiency and sucrose feeding upregulated *Ugp* expression in all conditions. Growth of *aba2-1*, *aba3-1* on -P medium increased *Ugp* expression in leaves, similarly to wt plants. Feeding leaves of different *aba/abi* mutants with mannose, which acts as a sink for cytosolic Pi, mostly induced *Ugp* expression, similarly to wt. Growth of *etr1-3*, *ein2-1* or *eto1-1* mutants on -P medium upregulated *Ugp*, like in wt leaves. *Ugp* expression increased in a similar manner after feeding wt and *pho1* leaves with sucrose, with and without ABA, and *aba1-1*, *aba1-3*, *abi1-1*, *abi2-1* mutants leaves with sucrose. The data suggest that Pi deficiency and sucrose signaling leading to upregulation of *Ugp* gene act independently of ABA and ethylene status.

Okadaic acid (inhibitor of protein phosphatases 1 and 2A) potently blocked the P-starvation and sucrose-dependent expression of *Ugp* in excised leaves, whereas staurosporine (protein kinase C inhibitor) had no significant effect, suggesting that P-starvation and sucrose effects on *Ugp* are transmitted by pathways that may share similar components with respect to their (in)sensitivity to both inhibitors.

This work was supported by grant 6-P04C-019-20 from State Committee for Scientific Research (KBN, Poland) given to I.C. and by Swedish Research Council (to L.A.K.)

P5.25 Hydroxyl radicals – a new and effective blocker of water channels (aquaporins) in *Chara*

Q. Ye, T. Henzler and E. Steudle, Department of Plant Ecology, University of Bayreuth, Germany

Hydroxyl radicals as produced in the Fenton reaction ($Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + \cdot OH$) have been used to reversibly inhibit the activity of aquaporins in the plasmamembrane of *Chara corallina*. Different from conventional blockers of aquaporins such as mercurials (e.g. $HgCl_2$), hydroxyl radicals turned out to be more effective and less poisonous. When *Chara* internodes were treated by hydroxyl radicals for 30 min, cell Lp decreased by about 90%. After removing the hydroxyl radicals from the medium, the effect was reversed within a few minutes indicating repair mechanisms which are not yet known. Blockage of water channels with hydroxyl radicals reduced the permeability of small test solutes (ethanol, acetone, 1-propanol and 2-propanol) indicating

some slippage of the solutes across aquaporins. For the rapidly permeating test solutes, channel closure caused anomalous (negative) osmosis, i.e. cells had negative reflection coefficient and were swelling in a hypertonic medium. Inhibition of aquaporins reduced the diffusive permeability of isotopic water (HDO) by about 30%. From the ratio of bulk to diffusive permeability of water, the number (N) of the water molecules that align in water channels was calculated ($N = 35$ to 60). Treatment with hydroxyl radicals decreased N by 70%. However, when water channels were closed by high external concentration (1200 mM) of acetone and triethylene glycol monoethyl ether, N decreased by 30% to 50%. The results are in line with the idea that there is a population of water channels present in *Chara* rather than just one type of channel.

P5.26 The relative contribution of the apoplastic and cell-to-cell paths to the overall hydraulic conductivity of the outer part (periphery) of young rice (*Oryza sativa* L.) roots

Kosala Ranathunge, Lukasz Kotula and Ernst Steudle, Department of Plant Ecology, University of Bayreuth, Germany

The relative contribution of the apoplastic and cell-to-cell paths to the overall hydraulic conductivity of the outer part (periphery) of young rice roots (L_{OPR}) was estimated using a pressure perfusion technique for 30-d-old rice plants (lowland cultivar, IR64 and upland cultivar, Azucena). The outer part of rice roots (OPR) comprised an outermost rhizodermis, an exodermis, sclerenchyma fibre cells, and an innermost unmodified cortical cell layer. Aerenchyma of root segments from two different root zones (20–50 mm and 50–100 mm from the root apex) were perfused with aerated nutrient solution using precise pump rates. No root anatomical differences were observed for the two cultivars used. Development of apoplastic barriers such as Casparian bands and suberin lamellae in the exodermis were highly variable. Matured apoplastic barriers were observed at around 50–80 mm from the root apex. Lignification of the exodermis was completed earlier than that of sclerenchyma cells. Radial water flow across the OPR was impeded either by partially blocking off the porous apoplast with China ink particles (diameter 50 nm) or by closing water channels in cell membranes with 50 μM HgCl_2 . The reduction of L_{OPR} was relatively larger in the presence of the apoplastic blocker ($\approx 30\%$) than in the presence of water channel blocker ($\approx 10\%$), suggesting a relatively larger apoplastic water flow. Better apoplastic blockers, such as small insoluble salt crystals, precipitated in apoplastic pores of the OPR, resulted in a decrease of L_{OPR} by a factor of 3–4. Reflection coef-

ficients of the OPR (σ_{sOPR}) for mannitol significantly increased during treatments. It was larger when apoplastic pores were closed, but absolute values were low (overall range of $\sigma_{\text{sOPR}} = 0.1$ – 0.4). The strongest evidence in favour of a predominantly apoplastic water transport came from the comparison between diffusional (P_{dOPR} , measured with heavy water, HDO) and osmotic water permeability (P_{fOPR}) or hydraulic conductivity (L_{pOPR}). P_{fOPR} was larger by a factor of 600–1400 (depending on the position along the root) compared with P_{dOPR} . The development of OPR along roots resulted in a decrease of P_{dOPR} by a factor of three (segments taken at 20–50 mm and 50–100 mm from the apex, respectively). Even though both pathways (apoplastic and cell-to-cell) contribute to the overall water flow, the findings indicate predominantly apoplastic water flow across the OPR, even in the presence of apoplastic barriers. The OPR of rice roots is a useful object for studying water flow across root tissues, namely the effect of apoplastic barriers (Casparian bands and suberin lamellae) on the partitioning between pathways.

P5.27 Water transport across the pedicels of cherry (*Prunus avium* L.) fruit and grape vine (*Vitis vinifera* L.)

Lukasz Kotula¹, Joanne Tilbrook², Steve Tyerman² & Ernst Steudle¹

¹ Department of Plant Ecology, University of Bayreuth, Germany

² Department of Wine and Horticulture, University of Adelaide, Australia

The effect of fruit development on water uptake by sweet cherry fruits was investigated over a time period of about one month. The objective was to quantify the hydraulic conductance of the berry pedicel and the forces driving water into the berry across the xylem.

The driving forces in the xylem of developing fruits were determined by measuring the water potential of pre and post veraison fruits using a pressure bomb. The diurnal courses of water potential of immature and mature fruits showed the same fluctuations, being the lowest (more negative) at midday, when the transpiration (and radiation) was the highest, and the highest (less negative) early in the morning and late at night. For immature fruits, the water potential was higher (less negative) than that of the mature fruits, being -1.4 ± 0.04 MPa and -2.2 ± 0.4 MPa at midday, respectively. The lower water potential of the mature fruits was probably caused by high sucrose content.

Hydraulic resistances of pedicels were measured using a perfusion technique (driving force for water flow about 0.2 bar). The hydraulic resistance of the cherry pedicel determined by steady state experiment was found to be very high. To determine the position of the highest

resistance of the pedicel a series of experiments were performed whereby the pedicel was successively cut and hydraulic resistances measured. Results showed that the highest resistance was offered by the brush of the pedicel. The resistance decreased as the pedicel was cut back from the brush. The comparison of hydraulic resistances of cherry pedicel, leaf petioles and twigs showed that the pedicel resistance was substantially higher than that of leaves and twigs. The hydraulic resistance of the pedicel thus controls the water uptake by the fruit which is pretty much isolated from the rest of the shoot. Similar results were obtained for grape wine.

P5.28 Effects of low root temperature on water uptake in cucumber and figleaf gourd root systems: cell and root levels

Seong Hee Lee¹, Ernst Steudle¹, Gap Chae Chung²

¹ Lehrstuhl Pflanzenökologie, Universität Bayreuth, Universitätstraße 3, D-95440 Bayreuth, Germany

² Agricultural Plant Stress Research Center, Division of Applied Plant Science, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757 Korea (South)

Exposure to low temperature (LT) had a different effect on root hydraulics of a temperature-sensitive (cucumber; *Cucumis sativus* L.) and -tolerant (figleaf gourd; *Cucurbita ficifolia* Bouché) plant species, respectively. Steady-state hydraulic conductivity of young root systems was measured using a pressure chamber. Preliminary data were obtained at the cell level (cell pressure probe). When the two species in hydroculture were grown at moderate temperature 20–23°C, root hydraulics of two species were similar. When LT of 8°C was applied for 3 to 6 d, there were visible changes in root anatomy of cucumber, but not in figleaf gourd. Cross sections suggested that the low overall hydraulic conductivity of cucumber roots was caused by strongly developed apoplastic barriers (suberin lamellae) at the endodermis. After short term exposure to LT (1 d), hydrostatic and osmotic L_{pr} of cucumber root systems were severely reduced, which was probably due to an inhibition of transpiration and closure of water channels. With figleaf gourd, there was only a reduction in hydrostatic but not in osmotic L_{pr} suggesting that water channels were not much affected at 8°C. When temperature was kept at 8°C for 1 d and then back to normal temperature of 20°C, reduction in osmotic as well as in hydrostatic L_{pr} was not completely recovered in cucumber. In figleaf gourd L_{pr} remarkably increased. The effect was bigger in osmotic than in hydrostatic L_{pr} . After 1 d exposure to low temperature, followed by 1 d at normal temperature, hydrostatic L_{pr} of both species was almost completely recovered. However, osmotic

L_{pr} of both species increased as compare to the original values due to the activity of water channels. It is concluded that the activity (open/closed state) of aquaporins and changes of root anatomy determine the water uptake and temperature sensitivity of root systems.

P5.29 In Vitro Propagation of aromatic and medicinal plant of Salvia Genus

B. Preci, E. Kongjika and E. Caushi, Institute of Biological Research, Tirana, Albania

Salvia officinalis, *Salvia verbenaca*, *Salvia viridis* and *Salvia fruticosa*, species well known as aromatic and medicinal properties, were propagated in vitro.

Collected buds after a sterilization with HgCl₂ (1% for 20 min) and ethanol (70% for 1 min) were placed in MS nutrient media supplement with cytokinin 6-benzylaminopurine (BAP 0.7 mg l⁻¹), ANA (0.1 mg l⁻¹) and GA₃ (0.1 mg l⁻¹). Shoot formation were obtained in 30 days after the first subculture, from which new shoot segments were taken. The segments subcultured on the same medium, were rooted alone or after supplementing the medium with different concentrations of IBA or NAA. Differences in *in vitro* growth rate and in propagation rate were observed between the species of Labiatae family. The plantlets were successfully acclimatized to ex vitro condition. The data from our experiments will permit us to evaluate the possibility for the improvement of medicinal and aromatic values by intergeneric hybridization within European Labiate.

P5.30 Abstract not supplied

P5.31 Abstract not supplied

P5.32 Abstract not supplied

P5.33 Abstract not supplied

P5.34 Responses of a Range of Broad-leaved Tree Species to Applications of Paclobutrazol

P.J. Lumsden and R. Allingham, University of Central Lancashire; M. Bloye, D. Hotchkiss and P. Houslay, Myerscough College, Lancashire

Trees planted in urban environments often suffer high mortality rates, largely due to poor root development (transplant shock). Once established though, such trees may require regular maintenance to avoid interference with overhead transmission lines. In America, management programmes of utility companies have increasingly incorporated tree growth regulators (TGRs) including Paclobutrazol (PBZ), under the brand names 'Clipper' or 'Profile'. PBZ, which inhibits cytochrome P450-dependent steps in gibberellin synthesis, is not licensed in the UK for control of mature trees. We have used

PBZ among a number of compounds to evaluate the effects of soil treatments on reducing transplant shock in beech, birch, lime and sycamore. In a separate project we made soil applications of PBZ to established plots of leyland cypress, lime, sycamore and ash to determine its potential for control of growth. Finally, we have used *in vitro* cultures of birch and poplar to determine dose response information and to determine whether PBZ has effects on sterol composition, as reported for cell cultures. We have found significant effects of PBZ on growth rates of established trees, which persist for at least three seasons after application, indicating the potential for PBZ to be used as a commercial TGR. In newly transplanted trees PBZ also reduced growth, while chlorophyll fluorescence measurements indicated an improvement in plant 'health'. Results from *in vitro* cultures have allowed us to compare effects of dose on stem and leaf expansion in two different species, and to determine effects on sterol composition.

P5.35 Abstract not supplied

P5.36 Abstract not supplied

P5.37 Cloning, Functional Expression and Characterisation of Sesame Seed Cystatin

Doulgas J.H. Shyu and Jason T.C. Tzen

Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan 40227, Republic of China

A cDNA fragment encoding cystatin, a cysteine protease inhibitor, was isolated from maturing sesame seeds, and the deduced sequence comprised of 199 amino acid residues coding for a 22 kDa polypeptide, contains no cysteine residue, and its calculated isoelectric point is pH 6.2. The clone was constructed in a non-fusion and a fusion vector, and then over-expressed in *Escherichia coli* BL21(DE3). The recombinant cystatins were presumably found in the soluble fraction of cell extract, and demonstrated to be functionally active in a reverse zymography. Low abundance of the corresponding endogenous cystatin in mature seeds was purified to homogeneity *via* a papain-coupling affinity column, and confirmed by western blotting with antibodies raised against the recombinant cystatin. Both endogenous and recombinant cystatin proteins showed effective inhibitory activities toward papain with K_i values of 7.89×10^{-8} M and 2.77×10^{-8} M, respectively. Immunoblotting analyses revealed that sesame cystatin was specifically expressed in maturing seeds and rapidly degraded after germination. Moreover, zymography and inhibition analyses indicated that the *de novo* synthesized proteases in germinating seeds could not be inhibited by sesame cystatin. The results imply that sesame cystatin may play crucial roles in the regulation of

endogenous cysteine proteases during seed maturation and germination.

P5.38 Elucidating the physiological nature of QTLs for drought resistance in rice using near isogenic lines

F.S. Khowaja, K. MacMillan and A.H. Price

Previous mapping studies have identified drought-related QTLs using a mapping population of recombinant inbred lines (RILs) derived from a cross between Bala (upland indica variety) and Azucena (upland japonica variety). The results indicated that an Azucena allele on chromosome 7 reduces leaf drying in field drought screens while another Azucena allele on chromosome 9 increases root length and root thickness in pot experiments. To investigate the physiological nature of QTLs on chromosome 7 and 9, 2 pairs of near isogenic lines (NILs) for the former and 1 pair of NILs for the latter were developed from RILs which contained residual heterozygosity using microsatellite markers. The NILs were grown in 75 cm long tubes (10 cm diameter) under two water regimes in greenhouse conditions and shoot and root physiological measurements were taken. Stomatal conductance, water use and shoot height growth were measured throughout the experiment. After 6 weeks, specific leaf area, relative water content, leaf fluorescence, shoot dry weight, root dry weight (divided into 3 depths), maximum root length and root thickness were measured. No clear differences were observed between matching NIL pairs on chromosome 7 which could account for the drought avoidance QTL originally detected there. However, differences in maximum root length and root thickness for chromosome 9 NILs match the original QTL results. The results for the QTL on chromosome 7 in particular illustrate the complexity of the genetics of drought resistance, and the difficulties still to be overcome for effective exploitation of genetic variation in quantitative traits.

P5.39 Parameters influencing *Agrobacterium*-mediated transformation of Bangladeshi Indica rices

M. Al-Forkan, P. Anthony, K.C. Lowe¹, M.R. Davey and J.B. Power

Biosciences & ¹Biology, University of Nottingham.

A reproducible and efficient *Agrobacterium*-mediated transformation system was developed for Bangladeshi Indica rices. Morphologically normal, fertile transgenic plants were obtained by co-culturing mature seed scutellum-derived embryogenic calli of the cultivars BR26 and Binni with *Agrobacterium tumefaciens* strain

LBA4404 carrying the super-binary vector pTOK233. Inclusion of acetosyringone (100 μ M) in the medium during co-cultivation of tissues with *Agrobacterium* (25–28°C) and selection with hygromycin B (50 mg l⁻¹) were essential for efficient transformation. Sonication-assisted, *Agrobacterium*-mediated transformation of embryogenic calli increased transient β -glucuronidase (GUS) activity up to 6-fold over control. Stable integration and expression of *gus*, neomycin phosphotransferase (*nptII*) and hygromycin phosphotransferase (*hpt*) genes in regenerated plants were confirmed by GUS histochemical and fluorometric assays, NPTII ELISA and Southern blot analysis. GUS activity and NPT protein varied between transformed plants. Southern analysis confirmed the integration of 2–3 copies of T-DNA into most of the regenerated plants, although transgene expression did not correlate with gene copy number. Plant height, number of panicles and spikelets/panicle, secondary branches/panicle, seeds/panicle and grain weight/panicle of transformed plants were all significantly ($P < 0.05$) increased, compared to controls. Mendelian segregation of transgenes was observed in the T1 seed generation. This reproducible, efficient protocol developed for *Agrobacterium*-mediated transformation will be exploited for the introduction of useful traits into these commercially-important Bangladeshi rices.

P5.40 Haemoglobin stimulation of somatic embryogenesis in Peanut cultures

N. Jayabalan, K.C. Lowe¹, P. Anthony, M.R. Davey and J.B. Power

Biosciences & ¹Biology, University of Nottingham

Somatic embryogenesis is a developmental process in plants that generates large numbers of embryos from somatic cells that can be exploited for mass propagation, often under bioreactor conditions via so-called artificial seed technology. Critical parameters influencing somatic embryogenesis include growth regulators and an adequate and sustained oxygen supply. Consequently, the present investigation has focused on optimisation of a somatic embryogenic system for peanut (*Arachis hypogaea* L.) through media supplementation with the auxin, picloram (4-amino-3,5,6-trichloropicolinic acid). The latter at 30 mg l⁻¹ was optimal for inducing regeneration of somatic embryos from embryonic axes of cultured zygotic embryos. Somatic embryogenesis did not occur in the absence of this growth regulator. An assessment was also made of the beneficial effect on somatic embryogenesis and plant regeneration of supplementing culture medium with the commercial bovine haemoglobin (Hb) solution, *Erythrogen*TM (Biorelease Corporation, USA). Hb at 1:50 and 1:100 (v:v) stimulated increases in mean fresh weight (up to a maximum of 57% over control), mean number of explants producing somatic embryos (by 15%) and mean number of somatic

embryos per explant (up to 29%). These results demonstrate that medium supplementation with Hb enhances somatic embryogenesis *in vitro*, probably through facilitating oxygen availability to growing cells.

P5.41 Purification of ILL2, an amidohydrolase in *Arabidopsis*

N.K. Bains^{1,2}, A.J. Thompson¹, R. Pickersgill² and R.M. Napier¹.

¹Horticulture Research International, Wellesbourne, Warwick, CV35 9EF

²Queen Mary, University of London, Mile End Road, London, E1 4NS

Several naturally occurring auxins are found in plants, the most abundant being indole-3-acetic acid (IAA). IAA is involved in most aspects of plant growth and development, for example cell enlargement, cell division and leaf senescence. IAA is found both as a free acid and as conjugates; it is thought that the free form is the active form (Bandurski *et al*, 1995). The conjugates represent 95% of the IAA pool of which 80% is amide linked. A number of amidohydrolases have been identified in *Arabidopsis*, one of which is ILL2, which is the most active in cleaving amino acid conjugates.

The aim of this project is to solve the crystal structure of ILL2 to give a better understanding of substrate recognition and mechanism of action. ILL2 has been cloned into a vector incorporating a GST tag (construct provided by Bonnie Bartel, Texas), and expressed in *Escherichia coli*. Induction conditions have been optimised and tag cleavage with thrombin protease has been successful. However attempts to further purify using FPLC have led to low yields of ILL2 protein. At present ILL2 is being cloned into several different vector systems incorporating an epitope tag(s) such as His and maltose binding protein tags. This should overcome the problem associated with low yields of ILL2. In 2002 Jozic *et al* solved the crystal structure of an aminopeptidase PepV from *Lactobacillus delbrueckii*, this has shown to have close homology with ILL2. The structure of PepV will therefore be used to model ILL2 using the modeller 4 system (Sali and Blundell, 1993).

P5.42 Effects of nitrogen in the production and development of in vitro potato microtubers

A.M. Kalifa and P.G. Alderson, Agriculture and Environmental Sciences, Nottingham University, UK

The effects of inorganic nitrogen nutrition on the induction and development of microtubers from nodal cuttings of four potato (*Solanum tuberosum* L.) genotypes, i.e. Kennebec, Cara, Desiree and Russet Burbank, was

investigated by changing the NO_3^- : NH_4^+ ratio in Murashige and Skoog (1962) culture medium. Five NO_3^- : NH_4^+ ratios, i.e. 20:40, 40:20 (control), 30:30, 50:10 and 10:50 mM were studied at a constant level of nitrogen (60 mM). Changing the NO_3^- : NH_4^+ ratio in the culture medium caused a major change in the weight and size (diameter and length) of microtubers *in vitro*. High ammonium ions (10:50 mM) in the culture medium dramatically reduced the growth parameters of microtubers in all of the genotypes. Compared with the control, the best growth of microtubers occurred in all of the genotypes when nitrate (NO_3^-) and ammonium (NH_4^+) were both present in the medium at the same concentration (30:30 mM). The genotypes showed differences in their response, i.e. Desiree and Russet Burbank were less affected than Kennebec and Cara.

P5.43 Regulatory phosphorylation of phosphoenolpyruvate carboxykinase in plants

K.J. Bailey, R.P. Walker, J.E. Gray and R.C. Leegood, University of Sheffield

Phosphoenolpyruvate carboxykinase (PEPCK) catalyses the ATP-dependent decarboxylation of oxaloacetate to phosphoenolpyruvate in plants. This is a key step in photosynthetic CO_2 concentrating mechanisms in some C4 plants, Crassulacean Acid Metabolism plants (and algae). It also plays a key role in gluconeogenesis in C3 plants. We are working with the C4 plant, *Panicum maximum*, to investigate the regulatory phosphorylation of PEPCK. Previously, this group has shown PEPCK is phosphorylated and that the response of PEPCK to its substrates, PEP and OAA, depends strongly upon the adenylate ratio in the assay and changes with its phosphorylation state, resulting in its activation by dephosphorylation in the light and inactivation by phosphorylation in darkness. We have obtained the sequence for PEPCK from *P. maximum* by using RACE PCR and primers corresponding to highly conserved regions downstream of the N-terminus. The PEPCK gene in *P. maximum* is similar to known C4 PEPCK genes. PEPCK has one potential phosphorylation site which shares homology with sites recognised by cAMP-dependent protein kinases. We have investigated the phosphorylation response of PEPCK to light intensity and CO_2 concentration by feeding leaves with ^{32}P and subsequent quantification by densitometry of autoradiograms from SDS-PAGE gels. The phosphorylation state of PEPCK has been confirmed by measurements of relative activity using a selective assay. Increasing light intensity and CO_2 concentration results in a lower relative phosphorylation state. We have also investigated the effect of metabolite effectors on the phosphorylation state of PEPCK.

P5.44 Carbon and nitrogen interactions in the carnivorous plant *Sarracenia purpurea*

K. Clark and R.C. Leegood, Department of Animal and Plant Sciences, University of Sheffield

Carnivorous plants tend to inhabit sunny, nutrient poor environments. They supplement the low nutrient uptake through their roots by trapping and digesting invertebrates, mainly insects, in modified leaves and absorbing the products of this digestion. The genus *Sarracenia*, commonly known as pitcher plants, are known to absorb amino acids through their pitcher surfaces but little is known about the fate of these amino acids, in terms of metabolism, once they have been absorbed.

Sarracenia purpurea pitchers were fed for 24 hours with a variety of amino acids including ^{13}C aspartate, ^{15}N glutamate, asparagine and glutamine, the phloem sap was then collected and subjected to HPLC analysis to quantitatively determine the amino acid content. Those fed ^{13}C or ^{15}N labelled compounds were also subjected to mass spectrometry analysis of both the phloem sap and tissue.

Feeding amino acids to pitchers significantly increased the amino acid concentration in the pitcher phloem sap and metabolism of some amino acids was seen to occur before they were transported into the phloem. Labelling of ascorbic acid, glucose and sucrose in the sap was seen as a result of feeding ^{13}C aspartate, and histidine and glutamate in the pitcher sap became labelled after feeding ^{15}N glutamate.

P5.45 The radial transport of abscisic acid (ABA) in maize roots under conditions of nutrient deficiency

Daniela Schraut and Wolfram Hartung

Roots of plants that have to grow in nutrient deficient soils of extreme habitats send abscisic acid (ABA) as a hormonal long distance stress signal to the shoot. To study the importance of radial ABA transport for the development of the root to shoot ABA signal, pressure gradients were applied to the cut surface of the mesocotyl of maize seedlings cultivated under N-, P- and K-deficiency and lateral flows of water and ABA were measured and related to the internal ABA concentration of the root. Nitrogen and phosphorus deficiency reduces both, the water and ABA flows. When potassium was replaced by sodium, water and ABA flows were significantly increased. The reduction of lateral ABA flows in N and P deficient roots is accompanied by an increased suberisation of the radial cell walls of the endodermis and hypodermis. The relation of internal ABA concentrations of the seedling roots to water and ABA flows

is shown. The data support earlier findings of an apoplastic bypass flow of ABA through the endodermis of maize roots. They also describe how nutrient deficiency can modify the intensity of the ABA stress signal.

P5.46 The haustorium of the parasitic association *Rhinanthus minor* / *Hordeum vulgare*

Jiang Fan, W. Dieter Jeschke, Wolfram Hartung

Rhinanthus minor is a hemiparasite that attaches to a wide range of hosts. After formation of haustoria it exploits the xylem sap of the host. Nutrients and hormones of the xylem sap are needed to establish an optimal growth and development of the parasite. The haustorium first penetrates the phloem of the host roots before puncturing a xylem vessel. As a reaction to infection, the endodermis of the host is converted to the tertiary stage. Abscisic acid of the haustoria reaches the highest concentrations within the whole *Rhinanthus minor* / *Hordeum vulgare* association. The distribution of ABA within the haustorium has been studied and a possible physiological function of ABA in the haustorium is discussed.

P5.47 Using infrared thermography to isolate *Arabidopsis* stomatal mutants that display lesions in the response to elevated CO₂

C.P.P. Tagliavia¹, A.M. Hetherington, G.H. Holroyd, W.J. Davies, and M.R. McAinsh

LEC, Lancaster University, Bailrigg, Lancaster, LA1 4YW, UK. E-mail address: ¹c.tagliavia@lancaster.ac.uk

Infrared thermal imaging has previously been used to isolate stomatal mutants that carry lesions in the ABA signalling pathway (Merlot S., Mustilli A. C., Genty B., North H., Lefebvre V., Sotta B., Vavasseur A., and Giraudat J., 2002, *Use of infrared thermal imaging to isolate Arabidopsis mutants defective in stomatal regulation*, Plant Journal 30 (5), 601–609; Wang, Holroyd, Leyser and Hetherington unpublished). The aim of this investigation is to use infrared thermal imaging to identify mutants that are compromised in their response to elevated CO₂. Stomata exhibit two distinct responses to elevated carbon dioxide. In the majority of *Arabidopsis* ecotypes and many other species, growth at elevated CO₂ is associated with a reduction in stomatal index. For example the product of the HIC gene (encoding a putative beta ketoacyl CoA synthase) is involved in this developmental signalling pathway, linking CO₂ perception to stomatal development (Gray J.E., Holroyd G.H., van der Lee F.M., Bahrami A.R., Sijmons P.C., Woodward F.I., Schuch, W. and Hetherington A.M., 2000, *The*

HIC signalling pathway links CO₂ perception to stomatal development, Nature 408, 713–716). Elevated CO₂ also brings about reductions in stomatal aperture (Mansfield T.A., Hetherington A.M. and Atkinson C.J., 1990, *Some aspects of stomatal physiology*, Annual Review of Plant Physiology and Plant Molecular Biology 41, 55–75). Although we know rather little about the CO₂ signalling pathway there is evidence for a role for calcium ions (Webb A.A.R., McAinsh M.R., Mansfield T.A., and Hetherington A.M., 1996, *Carbon dioxide induces increases in guard cell cytosolic free calcium*, Plant Journal 9 (3), 297–304) and malate (Hedrich R., Marten I., Lohse G., Dietrich P., Winterm H., Lohaus G. and Heldt H.W., 1994, *Malate-sensitive anion channels enable guard cells to sense changes in the ambient CO₂ concentration*, Plant Journal 6(5), 741–748). Here we shall describe our progress in the construction of a chamber suitable for screening for *Arabidopsis* mutants compromised in their response to elevated CO₂.

P5.48 Distribution of the triterpenoids, asiaticoside and madecassoside, in Malaysian *Centella asiatica* (L.) Urban

Z.A. Aziz, P. Anthony, K.C. Lowe¹, M.R. Davey, J.B. Power and R.M. Smith²Biosciences & ¹Biology, University of Nottingham and ²Chemistry, University of Loughborough

Centella asiatica is an aromatic species used extensively as a medicinal plant, especially in South East Asia. Investigations on the chemical content of *C. asiatica* indicate that it contains triterpenoid compounds, including asiaticoside and madecassoside. Several therapeutic properties of *C. asiatica*, including treatment of diarrhoea, fever, leprosy and psoriasis, have been ascribed to these constituents. The distribution of asiaticoside and madecassoside was evaluated in 2 types [heavily fringed-leaf margin (F-line) and smooth-leaf margin (S-line)] of Malaysian *C. asiatica*. Leaves, petioles and roots were freeze dried, ground and extracted using methanol:water (9:1, v:v). Chemical analysis was performed using gradient HPLC with acetonitrile:water as the mobile phase. Both compounds were found to be at their maximum concentrations in leaves, with $0.79 \pm 0.05\%$ and $1.15 \pm 0.15\%$ d. wt. asiaticoside and $0.97 \pm 0.08\%$ and $1.65 \pm 0.02\%$ d. wt. madecassoside in F- and S-lines, respectively. In the F-line, roots contained the lowest concentrations of both compounds ($0.12 \pm 0.02\%$ d. wt. asiaticoside and $0.11 \pm 0.02\%$ d. wt. madecassoside), whereas the petioles of S-line plants contained the lowest concentrations of these compounds ($0.16 \pm 0.02\%$ d. wt. asiaticoside and $0.17 \pm 0.02\%$ d. wt. madecassoside). These results are relevant to the commercial production and exploitation of such secondary plant products.

P5.49 Gravitational influences on the *Arabidopsis* genome

R. Hampp, M. Martzivanou, P. Anthony¹, K.C. Lowe², M.R. Davey¹ and J.B. Power¹

Physiological Ecology of Plants, University of Tübingen and ¹Biosciences & ²Biology, University of Nottingham

Gravity is a ubiquitous, uniform and inescapable force on the Earth's surface that influences the development of living organisms. Experimental systems for studying gravitational effects on biological processes have relied upon laboratory-based clinostats, space orbiting stations and free-fall conditions, all of which present specific limitations. To overcome constraints associated with these approaches, a high-field, closed-cycle, liquid helium-cooled, super-conducting magnet system, unique in the UK, is being exploited at Nottingham. Experiments are being conducted to investigate the effects of (1) zero ($0\times g$; diamagnetic levitation), normal ($1\times g$), or enhanced ($2\times g$) gravity, and (2) intense magnetic fields on gene expression in cultured cells of the model plant, *Arabidopsis thaliana*, Ecotype Columbia. In short-term experiments, cells were held for 1–2h in culture vessels at specific locations, corresponding to the above gravity conditions, within the magnet bore. Cells were harvested and flash frozen prior to RNA extraction and array analyses for genes encoding enzymes involved in major metabolic pathways (e.g. glucose-6-phosphate dehydrogenase) and signalling factors (e.g. calcium binding protein). Array analyses in these preliminary studies indicate that short-term exposure to microgravity does not induce major changes in gene expression. Future experiments will determine the effects of longer-term exposure on gene expression, cell metabolism and development.

P5.50 A pivotal role for the peptide transporter HvPTR1 in barley grain germination

W.M. Waterworth, M.K. Ashley, C.E. West and C.M. Bray

School of Biological Sciences, University of Manchester, UK

Peptide transport, mediated by the HvPTR1 transporter localised to the plasma membrane of the scutellar epithelium, plays a crucial role in the mobilisation of organic nitrogen from the endosperm to the embryo during barley grain germination. Peptide transport activity across the scutellum is regulated at the post-translational level by metabolites (inhibited up to 70% by amino acids and stimulated by glucose) at concentrations comparable with those found in the endosperm of the germinated barley grain. We have shown that HvPTR1 becomes heavily phosphorylated in response to amino

acids by *in vivo* [³²Pi]HvPTR1 labelling studies and that control of HvPTR1 transport activity is effected through changes in the phosphorylation status of specific serine residues (Ser 183) in the transporter protein. Thus peptide transport activity across the scutellum during germination is regulated at the post-translational level in response to rising levels of amino acids emanating from the endosperm as a result of storage protein breakdown and mobilisation. This is potentially an important element in balancing the flux of organic nitrogen and carbon from the endosperm to embryo during germination and seedling establishment. Ongoing studies will characterise the components which link metabolite sensing to HvPTR1 transporter activity. Additionally we have experimentally defined the topology of HvPTR1 using an epitope tagging approach and determined the location, orientation and number (11) of transmembrane spanning domains, and orientation of the N and C termini in the plasma membrane.

P5.51 Adenosine kinase of tobacco BY-2. Cloning and gene expression

Z. Kwade¹, A. Goossens², A. Azmi¹, and H. Van Onckelen¹

1 Laboratory of Plant Physiology and Biochemistry, Department of Biology, University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium

2 Department of Plant System Biology, Flanders Interuniversity Institute for Biotechnology, Ghent University, Technologiepark 927, B-9052, Gent, Belgium

Adenosine kinase (ADK; EC 2.7.1.20; ATP: adenosine 5'-phosphotransferase) is a housekeeping enzyme responsible for the conversion of adenosine (Ado) to adenosine monophosphates (Anderson, 1977). ADK is a component of the adenylate metabolic network and a key enzyme in the regulation of the intracellular level of Ado. It has been reported that the purine plant hormones, cytokinins, can also serve as substrates for adenine salvage enzymes *in vitro*. Through its potential role in conversion of cytokinin bases and possibly ribosides to nucleotides, which are thought to be the inactive forms of cytokinins, ADK may be important in regulating the level of 'active' cytokinins in plant cells (Auer, 2002).

Using a zeatin affinity column Laukens *et al.* isolated a 40-kDa protein from tobacco (*Nicotiana tabacum*) Bright Yellow 2 (TB-Y-2) cells, further identified by mass spectrometry as ADK.

In our experiment we have identified and characterized the full-length complementary DNAs (cDNAs) encoding four ADK isoforms of *Nicotiana tabacum* BY-2 cells and made an attempt to unravel the expression pattern of *adk* genes during the cell cycle.

P5.52 Post-translational regulation of AGPase activity wheat endosperm

M.M. Burrell¹, I.J. Tetlow¹, M.M. Burrell², and M.J. Emes¹

¹Department of Botany/MBG, University of Guelph, Ontario, Canada;

²Animal and Plant Sciences, University of Sheffield, UK

ADPglucose pyrophosphorylase (AGPase, E.C. 2.7.7.27) catalyzes the synthesis of ADPglucose which is the immediate soluble substrate for the synthesis of starch. The enzyme is a heterotetramer of two large subunits and two small subunits, and in monocotyledonous storage tissues is located inside and outside the starch-synthesizing amyloplasts.

In addition to the well-characterized allosteric regulation of AGPase activity by metabolites, recent work has indicated post-translational modification of higher plant AGPases involving thioredoxin. It is thought that partial inactivation of AGPase occurs by the formation of intramolecular disulphide bonds between the N-termini of the small subunits of AGPase, producing an inactive dimer. Work with wheat endosperm amyloplasts indicates that dimer formation is dependent on redox state. We are currently characterizing the dimeric forms of wheat endosperm AGPases from plastidial and whole cell extracts, the latter containing the more abundant cytosolic form of AGPase.

P5.53 Purification and characterization of enzymes involved in lysine metabolism in sorghum

R.R. Ferreira¹, R.F. Fornazier¹, S.A. Gaziola¹, L.W. Meinhardt², P.J. Lea³, R.A. Azevedo¹

¹ESALQ-USP, Brazil, ²UNICAMP, Brazil, ³Lancaster University, UK

It is necessary to increase our understanding of the regulation of lysine metabolism, in order to improve the nutritional quality of plants by genetic manipulation. Among the enzymes involved in lysine metabolism, aspartate kinase and dihydrodipicolinate synthase are important in lysine biosynthesis, whereas lysine 2-oxoglutarate reductase and saccharopine dehydrogenase play a key role in lysine catabolism. We have isolated and partially purified by ion exchange and gel filtration chromatography, aspartate kinase, homoserine dehydrogenase, lysine 2-oxoglutarate reductase and saccharopine dehydrogenase from sorghum seeds. Two isoenzymes of aspartate kinase were detected, one sensitive to lysine inhibition and the other sensitive to threonine inhibition. The lysine-sensitive aspartate kinase was predominant, whereas the threonine-sensitive form appeared to co-purify with the threonine-sensitive homoserine dehydrogenase, suggesting the existence of a bifunctional

threonine-sensitive aspartate kinase/homoserine dehydrogenase as already detected in other plant species. The activities of lysine 2-oxoglutarate reductase and saccharopine dehydrogenase were the lowest observed for any other plant species, including the high-lysine maize mutant opaque-2. The presence of a bifunctional lysine 2-oxoglutarate reductase and saccharopine dehydrogenase enzyme was confirmed, however others isoforms were also detected. Analysis of lysine concentration in the seeds of the sorghum variety used revealed an accumulation of lysine, which may explain the very low activity levels of the enzymes involved in lysine catabolism observed. (Financial support from FAPESP, Brazil, and the British Council)

P5.54 Cadmium induced oxidative responses in higher plants

P.L. Gratão¹, G.J.G. Pereira¹, R.A. Gomes-Junior¹, R.R. Ferreira¹, R.F. Fornazier¹, P.F. Cardoso¹, F.R. Capaldi², V.A. Vitorello², S. Filipi³, P. Mazzafera³, R.J. Smith⁴, P.J. Lea⁴, A.P. Vitória⁵, R.A. Azevedo¹

¹ESALQ-USP, Brazil, ²CENA-USP, Brazil, ³UNICAMP, Brazil, ⁴Lancaster University, UK, ⁵UENF, Brazil

Cadmium (Cd) is a very toxic heavy metal and occurs in low concentrations in soils but the concentration may be high in areas that have been subjected to mining or application of sewage sludge. Cd enters the plant system rapidly where it accumulates mainly in the root leading to inhibition of plant growth. Cd can also induce the production of reactive oxygen species (ROS). We studied the antioxidant responses of distinct higher plant species to Cd exposure, using seedlings, and in some cases in vitro cell cultures. Experiments were carried out using concentrations of CdCl₂ ranging from 0.01-3 mM for up to 96 h. Analysis of Cd uptake indicated that most of the Cd accumulated in the roots, but some was also translocated to the leaves. Roots and leaves were analysed for catalase (CAT), glutathione reductase (GR), glutathione-S-transferase (GST), guaiacol peroxidase (GPx) and superoxide dismutase (SOD) activities. GR activity increased considerably in the roots of all plant species exposed to Cd. CAT activity increased in roots but to a much lesser extent when compared to GR, and varied depending upon the plant species. SOD activity staining revealed several isoenzymes in the leaves of all plant species, however, only in radish was there a clear increase in enzyme activity. The activity of GPx was also shown to increase in tobacco in vitro cell suspension cultures grown in the presence of Cd. The results suggest that the main response may be via the activation of the ascorbate-glutathione cycle for the removal of hydrogen peroxide, or to ensure the availability of reduced glutathione for the synthesis of Cd-binding peptides. (Financial support from FAPESP, Brazil, and the British Council)

P5.55 Characterization of storage proteins of maize endosperm mutants

A.A. Toro¹, C.M. Bellato², L.W. Meinhardt³, F. Mestrinelli², B.D.A. Berdejo¹, P.J. Lea⁴, R.A. Azevedo¹

¹ESALQ-USP, Brazil, ²CENA-USP, Brazil, ³ UNICAMP, Brazil, ⁴ Lancaster University, UK

The capacity of two maize opaque endosperm mutants (o1 and o2) and two floury (fl1 and fl2) to accumulate lysine in the seed in relation to their wild type counterparts Oh43+ was examined. The highest total lysine relative content was 3.78% in the o2 mutant and the lowest 1.87% in fl1, as compared to the wild type (1.49%). For soluble lysine, o2 exhibited over a 700% increase, whilst for fl3 a 28% decrease was encountered, as compared to the wild-type. In order to understand the mechanisms causing these large variations in both total and soluble lysine content, a quantitative and qualitative study of the N constituents of the endosperm has been carried out and data obtained for the total protein, non-protein N, soluble amino acids, albumins/globulins, zeins and glutelins present in the seed of the mutants. Following two dimensional PAGE separation, a total of 35 different forms of zein polypeptides were detected and considerable differences were noted between the five different lines. In addition, two enzymes of the aspartate biosynthetic pathway, aspartate kinase and homoserine dehydrogenase were analysed with respect to feedback inhibition by lysine and threonine. The activities of the enzymes lysine 2-oxoglutarate reductase and saccharopine dehydrogenase, both involved in lysine degradation in the maize endosperm were also determined and shown to be reduced several fold with the introduction of the o2, fl1 and fl2 mutations in the Oh43+ inbred line, whereas wild-type activity levels were verified in the Oh43o1 mutant. (Financial support from FAPESP, Brazil, and the British Council).

P5.56 Abstract not supplied

P5.57 Does a future high CO₂ environment alter leaf senescence in a temperate forest exposed to free air CO₂ enrichment (POPFACE)? Evidence from leaf, canopy and remote measurements

M.J. Tallis, G.J.J. Clarkson, M. Pecchiari, F. Miglietta and G. Taylor, Biology, University of Southampton

Long-term phenological studies have detected a lengthening growing season of mid-latitude plants, a change

generally attributed to climate warming. After 5 years of free air CO₂ enrichment (FACE) at the POPFACE experiment, with the plantation characterized by a closed canopy a delay in autumnal senescence was evident for *Populus nigra* and *Populus x euramericana*

Canopy Normalised Difference Vegetation Index (NDVI) calculated from air-borne and ground-based reflectance spectroscopy was significantly increased in elevated CO₂. Plant Senescence Reflectance Index (PSRI) a measure of canopy carotenoid to chlorophyll ratio was significantly reduced indicating retention of chlorophyll in elevated CO₂, a finding supported by leaf level chemical extraction of chlorophyll. Leaf area index (LAI) was significantly stimulated by FACE throughout the growing season for *P. x euramericana* with a 33–37% stimulation, the largest stimulation, occurring late season after bud set. Post hoc analysis highlighted evidence that nitrogen fertilization had little influence on the timing of senescence in elevated CO₂ but appeared to promote senescence at ambient CO₂.

This study suggests that elevated CO₂ alone may contribute to the lengthened growing season generally attributed to global warming.

P5.58 Abstract not supplied

P5.59 Towards Measuring the Metabolic Flux of Carbon into Fatty Acids in Developing Oilseed Rape Embryos in vivo

M.J. Pike, E. Morley-Smith, ¹S. Troufflard and S. Rawsthorne

Metabolic Biology, John Innes Centre, Norwich, UK and ¹Universite Picardie Jules Verne, Amiens, France.

A novel in vivo method for measuring carbon flux into fatty acids in embryos of oil seed rape has been developed. Previous methods have usually involved invasive techniques whereby embryos are removed from their siliques and testas before incubating in a feeding media which leads to disruption of their light, oxygen and metabolite environments [e.g. 1, 2]. We have devised a viable method of incubating whole excised siliques in a media containing ¹⁴C-sucrose and allowing experiments to run for extended periods of time before harvesting the embryos and extracting and quantifying the incorporation of ¹⁴C into fatty acids. This method is shown to have no detrimental effects on seed metabolism. Techniques to measure the dilution effect that occurs in the

embryo when the supplied ^{14}C -sucrose becomes diluted by endogenous carbon sources are also being developed to allow absolute flux to be determined using the measured specific radioactivities. Preliminary experiments suggest that this technique could be used to investigate and compare fluxes through other metabolic pathways under different physiological conditions. Results from preliminary experiments will be presented.

1. Schwender and Ohlrogge, (2002) *Plant Physiol.* 130, 347–361.
2. Eastmond PJ and Rawsthorne S (2000) *Plant Physiol.* 122, 767–774.

P5.60 Why hide in the shade? Factors determining distribution of epiphytes

Casandra Reyes, Kate Maxwell and Howard Griffiths, Cambridge University, UK

Having a gradient of climatic factors along the height of a canopy, it has always intrigued us how different species of epiphytes are distributed, and what factors determine stratification. We studied the distribution of epiphytic bromeliad species in the dry forest of Chamela, Mexico, and found a counterintuitive distribution. In this environment, the tank lifeforms, which have a water impounding reservoir, were distributed at the upper, drier canopy strata, and the atmospheric lifeforms, which are documented to be more drought resistant and function with a high density of water absorbing trichomes, were found to have representatives at both upper and lower canopy. We studied the exposed tank *Tillandsia fasciculata* and the shaded atmospheric *Tillandsia pseudobaileyi* in order to determine if light or water were determining distribution factors. We found that the atmospheric had lower saturated rates of photosynthesis, characteristic of shade plants. After a prolonged drought, individuals of the atmospheric species that were under high light were not able to recover their photosynthetic capacity. This indicates that if left in exposed sites, this species would undergo serious and irreversible photodamage. The tank species showed the opposite, with high saturation points and rapid recovery of photosynthetic capacity in both exposed and shaded individuals. Water was also a determining factor, the tank species maintained high water content during the early dry season (after three months of drought), while the atmospheric quickly dehydrated at the beginning of the drought season. These results are mirrored by the $\delta^{18}\text{O}$ of leaf water, which indicate transpiration.

P5.61 Biochemical and structural characterisation of novel aldo-keto reductase (AKR) genes in *Arabidopsis* indicates possible roles in detoxification and/or steroid metabolism

Anna M Reed, Dr Jonathan P Ride, Dr Christopher M Bunce, Dr Owen C Mather, Dr Scott A White

Screening of the model plant *Arabidopsis thaliana*'s genomic sequence has revealed four potential genes that have significant homology to a human gene that encodes a member of the aldo-keto reductase (AKR) family. This well-characterised human AKR (AKR1C3) is implicated in steroid metabolism, detoxification and cell differentiation. Plants are also now known to contain steroid hormones, and it was of interest to determine whether these plant AKRs had steroid metabolising activity.

RT-PCR has revealed evidence for expression of the genes (denoted AtAKR1-4) in *Arabidopsis* tissue. All four of the genes have been successfully cloned into bacterial cells & recombinant proteins have been expressed and purified. The purified proteins show differing activity in enzyme assays with a wide range of substrates including steroids and sugars and using the pure protein we have solved the crystal structure of one of the AtAKRs. The real challenge of this study will be attempting to elucidate the precise function of these novel AKRs in *Arabidopsis* using a variety of techniques. Not many AKR enzymes have been characterised in plants thus far but studies on related proteins in alfalfa and foxglove have indicated the possibility of both detoxification and steroid-modifying roles. There is also the fundamental question to consider of whether there is a common role of AKR's in cell differentiation and tissue development amongst all multicellular organisms.

P5.62 Investigating the roles of the *Arabidopsis* MAX genes in shoot branching control

Katherine Bainbridge and Ottoline Leyser, University of York

The *MAX* genes of *Arabidopsis* define an important pathway involved in the auxin mediated inhibition of axillary bud outgrowth. Although the *MAX* genes have been cloned, much remains to be learnt about their detailed function.

Grafting data presented here, demonstrate that wild-type roots are able to rescue the branching phenotype of a *max4* shoot. This provides evidence that the *MAX4* gene is responsible for the production of a mobile signal, able to move acropetally to inhibit bud outgrowth and that

expression in either root or shoot tissue is sufficient to inhibit branching.

Detailed metabolite profiles of the *max* mutants have been created. It is hoped that, through careful analysis, candidate signalling molecules can be identified and tested for their ability to restore a wild-type phenotype to *max* mutant plants.

To investigate whether shoot branching might be regulated by changes in *MAX* gene expression, expression patterns of *MAX4* were characterised using a promoter::GUS construct. The reporter gene is expressed in root tips and in nodal tissue surrounding buds, but not in the buds themselves. Following 8 hours auxin application, staining in the elongation zone of the root becomes apparent, indicating auxin acts to up-regulate *MAX4*.

P5.63 Processing Of Reporter Proteins in Plants Using a Polyprotein Approach

S.E. Davenport¹, J.A. Tuerck¹, M.J. Maunder¹ and P.J. Lea².

¹Advanced Technologies Cambridge; ²Lancaster University

Very often genetic engineering of plant metabolic pathways involves the co-ordinate expression of several transgenes. A number of strategies have been used for the expression of multiple genes in plants; retransformation, crossing, cotransformation and transformation vectors containing multiple expression cassettes. Such methods are time-consuming, labour-intensive and cannot reliably ensure co-ordinate expression. Novel technologies have been developed that use single transgenes to manipulate the expression of multiple genes. Internal ribosomal entry sites (IRES) from viruses have been used to direct cap-independent translation initiation in plant cells. This method does not work reliably in all plant cells and tissues. In a polyprotein approach, a number of coding sequences are fused together as a single transcriptional unit and expressed from a single promoter. Translation of the transcription unit leads to the formation of a polyprotein precursor which is proteolytically cleaved to release the individual proteins.

Potviruses use this polyprotein approach and the viral RNA is translated into a single polyprotein containing autocatalytic proteolytic activity. A viral protease must be expressed along with the proteins of interest and might have undesired proteolytic activity on endogenous proteins. Francois, Broekaert and Cammue (2002) used a naturally occurring plant linker peptide from *Impatiens balsamina* to connect the sequences coding for two small antifungal proteins from *Dahlia merckii* and *Raphanus sativus*.

This project wanted to investigate if it was possible to use the same linker peptide with two large reporter genes, *E. coli* β -Glucuronidase (GUS) and firefly lucif-

erase (LUC) in two plant species, *Arabidopsis thaliana* and *Nicotiana tabacum*.

P5.64 Genetic characterisation of the symbiotic interaction between *Lotus japonicus* and its symbiont

N. Rispaill, K.J. Webb, L. Skøt and P. Morris

Institute of Grassland and Environmental Research, Aberystwyth, Ceredigion, SY23 3EB, UK

Legumes can interact with specific soil bacteria called rhizobia in a symbiotic process. This interaction leads to the formation of a new organ, the nodule, within which the bacteria fix atmospheric nitrogen for the plant in exchange for carbohydrates. The initiation and maintenance of this symbiotic relationship implies a complex communication between plant host and its symbiont, which is genetically driven.

Recently, the use of the model legume *Lotus japonicus* allowed the identification of the first genes involved in this interaction mainly by map-based cloning techniques. To increase our understanding of the symbiotic process, we undertook in parallel a mutation project of *L. japonicus* by transgenesis using *A. tumefaciens*. The strategy was to create promoter-trapped lines by insertion of a T-DNA insert containing a promoterless *uidA* gene (encoding the β -glucuronidase enzyme) in order to tag novel genes involved in symbiosis. Seven different transgenic lines expressing GUS in roots and/or nodules were generated. These lines will be briefly presented, along with the isolation and characterisation of the tagged-gene from one particular line, T711. The identification of this gene and its implication in symbiosis will be further discussed.

P5.65 Characterisation of an *Arabidopsis thaliana* mutant with a reduced number of flowers

P.S. Saputhanthri, K. Beauchamps, S. Gaynor and R. Hooley

Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY, UK

An *Arabidopsis thaliana* mutant *purple patch inflorescence* (*ppi*) in the ecotype Ws-2 is recessive and affects the growth and development of the plant in both the shoot and the root throughout the entire life-cycle.

Mature flowering plants of *ppi* grown under long days have a pleiotropic phenotype in which plants are pale green, semi-dwarf with small leaves and have reduced apical dominance. A striking feature of *ppi* is that it produces a reduced number of flowers in each floral meristem along with an unusual pigmented zone just below the terminal flower. No defect in the timing of

transition to flowering in *ppi* was observed compared with the wild-type.

Some aspects of the phenotype suggest possible defects in the synthesis or signalling of plant hormones. Growth response and hormone bioassays have given some insights into the nature of the mutation, though are not conclusive at this stage. Seedlings of the mutant have shorter hypocotyls than wild-type in the light, and are partially de-etiolated in the dark. Mutant seedlings grown on nutrient agar show an altered root development with fewer root-hairs compared with the wild type. Seeds of the mutant were found to be hypersensitive to the gibberellin (GA) biosynthesis inhibitor paclobutrazol, suggesting defects in GA biosynthesis and / or signalling.

P5.66 Expression and characterisation of legume lipoxygenases and hydroperoxide lyases

Eric Belfield, Richard Hughes, Raquel Olias¹, Nicolas Tsesmetzis and Rod Casey

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

¹Institute of Food Research, Norwich Research Park, Norwich NR4 7UA UK

Lipoxygenases (LOXs) and fatty acid hydroperoxide lyases (HPLs) play key roles in the formation of biologically active oxylipins, such as short-chain aldehydes and wound hormone, from polyunsaturated fatty acids. We are over-expressing LOXs and HPLs in *Arabidopsis thaliana* in order to manipulate oxylipin metabolism and better understand their roles in plant defence. We have expressed the enzymes in *E. coli* and characterised them prior to plant transformation.

Pisum sativum (pea) seed LOX-2 and -3 were expressed as native proteins, and *Medicago truncatula* LOXB, HPLE and HPLF as His-tag fusions. The HPLs were expressed both with (+) and without (–) N-terminal sequences that have been proposed to serve a regulatory role.

LOX-3, which has similar properties to LOXB, is more stable, has more stringent substrate requirements, and narrower product specificity than LOX-2. HPLF+ had the highest expression level in *E. coli*. It is most active with the 13-hydroperoxides of linoleic and α -linolenic acids, but also metabolises 9-hydroperoxides. HPLE is a 13-HPL that is most similar to a previously-characterised alfalfa HPL and has only 34% amino acid sequence identity with HPLF.

HPLs are members of the CYP74 subgroup of cytochrome P450s that have extremely high turnover numbers and great potential as biocatalysts. Based on known P450 3D structures, a number of site-directed *M. truncatula* HPL mutants have been produced to aid understanding of their specificity and reaction mechanism.

The HPLs and LOXs are being introduced into *Arabidopsis*, under the control of leaf-specific (HPLs) or inducible (LOXs) promoters.

P5.67 Abstract not supplied

P5.68 Ethanol metabolism during menadione-induced oxidative stress in *Arabidopsis thaliana*

A.P. Garlick, G.S. Tunbridge, J.A. Turton and N.J. Kruger, University of Oxford

Treatment of an *Arabidopsis* cell suspension culture with menadione stimulates generation of reactive oxygen species and imposes a mild oxidative stress. This stress leads to inactivation of pyruvate dehydrogenase (PDH), an accumulation of pyruvate and enhanced accumulation of ethanol and acetaldehyde. Metabolism of [¹⁴C]glucose confirms that flux into the tricarboxylic acid cycle decreases and the proportion of label metabolised via ethanolic fermentation increases under these conditions. We suggest that the latter represents an attempt of the system to stimulate a metabolic bypass involving pyruvate decarboxylase, aldehyde dehydrogenase and acetylCoA synthase to allow continued synthesis of acetylCoA, via acetaldehyde and acetate, despite the decrease in PDH activity. To test this proposal we have examined the capacity of *Arabidopsis* cells to metabolise ethanol. This compound enters general metabolism only after oxidation to acetaldehyde and thus its utilisation is diagnostic for the PDH-bypass. In untreated cells, [1-¹⁴C]ethanol is metabolised principally to carbon dioxide, organic acids, amino acids, proteins and lipids consistent with its conversion to acetylCoA. However, the amount of radioactivity entering lipids and tricarboxylic acid cycle products decreases under oxidative stress and the distribution of label within and between these groups of compounds is altered. This implies that *Arabidopsis* has the capacity to catalyse the conversion of pyruvate to acetylCoA independently of PDH. Nevertheless, although this bypass may compensate for inactivation of PDH, the menadione-induced perturbations of ethanol catabolism indicate that other enzymes of the primary metabolic pathways are susceptible to modification and may contribute to the metabolic consequences of oxidative stress.

P5.69 Phosphate allocation and reproductive behaviour in *Arabidopsis thaliana* and *Silene dioica*

J. Richards and A.H. Fitter, University of York

Flowering time is critical to the reproductive success of a plant. Phosphate has previously been implicated in determining the flowering time of both *Holcus lanatus*

and the *pho* mutants of *Arabidopsis thaliana*. Most of our understanding of the transition to flowering comes from work on *A. thaliana* mutants and a number of genetic pathways have been identified which lead to flowering. To understand where the link between phosphate and flowering time fits into the recent advances in the understanding of the genetic control of flowering time we have studied the *pho2* mutant. The *pho2* mutant accumulates excessive amounts of phosphate (2–5 fold higher) into shoots compared with wild type plants and has an early flowering phenotype. How changes in photoperiod and ambient growth temperature affect the phosphate allocation and flowering time of *pho2* has been studied and the results will be presented. Double mutants of *pho2* and a range of other flowering time mutants are being created. We also measured gender differences in both flowering time and leaf phosphate concentration in a natural population of *Silene dioica*, in which male plants are known to flower earlier and reported to accumulate more phosphate than females. We found that there was a negative relationship between phosphate and flowering in males only and this relationship was habitat dependant.

P5.70 Modified cellular polarity in the *hydra* mutants of *Arabidopsis* is associated with multiple patterning anomalies throughout shoot development

Margaret Pullen and Keith Lindsey, The Integrative Cell Biology Laboratory, School of Biological and Biomedical Sciences, University of Durham

During *Arabidopsis* shoot development, cells in the young hypocotyl epidermis have a co-ordinated polarity

in line with the shoot axis, and axial polarity-dependent events around the meristem are associated with correct dorsiventral patterning in the emerging leaf primordium. Such directionally expanding cells show a polar arrangement of the microtubular cytoskeleton. Later in organogenesis, axial polarity-dependent cell division events are required in the formation of trichomes, and local (non-axial) polar cell division events are involved with the differentiation of stomatal complexes.

The *hydra* mutants result from single gene mutations in components of the sterol biosynthesis pathway; these produce a pleiotropic, seedling lethal phenotype with a variable morphology and multiple patterning defects (Topping et al 1997 *Development* 124, 4415–4424, Souther et al 2002 *Plant Cell* 14, 1017–1031). Hypocotyl epidermal cell files of *hydra* contain variably mis-shapen cells. Examination of a tubulin::GFP fusion protein shows that these cells have variable and poorly co-ordinated polarities during expansion. Leaf development in *hydra* demonstrates multiple patterning defects in both stomatal complex formation, and in trichome morphology. Transgenic promoter-reporter fusion markers of cellular patterning and dorsiventrality show mis-expression in *hydra* lateral organs, a number of which include the StART domain, considered to be sterol responsive (Ponting & Avarind 2000 *Trends Biochem. Sci.* 24, 130–132.).

A reduction in ethylene perception has been shown to produce a partial rescue of the *hydra* mutant phenotype, implicating ethylene in these patterning processes (Souther et al 2002). The effects of ethylene on cellular polarity and axiality in an altered sterol environment are discussed in relation to *hydra* cellular morphology.