



Society for Experimental Biology Annual Main Meeting 28th June – 1st July 2009, Glasgow, UK

P5 – YOUNG SCIENTISTS IN PLANT BIOLOGY (FORMERLY DEVELOPMENTS IN PLANT BIOLOGY)

P5.1

10:30 Monday 29th June 2009

The role of PIF3 in phytochrome regulation of chloroplast development

Matthew J. Terry (University of Southampton), Patrick G. Stephenson (University of Southampton), Christian Fankhauser (University of Lausanne)

The establishment of functional chloroplasts during the transition to photoautotrophic growth is a crucial stage in seedling development. As the seedling emerges into light, signals from the phytochrome and cryptochrome families of photoreceptors promote the development of chloroplasts and the photosynthetic apparatus. The phytochromes signal in part through direct interaction with a family of nuclear-localised, phytochrome-interacting factors (PIFs). One of these factors, PIF3, has previously been proposed as a positive regulator of chloroplast development. We have re-evaluated the phenotype of the *pif3* mutant and show that it is similar to the *pif1* mutant, which lacks a repressor of chloroplast development, PIF1. Consistent with this, a *pif1pif3* double mutant shows an additive phenotype in all respects. All mutants have elevated protochlorophyllide levels in the dark and etioplasts with smaller prolamellar bodies and more prothylakoid membranes than wild-type seedlings. Interestingly, the *pif* mutants also show a broader constitutively photomorphogenic phenotype with reduced hypocotyl elongation and increased cotyledon opening in the dark. Investigation of the molecular basis of the chloroplast phenotype revealed that the key tetrapyrrole pathway genes *HEMA1*, *CHLH* and *GUN4* all have elevated expression levels in dark-grown *pif* mutants. This was due in part to altered circadian regulation of these genes in the dark, although expression of the core clock components *TOC1*, *CCA1* and *LHY* was largely unaffected. We therefore propose that PIF1 and PIF3 are negative regulators that function to integrate light and circadian control in the regulation of chloroplast development.

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P5.2

11:00 Monday 29th June 2009

Phytochrome B and PIF4 regulate stomatal development in response to light quantity

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Stomata are pores on the surfaces of leaves that regulate gas exchange between the plant interior and the atmosphere. Plants adapt to changing environmental conditions in the short term by adjusting the aperture of the stomatal pores while longer term changes are accomplished by altering the proportion of stomata that develop on the leaf surface. Although recent work has identified genes involved in the control of stomatal development, we know very little about how stomatal development is modulated by environmental signals, such as light. Here we show that mature leaves of *Arabidopsis* grown at higher photon irradiances show significant increases in stomatal index (S.I.) compared to those grown at lower photon irradiances. Light quantity mediated changes in S.I. occur in red light suggesting that phytochrome photoreceptors, are involved. Using a genetic approach we demonstrate that this response is dominated by phytochrome B and also identifies a role for the transcription factor, *PHYTOCHROME-INTERACTING FACTOR 4* (*PIF4*). In sum, we identify a photoreceptor and downstream signalling protein involved in light-mediated control of stomatal development.

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P5.3

11:20 Monday 29th June 2009

Identification of regulators and targets of circadian calcium signalling

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California, San Diego), Soeng L. Baek (University of Cambridge), Zoe Rutterford (University of Cambridge), Matthew A. Stancombe (University of Cambridge), Michael J. Gardner (University of Cambridge), Carlos T. Hotta (University of Sao Paulo), Antony N. Dodd (University of York), Alex A. Webb (University of Cambridge)

The plant circadian clock is an endogenous timekeeper that regulates a wide variety of physiological processes. Circadian oscillations in the concentration of cytosolic free calcium ($[Ca^{2+}]_{cyt}$) occur in plants and we are elucidating the mechanisms involved in their generation and identifying the targets of circadian Ca^{2+} signalling. Circadian oscillations of $[Ca^{2+}]_{cyt}$ behave differently to other circadian outputs in *timing of chlorophyll A binding protein 1-1* (*toc1-1*) mutants (Xu et al., 2007) and when inhibitors of cyclic ADP ribose action are applied (cADPR, Dodd et al., 2007). We are using these conditions to identify transcripts that are co-regulated with $[Ca^{2+}]_{cyt}$ by performing time-series whole genome transcriptional analysis in *toc1-1* and in the wild type in the presence or absence of the cADPR inhibitor, nicotinamide. Circadian regulation of transcript abundance is severely disrupted in both the *toc1-1* mutant and in plants treated with nicotinamide. We are also using a reverse genetic approach to reconstruct the genetic network regulating circadian oscillations of $[Ca^{2+}]_{cyt}$ and have shown that the TIME FOR COFFEE protein is required for circadian rhythms of $[Ca^{2+}]_{cyt}$ whereas the pseudo-response regulator proteins PRR5, PRR7 and PRR9 regulate the period of these rhythms.

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P5.4

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Shaking the tree – Sequenced based identification and functional characterization of ABCB transporters in monocots

Anne S. Knoeller (Purdue University), Elizabeth Richards (Purdue University), Angus S. Murphy (Purdue University)

Plant ABCB transporters function in multiple physiological processes such as auxin transport, malate scavenging, and accumulation of alkaloids. Manipulation of ABCB transporters is potentially useful for enhancing crop plant productivity, as demonstrated for the maize auxin transport mutant *br2*. However, choosing specific ABCBs for manipulation is difficult, as the ABCB transporter family has functionally diverged among species. Some gene duplication events preceded monocot/dicot divergence, as observed for the auxin transporters AtABCB1 and AtABCB19. However, another cluster containing both a berberine importer and the auxin transporter AtABCB4 is distal to other auxin transporter clusters. We chose OsABCB10 from this clade for further analysis. OsABCB10 knock-out lines are hypersensitive to drought, and OsABCB10 expression increases eight-fold in wild-type plants under drought stress. *In situ* hybridization showed that OsABCB10 is expressed in monocot-specific silica cells in leaves and the epidermal layers of the husk. Reduced seed yield observed in the mutant suggests that OsABCB10 function protects the developing seed from abiotic stress. Transport studies of recombinant OsABCB10 in *Saccharomyces pombe* showed that OsABCB10 does not transport auxin. Growth assays demonstrated that these recombinants are more sensitive to ferulic acid. As ferulic acid has been proposed to reduce cell wall extensibility by binding to cell wall arabinoxylans, this suggests that OsABCB10 could function in drought-induced growth arrest. Although highly similar to AtABCB4, substrate affinity and function differ. We therefore conclude that sequence comparison alone is insufficient for predicting substrate

affinities. A detailed analysis of the substrate binding site, including three-dimensional modeling, is required.

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P5.5

13:40 Monday 29th June 2009

Role of the protein kinase CIPK8 in guard cell signalling

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Stimulus induced increases in the cytosolic concentration of free calcium ions (calcium signals) are involved in the regulation of a large number of responses in plants. Decoding these signals is carried out by a number of different calcium binding proteins such as calcium sensors, which either respond to the signal or relay it onwards. Calcineurin B-like proteins (CBLs) represent a unique family of plant calcium sensors that relay signals by interacting with a family of serine–threonine protein kinases (CIPKs). In *Arabidopsis*, 10 CBL calcium sensors interact with 25 CIPKs to decode temporal and spatial changes in Ca^{2+} , possibly by preferential complex formation of specific CBLs with discrete subsets of CIPKs. Interaction analyses revealed that some CBLs potentially interact with a wide range of CIPKs, while others (the majority) interact with a reduced subset of CIPKs and a minority only appears to have one or two CIPK partners. Current research is focused on identifying which CBLs respond to which extracellular signals and to working out the details of the CBL/CIPK interaction network. Recent work has implicated CIPK8 in plant nitrate signalling. Our current research has used infrared thermal imaging to help identify guard cell responses that CIPK8 may be involved in and these results will be discussed.

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P5.6

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A novel class of regulatory protein with one active site for kinase and dephosphorylase activities

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The pyruvate, orthophosphate dikinase (PPDK) regulatory protein (RP) has unusual enzymatic properties and may define a new class of protein kinase dephosphorylase.

It is bifunctional, catalysing the dark-dependent inactivation of PPDK via phosphorylation, and its light-dependent activation via dephosphorylation. Both activities proceed via atypical mechanisms, the phosphorylation activity requiring ADP as the phosphoryl-group donor instead of ATP, and the dephosphorylation mechanism proceeding via an orthophosphate-dependent and pyrophosphate-forming reaction, rather than typical anhydride bond hydrolysis. However, despite having such unusual properties, very little is known about the structure of the protein: it possesses none of the domains that characterise any other kinase or dephosphorylase proteins.

Here we investigate the location of the unidentified kinase and dephosphorylase domains in RP from *Arabidopsis*, and also how RP interacts with PDK. Site-directed mutagenesis of AtRP1 and generation of chimaeric AtRP1–AtRP2 proteins have provided insight into the location of individual residues and regions both associated with each activity, and with PDK interaction.

Results from PDK-RP yeast-two-hybrid studies alongside RP kinase and dephosphorylase assays indicate that a central region of AtRP is of critical importance to both interaction with PDK, and kinase and dephosphorylase activities. Our data do not support the proposal that separate sites are involved in RP kinase and dephosphorylase activities, or the currently held view that a putative P-loop is likely to be critical for kinase activity only.

This work provides an important insight into the mechanism of action of a regulatory protein which controls the activity of a key C₄ photosynthetic enzyme.

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P5.7

14:20 Monday 29th June 2009

Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein

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Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A new paradigm for such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking. The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in stem cell maintenance. A gain-of-function trafficking assay in *Arabidopsis* was developed to demonstrate that the KN1 homeodomain is necessary and sufficient for trafficking in vivo. This system provides a simple and tractable model to understand how proteins traffic and to isolate mutants defective in trafficking. As a proof of concept for our strategy, a mutant with attenuated KN1 trafficking has been identified as a chaperonin gene. This chaperonin appears essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between chaperonin and KN1. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. A functional characterization of chaperonins, the first ever factor so far known to be critical for KN1 PD trafficking will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein folding machinery, which is not well understood in any system at a molecular level.

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P5.8

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Comparison of cell wall mechanical properties of some *Arabidopsis thaliana* mutants

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Introduction

The relationship between the composition and architecture of the primary plant cell wall, and its mechanical properties is not fully understood. The elasticity, plasticity and strength of the primary plant cell wall are presumed to arise primarily from a network of the cellulose microfibrils, cross-linked with hemi-cellulose, and interpenetrated by pectin. It is known that changes in the composition and architecture of the cell wall lead to detectable differences in the mechanical properties. In this work, a comparison was made to the mechanical properties of cell walls of *Arabidopsis thaliana* wild type and a selection of cell wall mutants grown in suspension cultures. Relationships between suspension culture properties, shoot and root growth characteristics and wall composition were sought.

Cell wall mutants

Three cell wall mutations (IDA, Mur1 and Snakeskin) were investigated and compared to the Columbia wild type. Shoot and root growth were characterised to evaluate the effects of the mutations on plant growth.

To produce viable single cells for use in micro-compression, callus cells were grown from root sections and transferred to liquid culture media and kept at a constant temperature and rotated. The viability of the cells was proven using neutral red staining.

Compression testing by microcompression

Force-deformation data were obtained by compression testing using micro-manipulation. Mathematical modelling was used to estimate the low strain elastic modulus of the cell walls, and the (pseudo) stress at wall failure.

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P5.9

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How does the aphid *Myzus persicae* respond to feeding on droughted *Brassica nigra*: A transcriptomics analysis

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P5.10

15:50 Monday 29th June 2009

How does the aphid *Myzus persicae* respond to feeding on droughted *Brassica nigra*: A transcriptomics analysis

Laura H. Vickers (University of Birmingham), Jeffrey S. Bale (University of Birmingham), Jeremy Pritchard (University of Birmingham)

The responses of many plant–insect interactions to drought conditions are unknown. Regarding specialist insect feeders, such as aphids, there have been predictions that they will perform better due to the increased concentration of amino acids within the phloem caused by drought. Others predict that aphids will perform worse as they are faced with increased need for osmoregulation as phloem solutes increase.

To understand the aphid response to droughted plants at the genetic level, the gene expression of *Myzus persicae* exposed to two different drought regimes was analysed on the host plant *Brassica nigra* using microarrays. Changes in aphid gene expression are interpreted within the context of changes in the physiological status of the plant. These changes were assessed using water potential measurements, amino acid concentrations and fresh–dry weight ratios. This information will help to identify aphid's mechanisms of feeding under drought.

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P5.11

16:10 Monday 29th June 2009

Dynamic acclimation in *Arabidopsis thaliana*

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Dynamic acclimation of photosynthesis is the process by which some plants can alter their photosynthetic capacity (P_{max}) upon transfer to different light conditions. *Arabidopsis thaliana* L. cv. Wassilewskija (WS) is able to dynamically acclimate, whereas *A. thaliana* L. cv. Columbia 0 (Col 0) is not.

Microarray analysis identified a glucose 6-phosphate/phosphate translocator (GPT2) that is highly up-regulated during dynamic acclimation. A T-DNA insertion knockout of GPT2 in WS is unable to increase P_{max} when transferred from low to high light. GPT2 is therefore essential for dynamic acclimation.

To investigate the physiological importance of dynamic acclimation, plants of Col 0, WS and the GPT2 knockout were grown over the winter in an unheated greenhouse under naturally variable light conditions. Their seed production was compared to that of plants grown in the lab at $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ light/20 °C/8 hour days.

When grown in the lab, Col 0 produces far fewer seeds than WS. However, there is no difference in seed production between WS and the GPT2 knockout.

When grown under naturally variable light conditions, WS had significantly higher seed production than when grown in the lab. Both Col 0 and the WS GPT2 knockout had substantially lower seed production than their respective lab controls.

This indicates that dynamic acclimation of photosynthesis plays an important role in maximising the reproductive fitness of plants growing under naturally variable conditions.

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P5.13

09:00 Tuesday 30th June 2009

A real-time biosensor for plant hormones

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If we are to understand the timing, direction and extent of responses to hormonal stimuli we need to capture quantitative information from living, responding tissues. Previously, quantitation of plant hormones has tended to be either post-event, time fractionated assays of plant homogenates (mass spectrometry, HPLC, GC, ELISA etc.) or assays have been qualitative using, principally, promoter–reporter constructs. Much detailed physiology has been determined from interpretations of these reporters, but they remain largely qualitative and post-event with little or no temporal resolution. Quantitative biosensors are widely used in other areas of biology and, although no one system will suit all applications, their continued development and application to plant physiology is overdue. A novel configuration and application of a surface plasmon resonance instrument (Biacore 2000) is described such that it becomes a biosensor platform capable of measuring in real-time continuous changes of concentration of aqueous analytes from living tissue. The examples presented are for the plant hormones auxin and ABA. The biosensor gives time-resolved dataflow with a dynamic range of approximately four orders of magnitude. We will show how samples from live plant tissues are collected to flow continuously over the Biacore chip.

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P5.14

09:30 Tuesday 30th June 2009

Seasonal growth of temperate terrestrial orchids as affected by the old tuber

Alfred A. Darkwa (University of Sussex), Peter Scott (University of Sussex)

Many terrestrial orchids are rare or even endangered. They have a unique relationship with specialized mycorrhizae, require optimal growth conditions, and several of their genera do not propagate easily, all these contribute to their rareness. There is therefore an urgent need to put in place complementary conservation measures which require a good understanding of the life cycle of the orchids to save them. This understanding is presently unavailable. A United Kingdom orchid species (*Anacamptis morio*) was used as the model for this study, which looks at the growth of temperate terrestrial orchids under normal conditions and the influence of old tuber on growth. Findings revealed that the plants have well defined growth patterns and can grow even in environmental conditions that are unfavourable for the growth of other plants. The old tuber of these orchids may only support the initial establishment of the canopy and the subsequent growth of below-ground tissue. The leaves rapidly reach a point of self-sufficiency and support their own growth. Although the old tuber does not influence flowering directly, it does so indirectly because the planted tuber must be of certain weight to produce plants that can flower. Initial tuber size and final canopy size did relate suggesting that larger tubers yield larger canopies and enhance flowering. This implies that any damage to the canopy (through grazing) from October to December will severely affect aerial growth consequently leading to smaller plants that are unlikely to flower and yield enough tubers.

Keywords: *Anacamptis morio*;; Terrestrial orchids;; Old tuber;; Growth

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P5.15**09:50 Tuesday 30th June 2009****Xylem vulnerability to cavitation for ten miombo canopy tree species with varying habitat preference**

Royd Vinya (University of Oxford, Environmental Change Institute), Yadvinder Malhi (University of Oxford, Environmental Change Institute), Nick Brown (Oxford University Plant Sciences)

We investigated xylem vulnerability to cavitation for 10 miombo forests canopy tree species with varying habitat preference. The traditional bench drying technique was employed in inducing cavitation in plant segments. Vulnerability curves relating water potential versus percent loss in conductivity were fitted with a sigmoid function. Tree species with narrow habitat preference (confined to wet miombo) were more vulnerable to cavitation than species with wide habitat preference (occurring both in wet and dry miombo). The xylem safety margins for species with narrow habitat preference ranged from -1.3 to -2.2 MPa and for the wide habitat preference it ranged from -3.1 to -3.6 MPa. We also investigated xylem safety margin plasticity among the wide habitat preference conspecific species growing on two sites with contrasting mean annual rainfall. Differences in vulnerability to cavitation between conspecific species were insignificant at both community and intraspecific level. The relationship between vulnerability to cavitation and xylem long distance water transport efficiency (leaf specific conductivity) among the ten miombo tree species was also investigated. At community level there was significant correlation between vulnerability to cavitation and leaf specific conductivity. Species with efficient xylem pathways were more vulnerable to cavitation than species with low leaf specific conductivity. The coupling of vulnerability to cavitation and leaf specific conductivity suggests that vulnerability to cavitation controls species composition in the miombo forests and might have crucial implications on adaptations to climate change.

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P5.16**10:40 Tuesday 30th June 2009****Understanding the causes of lodging of the Ethiopian cereal teff (*Eragrostis tef* (Zuccagni) Trotter)**

Sander H. Van Delden (Wageningen University), Gerard Brouwer (Wageningen University), Tjeerd-Jan Stomph (Wageningen University), Jan Vos (Wageningen University)

Teff is a short day C4 cereal crop species, originating from Ethiopia. Teff grains and flour do not contain gluten and seem to contain a slowly digestible starch structure. Therefore teff could be beneficial for coeliac disease, diabetes, and obesity patients. This caused an interest in growing the crop outside Ethiopia for specialty food markets.

Teff can be grown in the temperate climates of NW Europe, such as in The Netherlands. Lodging, the permanent displacement of crop plants from their vertical due to root or stem failure, is the major yield constraint of teff in both Ethiopia and the Netherlands.

The objective of this paper is to explore the 'weak points' of the plant using biomechanical models. The model parameters were obtained from a field trial with two teff cultivars on a sandy soil using novel in situ and laboratory measurements. Results indicated teff is most sensitive to root lodging and that given its current morphology,

lodging of free standing plants is inevitable in the tested environment. However, if breeders would succeed in enhancing the anchoring properties of the root system, the stems of the crop would probably bend or break so simultaneous improvements of root anchorage and stem strength are advocated. Until now, the current paradigm in lodging research states that rain predominately acts by reducing the soil shear strength and therefore reduces the root anchorage strength. Our data showed an additional significant direct effect of rain on the lodging susceptibility of the teff plants.

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P5.17**11:00 Tuesday 30th June 2009****Mycorrhizal colonization does not seem to influence caesium uptake by *Medicago truncatula* grown under potassium-deficient conditions**

Lea Wiesel (Scottish Crop Research Institute), Martin R. Broadley (University of Nottingham), Philip J. White (Scottish Crop Research Institute)

Radiocaesium (Cs) contamination of soils is a worldwide problem that has arisen from human activities, such as intentional and unintentional discharges from the nuclear industry. The contamination of soils by Cs is of serious concern because of the long half-lives of the radionuclides ($^{134}\text{Cs} = 2$ yr, $^{137}\text{Cs} = 30$ yr) and the emission of harmful β and γ radiation during decay. Radio-caesium is rapidly incorporated into biological systems and enters the food chain through vegetation. Plants acquire Cs from the soil solution. Because of the chemical similarity of Cs and potassium (K), K transport proteins contribute to Cs uptake by roots and the delivery of Cs to the shoot. More than 80% of vascular plants live in symbiosis with arbuscular mycorrhizal (AM) fungi. The fungi gain carbohydrates from plants and, in return, they help supply plants with water and mineral nutrients. Because of the role of AM fungi in plant nutrition, it has been suggested that they might also affect Cs uptake by plants. To investigate the influence of AM fungi on Cs uptake by plants, *Medicago truncatula* was grown in association with *Glomus* sp. under *in vitro* conditions. Under K-deficient conditions, neither Cs in the media nor mycorrhizal infections influenced fresh weight of *M. truncatula*. The colonization of roots with *Glomus* sp. did not change K or phosphorus (P) concentrations in plant tissues. Neither the Cs concentration in shoots nor the Cs concentration in roots appears to be affected by mycorrhizal infection.

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P5.18**11:20 Tuesday 30th June 2009****Action potential-induced changes in photosynthetic pattern of a plant cell**

Natalia A. Krupenina (Lomonosov Moscow State University), Alexander A. Bulychev (Lomonosov Moscow State University)

Close relatives of land plants, Characean algae, have long been used as a model system for studying chloroplast–cytoplasm relations, photosynthesis, electric excitability and self-organization. Illuminated *Chara corallina* cells produce spatial patterns of chlorophyll (Chl) fluorescence

and extracellular pH. In the resting cell the periodic pH profile is parallel to the profile of non-photochemical quenching (NPQ) and antiparallel to that of effective photosystem II quantum yield ($\Delta F/Fm'$). Photosynthesis is higher in the acid zones compared to alkaline zones. Our previous measurements demonstrated that electrically-induced action potential (AP) smoothes the pattern of extracellular pH and induces a stronger AP-induced quenching of Chl fluorescence in alkaline than in acidic zones. Based on these observations, we suggested that the pattern of photosynthetic parameters, unlike the pH profile, is not smoothed after AP but becomes more pronounced. In this work, we examined the effects of *Chara* cell excitation on images of $\Delta F/Fm'$ and NPQ with high resolution Imaging-PAM technique. The results suggest that AP generation enhances the banding patterns of NPQ and photosynthetic electron transport in the chloroplast layer. The observation that AP generation intensifies the separation of chloroplast layer into areas with high and low photosynthesis adds to the list of documented phenomena induced by propagating electric signals in plants. The supposed primary cause for both temporal smoothing of heterogeneous pH profile and enhancement of functional heterogeneity in the chloroplast layer is many fold increase in cytosolic Ca^{2+} concentration during AP.

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P5.19

11:40 Tuesday 30th June 2009

Induction of organogenesis and somatic embryogenesis in seedling explants of some novel genotypes of chickpea (*Cicer arietinum* L.) – A recalcitrant crop

Anuradha Yadav (University of Delhi)

Chickpea has been recognized as one of the important crops all over the world and is extensively cultivated as one of the most important winter crop throughout India, especially in northern states mainly because of its seeds which are used as a rich source of dietary protein. Chickpea contributes 15% to the world's pulse harvest of about 58 million tones. Despite significant gains in world pulse production during the past two decades, with an average annual growth rate of 1.9%, chickpea production growth has been slow. Unfortunately, its productivity has been severely hampered due to several constraints like its cultivation on marginal lands, impacts of biotic and abiotic stresses, low research and management efforts. Incidentally, improvement of the crop employing technique of genetic engineering too, poses difficulty due to non-availability of the efficient tissue culture protocols. Significance of development of protocol for each genotype as well as selection of best genotype has been well documented in the light of the urgent need for the crop improvement all over the globe, employing techniques of genetic engineering. Present communication reports the (i) multiple shoots induction through seed explants, (ii) somatic embryogenesis through leaflet explants as well as (iii) successful plantlets regeneration through seed explants of three genotype of chickpea BG1101, BGD72 and BG1003 for improving the crop through biotechnological manipulations. A significant variation in embryogenic response has been noticed during culture of leaf explants of six genotypes under the same regime of growth regulators such as N^6 -benzyladenine (BA), kinetin (Kn), 2,4-d (2,4-dichlorophenoxyacetic acid), indole-3-butyric acid (IBA) and alone or in combinations. Of the various growth regulators BA at 5 μ M proved optimum for eliciting morphogenic response in seed and 20 μ M 2,4-d proved optimum for eliciting 100% embryogenic response employing leaflet explants in all the genotypes. A significant variation has been noticed in different genotypes with respect to average number of shoots per culture, maximum being 28 ± 0.76 in BG1101. For induction of roots, MS (1/2) + IBA proved best where 72.5% shoots developed an average of

18.10 ± 1.37 roots within 20 d. The plantlets have been hardened and transferred to soil. Significant variation has been noticed with respect to average number of somatic embryo per culture, maximum being 90.62 ± 3.47 in BG256. Such emblings subsequently transferred to growth regulator free medium for further elongation of shoots and roots. Plants with well developed roots were transferred to pots containing garden soil. Thus a remarkable influence of growth regulators has been documented on the different explants on the *in vitro* regeneration of different genotypes of *Cicer*. Based on the aforesaid intensive and extensive investigations it has been concluded that BG256 proved best for differentiating somatic embryos and BG1101 may prove best for average shoot production and used for further improvement of crop using genetic manipulations.

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The role of AtVAMP714 in *Arabidopsis* development

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The *siamese* mutant of *Arabidopsis* was identified in an activation tagging screen, exhibits defective meristem function and development. Sequencing the insertion locus revealed that the activation tag was positioned non-coding region between *At5g22360* and *At5g22370* genes. *At5g22360* is upregulated in the activation tagged line. This gene encodes the VAMP714 protein predicted to be the vesicle associated, and is a member of a protein family. We hypothesize that VAMP714 protein is involved in the targeting and/or fusion of transport vesicles. We used transgenic *Arabidopsis* lines to analyse the gene promoter activity and VAMP714 protein localization. The gene promoter is active in vascular tissues and the promoter is upregulated by auxin. VAMP714:GFP fusion protein is localized in vesicles. The overexpression of VAMP714 protein disrupts auxin transport and auxin mediated gene expression and development. In the overexpression phenotype PIN1 and PIN2 proteins are altered in abundance and localization, suggesting that VAMP714 protein may be involved in the efflux of auxin, possibly through the control of recycling of PIN proteins. We are focusing our experiments on the co-localization of VAMP714 with PIN proteins and analysing of loss-of-function mutants to further investigate the molecular role of AtVAMP714.

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P5.21

14:20 Tuesday 30th June 2009

Production of an allelopathic, rhamnogalacturonan-related oligosaccharin in cress seed mucilage

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The disaccharide lepidimoid [2-O-(4-deoxy- α -l-threo-hex-4-enopyranosyluronate)-l-rhamnose] is reported to be an oligosaccharin, i.e.

to exhibit 'hormone-like' biological activity. It was reported in cress (*Lepidium sativum*) root exudates, exerting allelopathic effects on neighbouring *Amaranthus* seedlings. It stimulates excessive growth of *Amaranthus* hypocotyls and inhibits root growth (Hasegawa et al., 1992).

We are investigating the natural production of oligosaccharins, and whether novel oligosaccharins besides lepidimoide may occur in cress.

We found an active principle with biological effects similar to those of lepidimoide to be more abundant in cress seed-coat mucilage than in root exudates. The active principle peaked 24 h after seed soaking, and thereafter plateaued. Surface-sterilising seeds did not diminish production of the active principle, suggesting that the enzyme responsible was of plant origin.

Active principle from mucilage partitioned into the aqueous phase when shaken with ethyl acetate at pH 2, 6.5 and 12, showing it to be hydrophilic, unlike auxins and gibberellins. Gel-permeation chromatography on Bio-Gel P-10 and P-2 suggested that the active principle had M_r ~500–750, compatible with oligosaccharide(s). Analysis of active versus inactive Bio-Gel fractions by TLC and HPLC suggested a particular oligosaccharide which may be the active principle. We are also testing the ability of seeds to release radioactive lepidimoide from [^{14}C]rhamnogalacturonan-1.

Reference: Hasegawa, K. et al. (1993) *Plant Physiol.* 100: 1059–1061.

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Ecophysiological effects of Cr and Zn on *Iris pseudacorus* grown under semi-controlled conditions

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The tolerance of *I. pseudacorus* to Cr and Zn, and the specific physiological response induced by each of them was examined. Plants were grown hydroponically in ZnCl_2 or $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ at 0, 10, 50, 100, and 200 $\mu\text{g mL}^{-1}$ for five weeks. Metal concentration in all plant tissues increased with increasing supply, and the highest accumulations were found in roots, followed by rhizomes. Leaves retained a remarkable amount of Zn. Both Cr and Zn decreased growth and increased root to shoot dry matter ratio (R/S). Dry weight was markedly reduced on leaves under Zn treatment, and on roots under Cr treatment. Element content and distribution were severely affected by both metals, especially Cr. The most remarkable alterations were found in roots, where the mineral content decreased in response to Zn (Al, Ca, Mg, Mn and S), and Cr (Al, Ca, K, Mg, Mn, and S). Both metals increased P concentration, and Zn incremented K. Cluster analysis on element content clearly separated controls from treatments, and Zn from Cr. No significant effect was noted on leaf chlorophyll fluorescence kinetics (F_v/F_m and Φ_{PSII}), or on photosynthetic pigments content, and carbon isotope composition ($\delta^{13}\text{C}$) was only slightly incremented in leaves and roots in response to both metals. In conclusion, the reduction of growth observed in *I. pseudacorus* in response to Cr and Zn should be attributed to root damage and biomass allocation, rather than to photosynthetic inhibition. This species is a good candidate for Cr rhizofiltration and Zn phytoextraction.

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Prospect of *Canna* (*Canna edulis* Ker.) rhizome as a feedstock for bioethanol production

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The utilization of bioethanol as an additive to gasoline increases in Indonesia during the last few years due to an increase in petroleum price and public awareness of environmental problems. Bioethanol can be produced from cellulose, starch or sugar containing agricultural products, such as rice straw, cassava, or sugar cane. Controversial opinions emerged when food resources are used as feedstock on bioethanol production. Thus finding alternative feedstocks become the major concern in our research. There are many underutilized tubers and rhizome that can be processed for bioethanol, such as rhizomes of *Canna edulis* Ker. Bioethanol production from canna rhizome has been evaluated both at laboratory scale and home industry scale using simple liquefaction, saccharification and fermentation procedure. It was found that based on laboratory scale experiments, one kilogram of canna rhizome can be converted to 110–120 mL of 75% bio-ethanol. Higher concentration of bioethanol can be obtained through redistillation process followed by molecular sieve purification. Cultivation technique to increase rhizome yield and several benefits in using canna rhizomes as feedstock of bioethanol production will be discussed.

Keywords: *Canna edulis* Ker. rhizome; Bioethanol

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P5.24

Poster Session – Monday 29th June 2009

Effects of light intensity and photoperiod on the growth rate, chlorophyll a and β -carotene of freshwater green micro alga *Chlorella vulgaris*

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The freshwater green micro-alga, *Chlorella vulgaris*, is known as one of the most useful photosynthetic microorganisms due to its ability to produce highly valuable biochemical or health food. The biochemical content is highly influenced by light quality and quantity, both of which are required to optimize algal growth in mass culture systems. This study provides information on the effect of light intensity (37.5, 62.5, and 100 $\text{mE/m}^{-2} \text{s}^{-1}$) and photoperiod (16/8, 12/12, 8/16 Light/Dark) on the growth efficiency and pigment content of *C. vulgaris* at a constant temperature of 25 ± 0.5 . The maximum growth rate and optimum light intensity were of 1.22 d^{-1} and 100 $\text{mE/m}^{-2} \text{s}^{-1}$ for this micro-alga. The optimum photoperiod was found to lie in (L/D) 16/8 for maximum biomass production. The chlorophyll a content was lower at high light intensity, while β -carotene showed inverse trend. The changes in pigments are considered to be an adaptation mechanism against high light stress. Low-light synthesized larger photosynthetic units, presumably to aid light harvesting, while high-light synthesized smaller photosynthetic units, presumably to prevent photo damage.

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P5.25**Poster Session – Monday 29th June 2009****Photosynthetic efficiency and carbon sequestration in evergreen and deciduous oaks in relation to different environments**

Demelza J. Carne (University of Plymouth), Stuart Lane (University of Plymouth), Miguel Franco (University of Plymouth), Maria Donkin (University of Plymouth)

Trees store around 10% of the global land-based carbon sink. At smaller scales the amount of carbon sequestered depends on species and environment. To achieve efficient United Nations endorsed carbon sequestration projects in the UK means finding suitable species for a range of urban and rural conditions.

Field-based experiments, running between 2009 and 2011, are measuring growth parameters and photosynthetic performance indicators to ascertain carbon sequestration potential of two species of oak, with contrasting leaf habits, in relation to environmental variables.

The deciduous species *Quercus robur* is an important British hardwood timber tree and the Mediterranean evergreen, *Quercus ilex*, is also an important European hardwood but considered invasive in the UK. Although both species are considered drought sensitive, *Q. ilex* is also constrained by minimum temperatures whereas *Q. robur* requires cold periods for successful germination and growth.

Preliminary data from one urban and one rural site showed that *Q. ilex* seedlings planted at the urban site had significantly higher photochemical performance (Fv/Fm) than those at the rural site ($p < 0.05$), although net assimilation rates (A_N) were significantly lower for seedlings grown in 10 L pots for the same urban site ($p < 0.005$).

Prudent site and species choice are important for afforestation projects that have to work alongside agriculture and biodiversity targets. This research will provide data for carbon modeling, aid evaluation of UK carbon pools and thus provide information for effective use of the license given by the United Nations for afforestation and reforestation practices.

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P5.26**Poster Session – Monday 29th June 2009****A comparison among *Impatiens* species with different invasive capacities**

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Phenotypic plasticity is believed to play an important role within exotic species, as it may increase their invasiveness.

To test this hypothesis we germinated seeds of five plant species of the genus *Impatiens* that are both invasive and native to Europe. *Impatiens noli-tangere* is native to continental Europe and Britain. *Impatiens glandulifera* is currently one of the dominant invasive plant species in Europe. In addition, we selected other exotic *Impatiens* that are naturalized in Europe and vary in their invasive capacity (*I. balfourii*, *I. parviflora* and *I. capensis*). *I. glandulifera* showed the highest germination rate. No germination occurred in *I. noli-tangere* seeds. Seedlings of the four germinated species were potted and measurements of growth were taken weekly. The photosynthetic capacity of 10 individual plants per species was assessed using an infra-red gas analyzer. Light response curves for each individual of each species were obtained weekly for 5 weeks, which followed the leaf's life cycle. CO₂ response curves also were obtained for 10

individuals per species. Plants had been harvested and growth parameters had been determined. These results will be combined with results of a further experiment where individuals of different *Impatiens* species will be grown in different levels of water stress.

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P5.27**Poster Session – Monday 29th June 2009****Engineering mine water treatment wetlands for the conservation of biodiversity**

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Traditionally constructed wetlands designed to passively treat mine water are dominated by *Phragmites australis* (Norfolk or Common Reed) and *Typha latifolia* (Bull Rush), often constituting a monoculture. Greater plant diversity within other habitats is known to increase diversity in other organisms such as microorganisms, invertebrates, birds and mammals. However, the potential for maximising this potential ancillary benefit of minewater treatment wetlands has not previously been assessed.

This study aimed to determine whether alternative plant species could be used within metal contaminated waters in order to increase plant diversity in treatment wetlands. Growth of, and metal accumulation in plant tissues by, *Lysmachia vulgaris* (Yellow Loosestrife), *Sparganium erectum* (Branched Bur-Reed), *Hippurus vulgaris* (Mare's Tail) and *Phragmites australis* (Control) was investigated.

Plants were grown in three synthetic mine waters based chemically upon known minewater discharges in the United Kingdom with a nutrient solution control. The plants were grown using hydroponics and in a commercial substrate used in constructed minewater treatment wetlands. Root length, shoot length, number of leaves and leaf length were recorded over time.

Iron plaques were removed using a DCB extraction and the metal content examined using Atomic Absorption Spectrometry (AAS). The roots, shoots and leaves were acid digested and metal accumulation determined through AAS (GFAAS and FAAS).

Results will be discussed in relation to plant metal tolerance, accumulation and growth. It is intended that the results presented could offer an alternative to the traditional use of monocultures, increase biodiversity and maintain wetland treatment performance.

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P5.28**Poster Session – Monday 29th June 2009****Effect of nitrate reductase deficiency on the accumulation of phenylpropanoids in *Arabidopsis thaliana* leaves**

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Nitrate reductase (NR) catalyzes the conversion of nitrate to nitrite, playing a pivotal role in plant nitrogen assimilation. Nitrite

derived from NR activity is also a substrate for the synthesis of nitric oxide (NO), an important signaling molecule in plants. Indeed, the *nia1 nia2* mutant of *Arabidopsis thaliana*, that has a very low NR activity, presents reduced levels of amino acids and NO, what may affect the production of nitrogen-derived secondary metabolites. In the present work, we analyzed the accumulation of phenylpropanoids in leaves of the *nia1 nia2* mutant and compared to that of the wild-type. Methanolic leaf extracts were analyzed by reverse phase HPLC and the secondary metabolites were further identified by mass spectrometry. In wild-type leaves, only one major compound was detected, which corresponded to sinapoylmalate. *nia1 nia2* leaves presented much lower levels of this metabolite, but accumulated sinapoylglucose, the immediate precursor of sinapoylmalate. Recovery of amino acid content of *nia1 nia2* by irrigating the plants with glutamine did not change the metabolic profile of this mutant. On the other hand, treatment with nitrate, that enhanced NO production by *nia1 nia2* leaves, increased sinapoylmalate and decreased sinapoylglucose levels. These results suggest that the low levels of sinapoylmalate in *nia1 nia2* leaves are not resultant from the deficient nitrogen incorporation into amino acids and may be related to its reduced NO content. Moreover, these results indicate an unrevealed signaling role of NO in activating the sinapoylmalate synthesis, one of the last steps of phenylpropanoid pathway. *Supported by FAPESP.*

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P5.29

Poster Session – Monday 29th June 2009

A new method for identification the oligochitosan binding protein on tobacco plasma membrane

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Oligochitosan is a potent plant immunity elicitor, however, the mechanism on how oligochitosan induces the resistance of plants to pathogens is still unclear. To understand the molecular mechanism regarding how plant cells perceive the oligochitosan signal, identifying the receptors for the elicitors is very important. In this work, a convenient method for identification of the oligochitosan binding protein on tobacco plasma membrane was found. Oligochitosan labeled with the green fluorescent probe 2-aminoacridone (2-AMAC) was prepared and 2-AMAC was used as negative control. Tobacco plasma membrane was purified by using aqueous two-phase partitioning and separated by polyacrylamide gel electrophoresis. After electrophoresis, the gel was washed by Triton-100 several times to renatured the protein function. Then the gel was incubated with 2-AMAC-oligochitosan at 25 °C for 30 min and the unincorporated 2-AMAC-oligochitosan was removed by washing in the PBS buffer for at least 6 h with five changes. Binding of oligochitosan with tobacco plasma membrane was observed under ultraviolet detector. The bands which presented green fluorescence were recovered from the gel with ordinary electrophoresis apparatus and sequenced by MALDI-TOF MS. This method is quick and non-radioactive for carbohydrate-protein interaction and identification compared to general isotope labeling method.

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P5.30

Poster Session – Monday 29th June 2009

The relationship between nitric oxide production and plant hormone gibberelic acid during germination and growth of tomato exposed to osmotic stress

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The involvement of nitric oxide (NO) in relation to the plant hormone gibberelic acid (GA) in the defence reaction of tomato under osmotic stress was studied on a specific model of two genotypes of *Lycopersicon esculentum* Mill. A single gene, recessive, male sterile and photoperiod-sensitive tomato mutant *7B-1* is resistant to abiotic stresses including a high osmoticum, salinity and low temperatures. Under white light, we determined the seed germination, fresh weight, stem and root length of 10 days old tomato seedlings of tomato under physiological and osmotic stress conditions, in combination with exposure to gibberelic acid (GA), its inhibitor paclobutrazol and compounds modulating the production of reactive oxygen/nitrogen species: NO donor S-nitrosoglutathione, NO scavenger PTIO, NO synthase inhibitors L-NAME and aminoguanidine, NADPH oxidase inhibitor imidazol, ascorbic acid and rutin. Seed germination was stimulated by NO donor, while NO scavenger showed strong inhibitory influence. NOS inhibitor L-NAME significantly suppressed the root elongation. Osmotic stress decreased the germination and growth of both genotypes. The seed germination under osmotic stress was stimulated by GA and its effect could be inhibited by NO scavenger in both genotypes, and by paclobutrazol in WT but not in mutant *7B-1* seeds. Our results demonstrated NO as an essential regulatory molecule in the process of tomato seed germination and root development regulated by plant hormone gibberelic acid.

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P5.31

Poster Session – Monday 29th June 2009

Characterisation of saline tolerance in Swiss chard (*Beta vulgaris* *cicla* L.): A potential novel crop for production on saline soils

Catherine Keeling (Writtle College, Chelmsford Essex), Clive R. Ireland (Writtle College, Chelmsford Essex)

In a study of potential new crops for production on marginal saline soils, Swiss chard (*Beta vulgaris* L. subsp. *cicla*) has been identified as tolerant to moderately saline conditions, however, the physiological basis for this tolerance remains poorly understood. Initially the growth responses of Swiss chard to NaCl in the field were confirmed in a trial conducted at a coastal site (Abbott's Hall Farm, Essex). In a soil with an E_c of around 6dSm⁻¹, transplanted Swiss chard established and grew with no significant difference in dry weight accumulation to the control plots (<1dSm⁻¹). Conventional crops (direct sown) failed to establish. To inspect the hypothesis that

saline tolerance is conferred in Swiss chard through an effective regulation of leaf Na^+ uptake and/or compartmentalisation, a hydroponic study was conducted in an environmentally controlled glasshouse at Writtle College. Seedlings of Swiss chard and fodder beet (*B. vulgaris* L. subsp. *rapacea*), used as a comparative control, were transferred to nutrient solution containing 0, 60, 240 and 420 mM NaCl (with a controlled Na^+ to Ca^{2+} molar ratio of 15:1). Ion analysis (Na^+ , K^+ and Ca^{2+}), fresh and dry weight, leaf area, and chlorophyll content were measured in specific leaves in successive harvests, from seedlings to mature plants. Swiss chard was shown to accumulate relatively high levels of leaf Na^+ at moderate salinity levels, but displayed high physiological tolerance to this accumulation. The extent to which the results contribute to the understanding of the key physiological characteristics of saline tolerant crop types is discussed.

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P5.32

Poster Session – Monday 29th June 2009

Production of vacuolar cytokinin dehydrogenases from *Arabidopsis thaliana* in *Pichia pastoris*

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Vacuolar proteins in plants possess complex signals of low amino acid sequence homology and what precludes exact understanding of their targeting. When producing such proteins in a recombinant yeast system, problems may arise from misinterpretation of the signal sequence by the heterologous host. We study the family of seven cytokinin dehydrogenase enzymes (CKX, EC 1.5.99.12) that control the metabolism of plant hormones cytokinins in *Arabidopsis thaliana*. Two genes coding for vacuolar cytokinin dehydrogenase enzymes AtCKX1 and AtCKX3 were chosen for protein overproduction in *Pichia pastoris*. In order to facilitate purification, recombinant proteins were fused with His-tag domain. The fusion on the C-terminal end of the proteins, however, proved to be useless in affinity purification. Therefore a novel vector was prepared from pGAPZ α A by inserting a coding sequence for 10 \times His from pET16b between the α -factor coding sequence and the multiple cloning site. With both studied proteins, the sequence coding

for N-terminal fragment predicted by SignalP 3.0 as a leader peptide was removed before cloning into pGAPZ α A to obtain secretion from yeast using the N-terminal α -factor signal sequence. Initial experiments, however, did not lead to secretion of active proteins. More accurate amino acid sequence analysis of AtCKX1 and AtCKX3 revealed the presence of a region resembling N-terminal sequence-specific vacuolar sorting signal (ssVSS) that typically contains degenerate signal [N/L]-[P/I/L]-[I/P]-[R/N/S], also called NPIR consensus sequence, and targets proteins to lytic vacuoles. Only after deleting this motif, active plant vacuolar cytokinin dehydrogenases were obtained in *Pichia pastoris*.

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P5.33

Poster Session – Monday 29th June 2009

Comparing components of CO_2 assimilation in a variety of willow species

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We need to know the extent to which photosynthetic capacity varies between willow (*Salix*) genotypes in order to assess the potential for increasing carbon capture through selection of trait associated (photosynthetic) QTLs. If sufficient natural variation in photosynthetic capacity is apparent, then subsequent attempts to identify the underlying processes and genes may be justified. This preliminary work demonstrates the existence of significant variation in photosynthetic capacity (expressed on a leaf area or leaf dry matter basis) attributable to metabolic and/or anatomical characteristics within willow leaves, as distinct from stomatal limitations. We are currently attempting to identify metabolic processes which contribute to these differences in photosynthetic capacity. The inter-species variation in above ground biomass (leaves + wood) was more closely correlated to differences in total leaf area per plant, than in assimilatory capacity per unit leaf area. Nevertheless, the observed variation in assimilatory capacity per unit leaf area (of between 2 and 3-fold) implies that considerable gains in biomass production could be achieved by increasing the photosynthetic capacity per unit area in species which already produce many leaves.

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