



## P.10—GENERAL PLANT METABOLISM

Organised by N. Smirnov for the Plant Metabolism Group

### P10.1—Comparisons between UV-B radiation induced stress and developmental senescence in *Arabidopsis thaliana*

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In recent years, the importance of UV-B radiation and its effects on plant life have emerged. The emission of halogenated compounds, including CFCs is leading to the gradual depletion of the stratospheric ozone layer that protects the Earth's surface from UV-B radiation (280–320 nm). Plant exposure to UV-B can lead to DNA damage, effects on photosynthesis and altered gene expression, which lead to physiological changes that can seriously affect yield.

Senescence is often defined as being a reversible process as opposed to programmed cell death. In *Arabidopsis thaliana*, leaves treated with UV-B have been shown to exhibit an early response to stress, followed by a partial recovery, then premature death. Three days after treatment, many genes associated, not only with stress, but also with senescence are expressed. This is accompanied by a severe drop in chlorophyll and in the efficiency of photosystem II (measured as  $F_v/F_m$ ). This early response is followed by a phase of recovery, 7 days after the initial response, with expression of stress/senescence-enhanced genes declining and  $F_v/F_m$  levels rising before premature leaf death. The response seen in UV-treated leaves differs from developmental senescence by means of chlorosis,  $F_v/F_m$  and gene expression. However, similarities between the two responses can also be observed via gene expression and a recovery process. Results have also indicated that responses to UV-B differ between different leaves of *Arabidopsis*. It is not known whether the early UV response is a distinct stress/senescence response and it is likely that it is regulated by a combination of pathways.

### P10.2—The role of the plastidial glucose-6-phosphate transporter in oilseed embryo development

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Fatty acid biosynthesis occurs in the plastids of developing embryos of oilseed rape (*Brassica napus*) requiring a source of carbon (from glucose-6-phosphate, pyruvate, or phosphoenolpyruvate), ATP and reducing power.

The glucose-6-phosphate transporter (GPT) is thought to be important in controlling the supply of carbon precursors and reducing power via the oxidative pentose phosphate pathway. It has been proposed that the GPT is regulated by the concentration of unbound long chain fatty acyl-CoAs exported from the plastid (Johnson et al., 2000). Expression of the GPT gene has been characterized in a number of tissues of both oilseed rape and *Arabidopsis*. By taking a transgenic approach to decrease the expression of the Glc6P transporter, we aim to determine whether the availability of Glc6P is an important factor in determining the yield of fatty acids produced by the plastid, and therefore, the accumulation of oil by embryo. Preliminary analysis of antisense plants shows a large reduction in numbers of mature seeds produced by a plant compared to wildtype. Seeds that reach maturity are small and wrinkled. Germination rates are lower than wildtype, and seedling cotyledons are reduced in size or absent.

The effect of increasing expression of this transporter is also being investigated using a full-length cDNA clone from pea. Analysis of T1 generation shows increased seed mass and size.

Johnson P.E., Fox S.R., Hills M.J., Rawsthorne S. (2000). Inhibition by long chain acyl-CoAs of glucose 6-phosphate metabolism in plastids isolated from developing embryos of oilseed rape (*Brassica napus* L.). *Biochem. J.* 384, 145–150.

### P10.3—Carbon metabolism in developing soybean embryos

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Soybean (*Glycine max*) is rich in seed protein (40%) and oil (20%). It is an important leguminous seed crop that is ranked first in world oil production (48%) among the major oilseed crops [1]. Breeding efforts to increase soybean oil from a 20% level have been unsuccessful, and therefore, an understanding of what controls the

amount of storage oil accumulated in the seed is necessary in order to undertake a biotechnology approach. In plants, the *de novo* synthesis of fatty acids occurs primarily in the plastid [2]. Earlier studies by Eastmond and Rawsthorne [3] using plastids isolated from oilseed rape embryos have revealed major developmental changes in the pathways of carbon flux into fatty acids, oil and other major storage products. Using similar techniques, it has been possible to develop a method to isolate metabolically active plastids from developing embryos of soybean. The ability of soybean plastids to metabolise exogenous substrates into fatty acids and starch has been studied. Analysis of storage product accumulation in developing and mature embryos of soybean has also been carried out.

1. Singh J.J., Hymowitz T. (1999). *Genome* 42, 605–616.
2. Harwood P.J. (1988). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39, 101–138.
3. Eastmond P.J., Rawsthorne S. (2000). *Plant Physiol.* 122, 767–774.

#### **P10.4—Using mutants to investigate starch synthesis in cereal endosperms**

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In cereal endosperm, there are several different mechanisms of starch granule initiation, which result in starch granules of different shapes, sizes and number per plastid. In maize endosperm, for example, there is one granule per plastid and the granules are relatively uniform in size and shape. In oat endosperm, many plastids contain multiple, irregularly shaped granules. In the starch of barley endosperm, small spherical B- and large lenticular A-type granules are present. To investigate the factors controlling size, shape and granule number in cereal endosperm, we have studied several mutations in oat and barley that affect the initiation and synthesis of starch granules. For example, two of the barley mutants studied have more than the normal number of granules per plastid. In one of these, the mutation eliminates the enzyme isoamylase, suggesting that this enzyme may be important in starch granule initiation. However, in the second barley mutant with increased granule number, *Franubet*, isoamylase is not lacking, suggesting that other factors are also involved in controlling granule morphology and number. The nature of the mutations in *Franubet* and in mutants of oat with altered starch synthesis in the endosperm will be discussed.

#### **P10.5—The role of malto-oligosaccharides in starch metabolism in *Arabidopsis***

T. Niittylä, S. Zeeman, A.M. Smith, John Innes Centre, Norwich, UK

Malto-oligosaccharide metabolism has been implicated in recent models of starch synthesis, and is an essential part of starch degradation. Very little is known about the processes of malto-oligosaccharide (MOS) synthesis and degradation in any tissue. The aim of this project is to describe the synthesis and role of short  $\alpha(1-4)$ -glucans (malto-oligosaccharides) in starch metabolism of *Arabidopsis thaliana* leaves. MOS are present in very low amounts in *Arabidopsis* leaves, giving special importance to efficient isolation and detection methods. Novel methods for the analysis of MOS will be discussed and results will be presented on the discovery of a new mutant defective in maltose metabolism and on MOS metabolism during starch synthesis.

#### **P10.6—Genomics approach to starch metabolism in *Arabidopsis thaliana***

D. Thorneycroft, S.M. Smith, University of Edinburgh; S.C. Zeeman, A.M. Smith, John Innes Centre, Norwich, UK

The complete sequence of the *Arabidopsis thaliana* genome provides a resource base from which to define all the genes required for starch synthesis and breakdown, so that their functions can be determined. There are approximately 30 genes predicted to encode enzymes committed to plastidial starch metabolism, and another 14 genes predicted to encode extra-plastidial enzymes capable of metabolising starch. Knock-out mutations for approximately half of these genes have been identified and we have isolated several such KO mutants for analysis. Furthermore, we have isolated numerous new mutants with altered starch content or composition in which novel members of the 'starch proteome' might be discovered. We have produced a microarray employing oligonucleotides specific for approximately 100 genes encompassing all those predicted to encode enzymes of starch and sucrose metabolism. The array is being used to investigate differences in gene expression in mutants and potentially to help identify mutated genes in those isolated through phenotypic screens. It is also being used to profile changes in gene expression in leaves through the diurnal cycle and in response to environmental cues.

#### **P10.7—Probing plant mitochondrial metabolism using nuclear magnetic resonance**

A.M.O. Smith, S.A. Hill, R.G. Ratcliffe, Plant Sciences, University of Oxford, UK; R. Douce, DBMS, CEA-Grenoble, France

The flux through the TCA cycle is an integral part of plant cell metabolism, since it is essential both for the maintenance and utilisation of the mitochondrial proton electrochemical gradient and for the supply of key metabolic intermediates that are required for biosynthesis. Much can be inferred about the operation of the TCA

cycle from respiration rate measurements on isolated mitochondria, but it would be useful if the carbon fluxes that occur during the respiratory events detected by the oxygen electrode could be observed directly. In fact, nuclear magnetic resonance (NMR) spectroscopy can be used for this purpose, and this approach has now been used to investigate TCA cycle turnover in mitochondria isolated from potato (*Solanum tuberosum* cv. Desiree) tubers. Using the oxygen electrode, it is possible to deduce that the complete TCA cycle operates in state 3 when pyruvate is supplied to mitochondria that have been equilibrated with citrate above a threshold concentration. The carbon fluxes that support pyruvate-dependent oxygen consumption have been observed directly by using  $^{13}\text{C}$  NMR to follow the metabolism of either (4- $^{13}\text{C}$ )oxaloacetate or (3- $^{13}\text{C}$ )pyruvate. These experiments utilise an air-lift system to maintain the oxygenation of the mitochondria, and an ADP regeneration scheme, based on the action of hexokinase, to maintain state 3 conditions in the NMR tube. The results provide good evidence for the operation of the full TCA cycle in the isolated mitochondria under the chosen conditions, and they emphasise the importance of citrate in permitting the flux to occur.

#### **P10.8—Regulation of Rubisco during drought and heat stress in wheat**

P.D. Hobson, A.J. Keys, M.A.J. Parry, IACR-Rothamsted, Harpenden, UK; P.J. Lea, Biological Sciences, Lancaster University, UK

The regulation of Rubisco in terms of activation and the presence of inhibitors was assessed in the leaves of wheat plants, in response to drought and heat stress. A decline in the leaf relative water content by 25% caused little change in the initial and total Rubisco activity or activation state. There was, however, some evidence for a decreased amount of Rubisco protein in drought-stressed plants, shown by reduced maximal Rubisco activity and CABP binding. In the droughted plants, there was a significant reduction in the concentration of the 'daytime inhibitor' of Rubisco activity, indicating that it did not play an important role during drought stress in this species. High temperature stress caused a reduction in photosynthetic rate. As the temperature was increased from 25 to 40 °C, the photosynthetic rate was reduced by 100%, followed by a 90% reduction in stomatal conductance, although the internal  $\text{CO}_2$  content increased slightly. The initial and total Rubisco activity and activation state declined with increasing temperature, suggesting a possible perturbation of the heat-labile Rubisco activase activity. The amount of RuBP declined as temperature increased, suggesting a reduction in the flux through the Calvin cycle. The decrease in concentration of the 'daytime inhibitor' of Rubisco activity indicated that, as for drought, it did not play an important role during temperature stress in this species.

#### **P10.9—Carbon and nitrogen partitioning in the coastal plants *Plantago maritima* and *Armeria maritima*: substrate availability, secondary metabolism and plant survival in a changing environment**

M.P. Davey, R. Baxter, R. Edwards, University of Durham; T.W. Ashenden, CEH, Bangor

The two coastal plants *Plantago maritima* and *Armeria maritima* possess two different biochemical strategies to tolerate osmotic stress. *Plantago maritima* primarily accumulates the carbon-based polyhydric-alcohol sorbitol, whilst *A. maritima* accumulates the nitrogen-based quaternary-ammonium compound betaine. A source-balance model is being used to investigate how *P. maritima* and *A. maritima* will respond to altered atmospheric carbon dioxide and nitrogen availability. Both species were subjected to 360 and 600  $\mu\text{mol mol}^{-1} \text{CO}_2$  and two contrasting regimes of nitrogen availability (28 and 2.8  $\text{mg l}^{-1} \text{N-NH}_4\text{NO}_3$ ). Studies are underway to determine the changes in growth, carbohydrate and secondary metabolism in each plant species. The latter study includes the determination of changes in key compatible solutes and intermediates in phenylpropanoid metabolism in response to environmental perturbation of the two plant species.

#### **P10.10—The regulation of starch synthesis in non-photosynthetic tissues**

S. Dutton, S. Hill, University of Oxford; A. Greenland Syngenta, Jealotts Hill

Starch synthesis is regulated by the enzyme ADP-glucose pyrophosphorylase (AGPase), which converts glucose 1-phosphate and ATP into ADP-glucose, the precursor for starch synthesis. This enzyme is allosterically regulated by the ratio of 3-PGA/Pi, but the importance of the regulatory properties of the enzyme in non-photosynthetic tissue remains uncharacterised.

We are interested in the role of AGPase in non-photosynthetic tissues, and have investigated the role of 3-PGA and Pi using a variety of techniques, including feeding experiments and the characterisation of transgenic potato plants, containing a cytosolic *Arabidopsis* phosphoglycerate kinase gene in order to manipulate 3-PGA concentration.

The addition of a variety of substrates, such as mannose, which sequesters Pi, facilitates the manipulation of Pi levels in potato tuber discs. Results indicated that incubation with mannose did not increase flux to starch, but did lower total hexose phosphate and adenylate levels. Pre-incubation with mannose maintained comparable hexose phosphate levels to W.T tuber discs, but did not lead to an increase in the rate of starch synthesis. However, preliminary experiments using mannose plus ade-

nine have resulted in an increase in the rate of starch synthesis, suggesting that adenylate levels play a significant role in the control of the rate of starch synthesis in non-photosynthetic tissues.

#### **P10.11—The role of sugar signalling pathways in plant–pathogen interactions**

S.L. Potter, University of Sheffield; A. Greenland Syngenta, Jealott's Hill Research Station; O. Goddijn, Syngent Zeneca-MOGEN, Netherlands; S.A. Rolfe, J.D. Scholes, University of Sheffield

Biotrophic fungal pathogens rely entirely on the host plant for their nutrient requirements, and thus alter the regulation of host metabolism. We have examined the effect of *Cladosporium fulvum* (tomato leaf mould) on the carbohydrate metabolism of tomato to examine the hypothesis that altered amounts and fluxes of sugars may perturb host sugar signalling pathways, perhaps contributing to a down-regulation of photosynthetic gene expression. Infected leaves contained greater amounts of sucrose and hexose sugars and had elevated apoplastic and soluble invertase activity. Quantitative, real time PCR revealed a stimulation of host apoplastic invertase, and hexokinase II mRNA levels and a down-regulation of Rubisco gene expression in infected compared with control leaves. Experiments will also be reported which examine the effect of *C. fulvum* on sugar metabolism in tomato plants which over-express trehalose-6-phosphate phosphatase (TPP). Results will be discussed in relation to our current knowledge of sugar signalling pathways.

#### **P10.12—Altered fructose-2,6-bisphosphatase levels cause phenotypic changes and shift development in plants**

O. Toldi, Agricultural Biotechnology Center, Godollo, Hungary; S. Sorvari, Horticulture MTT, Piikkio, Finland; P. Scott, University of Sussex, Brighton, UK

Fructose-2,6-bisphosphate (F2,6P2) is an important intracellular signal metabolite in the control of carbohydrate metabolic fluxes in eukaryotes. Although the specific mechanism of F2,6P2 action varies between species and between tissues, most involve the allosteric activation of Pi-dependent fructose-6-phosphate kinase (PFK) and inhibition of cytosolic fructose-1,6-bisphosphatase (FBPase). These highly conserved enzymes regulate the fructose-6-phosphate/fructose-1,6-bisphosphate cycle, and thereby, determine the carbon flux. In theory, sucrose synthesis can be elevated by down regulation of the endogenous F2,6P2 level, while accumulation of primary starch becomes enhanced by its up regulation. When conventional procedures were followed during the recovery of transgenic plants, the

introduction of two modified forms of the rat liver-originated bifunctional enzyme 6PF2K/F2,6P2ase (one form possessing the capacity to synthesise F2,6P2 only, while the other catalyses its breakdown) to the tobacco, potato and *Kalanchoe daigremontiana* has resulted in dramatic changes in the carbon flux without resulting in phenotypic alterations. In contrast, when transgenic plants were grown under identical conditions, significant differences were found in growing and developmental parameters of strawberry, carnation and carrot. A model will be presented by which we try to explain the physiological background of our findings.

#### **P10.13—CP12-mediated dark/light regulation of photosynthesis is important for plant growth**

S.B. Britliff, J.C. Lloyd, C.A. Raines, Biological Sciences, University of Essex; N. Wedel, University of Osnabrueck, Germany

The chloroplast protein, CP12, has been shown to bind to the Calvin cycle enzymes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and ribulose-5-phosphate kinase (PRKase), in vitro. Both of these enzymes are activated in the light by the ferredoxin/thioredoxin system, and biochemical studies suggested that CP12 might be involved in the dark de-activation of GAPDH and PRKase. To determine the in vivo function of CP12 antisense, transgenic plants containing reduced levels of this protein were produced. Analysis of these plants revealed malformed leaves and flowers, reduced fertility and slower growth rates. Although photosynthetic CO<sub>2</sub> fixation rates and sucrose and starch levels are reduced in the CP12 antisense plants these are not sufficient to account for the severe phenotype observed. To test the hypothesis that CP12 functions in dark de-activation, we have grown the antisense plants in continuous light; however, the visual phenotype of these plants was identical to that of plants grown in normal dark/light regime. Although these data are preliminary, they suggest that dark de-activation of GAPDH and PRKase does not fully account for the role of CP12 in the plant.

#### **P10.14—Effect of over-expression of the Calvin cycle enzyme sedoheptulose-1,7-bisphosphatase on photosynthetic carbon assimilation, partitioning and growth**

S. Lefebvre, O.V. Zakhleniuk, N.J. Slee, J.C. Lloyd, C.A. Raines, Biological Sciences, University of Essex, UK

Sedoheptulose-1,7-bisphosphatase (SBPase) catalyses the dephosphorylation of sedoheptulose-1,7-bisphosphate in the regenerative phase of the photosynthetic carbon reduction (Calvin) cycle, producing the CO<sub>2</sub> acceptor

molecule ribulose-1,5-bisphosphate. It has been shown previously, using antisense technology, that SBPase has a high flux control coefficient, up to 0.75 over photosynthetic CO<sub>2</sub> assimilation. More recently, it has been shown that a small reduction (20%) in SBPase activity affects the allocation to sucrose and starch, growth rates and yield. These data suggest that it may be possible to increase carbon assimilation rates by increasing SBPase activity in transgenic plants. Transgenic lines have been produced in which the wild type SBPase content has been increased by expression of an *Arabidopsis thaliana* SBPase cDNA. Analysis of these plants revealed a small increase in the rate of photosynthetic CO<sub>2</sub> assimilation together with an increase in starch accumulation of up to 50%. An increase in yield of between 20 and 30% above the wild type was also evident. These results are remarkable and clearly demonstrate the potential for increasing plant productivity by improving photosynthetic carbon fixation.

**P10.15—Over-expression of *Escherichia coli* transaldolase in tobacco (*Nicotiana tabacum*)**

S. Shimizu, W.P. Quick, Animal and Plant Sciences, University of Sheffield

Transaldolase is an enzyme of the oxidative pentose phosphate pathway (OPPP). Transaldolase catalyses the reversible reaction converting sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate into fructose 6-phosphate and erythrose 4-phosphate, which is the precursor for many secondary metabolism including aromatic amino acid, lignin and flavonoid synthesis. Little is known about the regulation of the OPPP in plants and it is still uncertain whether there is a complete OPPP pathway in the cytoplasm. An incomplete cytosolic OPPP is suggested, as we have now cloned two genes from tomato that have plastidic signal peptides and no further genes can be identified by the BLAST search of the GenBank database (NCBI). To further understand the role of transaldolase, we have initiated a programme to heterologously over-express *Escherichia coli* transaldolase and to target expression to either the cytosol or the plastid in tobacco plants. We have subcloned the *Escherichia coli* transaldolase genes *talA* and *talB* in the sense orientation under the control of the CaMV 35S promoter with or without the plastidic transit peptide derived from glucose-6-phosphate dehydrogenase P2 isoform (Wendt et al., Plant J., 2000). Tobacco plants were subsequently transformed by the *Agrobacterium*-mediated method. Here, we report our current progress with screening of the tobacco transgenic lines.

**P10.16—Do *Sarracenia nigra* and *Dionaea muscipula*, two species of carnivorous plants, utilise phosphoenolpyruvate carboxykinase when absorbing amino acids from their prey?**

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

Carnivorous plants generally occur in habitats where nutrients, particularly nitrogen, are limiting. Carnivory in plants appears to have evolved to supplement the uptake of nutrients through the roots with the absorption of nutrients from captured and digested prey. Phosphoenolpyruvate carboxykinase (PEPCK) catalyses the decarboxylation of oxaloacetate to phosphoenolpyruvate in vascular plants. It has well-defined roles in gluconeogenesis and C<sub>4</sub> and CAM photosynthesis. It also has potential roles in the anaplerotic reactions associated with the Krebs cycle and amino acid metabolism. Therefore, a potential role exists for PEPCK in carnivorous plants, converting nitrogenous compounds from prey into suitable forms for use and transport within the plant. PEPCK was shown to be present in *Sarracenia nigra* (Northern pitcher plant) and *Dionaea muscipula* (Venus flytrap) through immunolocalisation and its presence was confirmed in *S. nigra* through SDS/PAGE gels and Western blots. In *D. muscipula* glands, PEPCK was induced, and present only with prey. However, in *D. muscipula* and in *S. nigra* phloem and the inner pitcher surface, PEPCK was constitutive, but with higher amounts apparently induced by prey.

**P10.17—Levels of carnitine/acylcarnitine produced by pea tissues in response to drought stress**

C. Masterson, C. Ginns, C. Wood, Biological and Nutritional Sciences, Newcastle upon Tyne

Metabolic profiling of carnitine/acylcarnitines in 2-week-old pea plants showed that in well-watered control plants (water content 95%), carnitine levels are 20-fold higher in the roots than in the shoots. In the shoots, carnitine exists entirely as free carnitine, whilst in the roots it is found esterified to activated acyl groups as both long and short-chain acylcarnitines. During drought (in which the water content was lowered to 78%), carnitine increased 10-fold in the shoot, 80% of which was found as long-chain acylcarnitine, whilst in the root, carnitine levels fell 10-fold and only free carnitine was detected. Following rehydration and recovery, a store of long-chain acylcarnitines was retained in both the root and shoot. Acylcarnitines provide ATP-independent reservoirs of activated acyl groups, which can be used for reacylation processes during acute periods of energy

depletion.<sup>1</sup> Arduini et al.<sup>2</sup> demonstrated a long-chain acylcarnitine pool which is used by erythrocytes for membrane repair. Drought leads to loss of lipids from the membranes of plant cells.<sup>3</sup> Carnitine could supply a reservoir of activated long-chain acyl groups for membrane repair in plants during drought and export of carnitine from the root to the shoot may occur.

1. Bremer (1983). *Physiol. Rev.* 63, 1420–1479.
2. Arduini et al. (1992). *J. Biol. Chem.* 268, 12673–12681.
3. Navariizzo et al. (1993). *Physiol. Plant.* 87, 508–514.

### **P10.18—The effect of carnitine on chlorophyll synthesis by pea seedlings**

C. Wood, M. Gillan, C. Masterson, Biological and Nutritional Sciences, University of Newcastle upon Tyne

Carnitine is involved in many aspects of fatty acid metabolism, membrane transport and activated acyl CoA conservation. It has been shown to influence chlorophyll biosynthesis in barley; this effect is thought to be a consequence of increased availability of acyl groups for plastid biosynthesis (1).

In this study, hydroponically dark-grown pea seedlings were used at 6 days old, which is the onset of greening in the newly emergent plumule. Maximum chlorophyll synthesis rates were recorded at 24 h in the light, after which net synthesis slowed down. Unsurprisingly, maximum greening was observed in the plumule of whole seedlings. Chlorophyll synthesis with part-seedlings constituted: plumule, 26%; plumule + radical, 32%; and plumule + cotyledon, 60% of whole seedling chlorophyll biosynthesis.

Chlorophyll biosynthesis was stimulated between 25 and 40% over the control rate with plants placed in 5-mM L-carnitine in the light for 24 h in all cases. Ten-mM L-carnitine also enhanced synthesis in the part-seedlings (above), but higher concentrations were inhibitory. After 48 h illumination, the chlorophyll content of all treatments were similar. Thus, carnitine appears to accelerate chlorophyll synthesis rather than increase the overall final quantity.

It is interesting to note that a massive increase in carnitine-dependent mitochondrial beta-oxidation has been observed in pea seedling cotyledons (2) at precisely this same developmental stage. The significance of carnitine-enhanced greening will be discussed in relation to its known involvement in metabolism.

1. Thomas et al., 1981. *Phytochemistry*, 20, 1241–1244.
2. Masterson and Wood, 2001. *Proc. R. Soc. Lond. B*, 268, 1949–1953.

### **P10.19—Peptide transport in the germinating and developing barley grain**

W.M. Waterworth, M.K. Ashley, C.E. West, C.M. Bray, Biological Sciences, University of Manchester, UK

Peptide transport plays a pivotal role in the mobilisation of nitrogen during cereal grain germination and is mediated by specific carriers localised to the plasma membrane of the scutellar epithelium. The barley scutellar peptide transporter *HvPTR1* has been cloned and functionally characterised by expression in *Xenopus* oocytes. In isolated barley embryos, peptide transport is rapidly inhibited by amino acids at concentrations (5 mM) comparable with those found in germinating barley grain. In vivo [<sup>32</sup>P]orthophosphate labelling studies with barley scutellar tissue demonstrate that *HvPTR1* is phosphorylated in the presence of amino acids and that phosphorylation occurs on phosphoserine residues. Ongoing studies are concerned with identifying further components of the regulatory mechanism and establishing the physiological significance of this control during germination.

Peptide transport may also contribute to the supply of nitrogen mobilised from the vegetative tissue of the mother plant to the developing barley grain during seed development. Here, we report that the non-hydrolysable peptide [<sup>14</sup>C]Gly–Sar can be transported through the plant vascular system and accumulates in the developing grain, predominantly in the embryo. Gly–Sar transport into the developing barley grain is active, and peaks at 15–20 dpa, immediately prior to seed storage protein deposition in the endosperm. Northern and Western analysis showed that *HvPTR1* is expressed in the developing barley embryo, from 3 dpa before declining between 20 and 30 dpa. The very early expression of *HvPTR1* raises the possibility that this peptide transporter may play an as yet undetermined role grain filling during seed development.

### **P10.20—Control of mannose metabolism in relation to ascorbate biosynthesis in plants**

J. Dowdle, S. Gatzek, N. Smirnoff, Biological Sciences, University of Exeter, UK

Ascorbate, which functions as an antioxidant and enzyme co-factor in plants, is synthesised from L-galactose (L-Gal) in a two step oxidation via L-galactono-1,4-lactone. The source of L-Gal is GDP-L-Gal. GDP-L-Gal is formed from GDP-mannose (GDP-Man) by a 3,5-epimerase and GDP-Man is in turn formed from Man-1-P by GDP-Man pyrophosphorylase. GDP-man is required for polysaccharide synthesis and protein glycosylation, so these processes potentially compete with ascorbate synthesis. Information on the extent to which

ascorbate synthesis is limited by GDP-Man availability will provide essential background information for attempts at metabolic engineering of the pathway or in producing high ascorbate plants by molecular breeding. We are, therefore, investigating the factors that control flux through this pathway, particularly the formation of GDP-Man from hexose phosphates. Two approaches are being taken. Firstly, transgenic tobacco plants that are over-expressing mannose metabolising enzymes (phosphomannose isomerase, phosphomannose mutase and GDP-mannose pyrophosphorylase) are being analysed. Secondly, the expression and activity of enzymes in the ascorbate biosynthesis pathway are being compared in leaves from *Arabidopsis thaliana* acclimated to low light intensity (small ascorbate pool size) and high light intensity (large ascorbate pool size). Preliminary results from these experiments will be presented.

#### **P10.21—The use of chlorophyll fluorescence JIP test to predict the performance of sunflower (*Helianthus annuus*) plants in a range of compost types**

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A polytunnel growth trial was set up at Rattery, near Dartington, Devon, in May 2001, in which sunflower plants were grown in a variety of composts. EcoSci Ltd., West Country Compost was tested alone and in combination with coir or silvafibre, while Levington's Compost was used as a control for comparison. In early May, soon after the plants became established, fluorescence parameters were measured on the first fully emerged leaf of each plant using a Hansatech PEA meter, and the full parameters for the JIP Test were calculated using the Biolyzer program (R. Rodriguez, University of Geneva, Department of Bioenergetics). The height, stem width and 'marketability value' of the plants was recorded on three separated occasions during the trial, and final results were compared with the initial predictions from the fluorescence parameters, in particular the Performance Index.

#### **P10.22—Ultrasound-induced physiological changes in cultured *Petunia* cells**

H. Böhm, P. Anthony, L.C. Garrett, E. Benes, L.G. Briarty, M.R. Davey, J.B. Power, K.C. Lowe, Biosciences and Life and Environmental Sciences, University of Nottingham

Ultrasound has several biotechnological applications in, for example, acoustic cell separators, cell immobilization systems, and, for plant cells, to potentiate *Agrobac-*

*terium*-mediated genetic transformation. However, a detailed understanding of the biological effects induced by ultrasound is essential to minimize any undesirable physiological perturbations and to optimize desirable outcomes utilizing this process. Therefore, *Petunia hybrida* cell suspension cultures were exposed to ultrasonic standing wave fields at 2.43 MHz for 40 min with mean sound pressures (within homogenous sound fields) from 0 (control) to ca. 1.1 MPa. Mean ( $\pm$ S.D.;  $n=6-9$ ) cell viability decreased to  $87\pm 10\%$  at 0.6 MPa and  $59\pm 23\%$  at 1.1 MPa, compared to an initial control value of  $92\pm 6\%$  ( $P<0.05$ ). Mean ( $n=3$ ) cell alkaline phosphatase (ALP) concentration increased linearly with sound pressure from a control value of  $0.006\pm 0.001$  to  $0.02\pm 0.01$  Sigma-Units  $\mu\text{g}^{-1}$  protein at 1.1 MPa ( $P<0.05$ ). Mean cell catalase activity also increased from a control value of  $0.020\pm 0.003$  to  $0.026\pm 0.008$  arbitrary units at 1.1 MPa. However, mean cellular lactate dehydrogenase (LDH) concentration was unchanged. These results indicate that cellular repair processes associated with increased ALP activity might be triggered by physical cell damage caused by ultrasound. The increase in cell catalase suggests increasing production of free radicals and other sonochemicals, which warrants further study. The absence of changes in LDH indicates that there was no major damage to respiratory pathways or to overall cellular integrity.

#### **P10.23—Naringenin promotes *Agrobacterium* transformation in *Passiflora* and tobacco**

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Naringenin (NAR) is a member of the class of phenolic compounds known as flavonols. Another flavonol, acetosyringone, is a potent inducer of virulence (*vir*) genes that promote T-DNA transfer from *Agrobacterium* to plant cells. There are no reports of NAR on *A. tumefaciens*-mediated transformation of plants. Therefore, the effects of supplementing, with NAR (100–300  $\mu\text{M}$ ), a co-cultivation medium for *A. tumefaciens* have been studied for promoting transformation of leaf explants of *Passiflora mollissima* and *P. giberti*, compared to *Nicotiana tabacum*. The bacterial strains used were 1065 with pVDH65 and EHA105 with p35SGUSINT. Both plasmids had neomycin phosphotransferase (*nptII*) and  $\beta$ -glucuronidase (*gus*) genes. Strain 1065 also harboured pTOK47, conferring a supervirulent phenotype. GUS activity was detected in explants of both *Passiflora* species exposed to the two bacterial strains. Maximum mean ( $\pm$ S.E.M.,  $n=9$ ) GUS activity following transformation with 100  $\mu\text{M}$  NAR ( $22.6\pm 2.9\%$ ) occurred with explants of *P. giberti* with strain EHA105 (1:2 v/v bacterial dilution) and was significantly ( $P<0.05$ )

greater than without NAR ( $14.0 \pm 0.1\%$ ). For *P. mollissima* and strain EHA105, maximum mean GUS activity with NAR ( $20.3 \pm 2.4\%$ ) was not significantly different to control ( $19.3 \pm 2.8\%$ ). With strain 1065, mean maximum GUS activity with 300  $\mu$ M NAR ( $6.0 \pm 0.6\%$ ) was greater than control where activity was undetectable. A similar finding occurred with *P. giberti* and strain 1065, where the maximum GUS activity with the same NAR concentration ( $5.0 \pm 2.0\%$ ) was also greater than without NAR where GUS activity was not detected.

#### **P10.24–Cryopreservation of potato germplasm**

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Cryopreservation of plant cells is employed routinely to minimise genetic changes and loss of morphogenic competence associated with long-term culture. Storage at ultra-low temperatures necessitates partial cellular dehydration, usually by pre-culture in medium with mannitol or sorbitol. There have been few studies to assess the benefits of other osmotically-active agents. Thus, suspension cells of potato (*Solanum tuberosum*) cv. Desiree were pre-cultured with sucrose (0.25–1.0 M), cryopreserved, and their viability and growth were assessed after thawing. Any further beneficial effects of culturing cells after thawing on filter paper supports overlaying medium were also studied. Mean ( $\pm$ S.E.M.,  $n=5$ ) cell absorbance (490 nm), assayed by triphenyltetrazolium chloride reduction, after 24 h pre-culture with 0.09 M sucrose (control) was  $0.48 \pm 0.06$ ; values with 0.25 M, 0.5 M or 0.75 M sucrose were 146%, 108% and 42% of control, respectively. Mean ( $\pm$ S.E.M.,  $n=10$ ) cell viability at 4 days post-thawing was significantly ( $P < 0.05$ ) lower for all treatments, with or without filter

paper supports, compared to their respective unfrozen controls. However, after 10 days of post-thaw culture, the mean cellular absorbance following pre-treatment with 0.75 M sucrose was significantly ( $P < 0.05$ ) greater for cells recovered on filter paper ( $0.62 \pm 0.05$ ) and lacking supports ( $0.54 \pm 0.08$ ), compared to corresponding unfrozen controls ( $0.33 \pm 0.06$  and  $0.34 \pm 0.08$ , respectively). Pre-culture with 0.75 M sucrose and recovery on filter supports also stimulated biomass production, at 28 days post-thawing, by 36% and 30% ( $P < 0.05$ ) over unfrozen controls and cells recovered without filter paper supports, respectively.

#### **P10.25 Carbohydrate metabolism in the grain of rice plants grown under NaCl salt stress**

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Rice (*Oryza sativa* L.) is a salt-sensitive species, and when grown under NaCl salt stress is known to have reduced grain yield. It is not clearly understood why this is so, but recent research has favoured a limited translocation of carbohydrate to the grain and a reduction in the starch synthesising enzyme starch synthase (Abdullah et al., 2001) over seed sterility (Khatun and Flowers, 1995). It is mainly sucrose that is translocated to the grain, some from stored carbohydrates in the upper leaves and culm (Cock and Yoshida, 1972), where it is thought to be broken down by enzymes such as sucrose synthase, neutral invertase and acid invertase. The resulting intermediates are eventually converted into starch, the major constituent of a mature grain, by enzymes such as starch synthase and ADP-glucose pyrophosphorylase. This project examines the activities of these enzymes in grain, from 5 days after anthesis to maturity. Seven varieties of rice plants were used, each known to produce different yields under NaCl salt stress.