P10–PLANT BIOTECHNOLOGY

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P10.1–The regulation of plant senescence

Vicky Buchanan-Wollaston, Tania Page,
Elizabeth Harrison, Chengguo Shen

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

Leaf senescence is the final stage of leaf development that marks the transition from net carbon and nitrogen assimilation to a period of catabolism followed by nutrient diversion to the developing sinks. Senescence is a programmed event responding to a wide range of external and internal signals, it requires de novo gene expression and protein synthesis and is controlled in a highly co-ordinated manner. Elucidation of the genes that control leaf senescence has been complicated by the complex combination of signalling pathways that appear to be involved in senescence. Much cross talk exists between senescence and stress or pathogen response pathways and also a number of hormonal and nutrient signals are implicated in the control of senescence.
We have isolated and characterised many genes that show enhanced expression during leaf senescence in *Brassica napus* and Arabidopsis. We are carrying out a functional analysis of senescence-enhanced genes that encode potential regulatory proteins by identifying insertion mutants and/or creating RNAi transgenics. These knock out mutants will be analysed by investigating the effects of the mutation on plant phenotype and gene expression patterns during senescence. We are using *Arabidopsis* microarrays to identify genes altered in expression in each mutant. This will give an idea of the downstream result of the expression of each transcription factor and will be helpful in elucidating the overall importance of that gene in the senescence process. We are also investigating the role and importance of stress response pathways involving ethylene, jasmonate and salicylic acid for gene expression during senescence.

P10.2–Environmentally friendly era in biotechnology: expression of traits for plant improvement and production of biopharmaceuticals and edible vaccines in transgenic chloroplasts

H. Daniell, Department of Molecular Biology and Microbiology, University of Central Florida, Orlando, U.S.A.

In order to address environmental concerns, our laboratory has pioneered the chloroplast genetic engineering approach, which overcomes concerns of gene containment (Daniell, Nature Biotechnology, 2002, 20: 581–586), low levels of transgene expression, impact on non-target insects or development of resistant insects and the presence of antibiotic resistance genes. Chloroplast transgenic approach has been successfully used to confer desired plant traits, including herbicide/ insect/ disease resistance, drought tolerance, phytoremediation (Daniell et al. 2002, Trends in Plant Science, 7: 84–91) and the expression of biopharmaceutical proteins. Assembly of several vaccine antigens, including cholera toxin β-subunit functional oligomers (Daniell et al. 2001, Journal of Molecular Biology, 311, 1001–1009), Anthrax Protective Antigen functional heptamers and hyper-expression of small antimicrobial peptides (De-Gray et al. 2001, Plant Physiol. 127: 852–862), human serum albumin (Fernandez San-Millan et al., 2003, Plant Biotechnology Journal), interferon and insulin like growth factor in transgenic chloroplasts will be presented. Hyper-expression of bacterial operons with exceptionally large accumulation of foreign proteins (up to 46% of insecticidal protein in the total soluble protein, DeCosa et al. 2001, Nature Biotechnology 19: 71–74) or to confer phytoremediation will be presented. In order for any transgenic approach for edible vaccine or oral delivery of biopharmaceuticals to be successful, it is essential to develop a selection system free of antibiotic selection. Such marker free chloroplast transformation systems have been accomplished recently (Daniell et al. 2001, Current Genetics 39: 109–116; Trends in Plant Science 6: 237–239). Recent developments in chloroplast genetic engineering will be presented.

P10.3–Global Transcript Analysis of Rice Leaf and Seed using SAGE Technology

J.M. Dunwell, J.G. Gibbins, Plant Sciences, University of Reading; B.P. Cook, M.R. Dufault, S.L. Madden, Genzyme, Framingham, USA

We have compiled two comprehensive gene expression profiles from mature leaf and immature seed tissue of rice (*Oryza sativa ssp. japonica* cultivar Nipponbare) using Serial Analysis of Gene Expression (SAGE) technology. Analysis revealed a total of 50 519 SAGE tags, corresponding to 15 131 unique transcripts. Of these, the large majority (~70%) occur only once in both libraries. Unexpectedly, the most abundant transcript (~3% of the total) in the leaf library was derived from a type 3 metallothionein gene. The overall frequency profiles of the abundant tag species from both tissues differ

greatly and reveal seed tissue as exhibiting a non-typical pattern of gene expression characterised by an over abundance of a small number of transcripts coding for storage proteins. A high proportion (~80%) of the abundant tags (≥9) matched entries in our reference rice EST database, with many fewer matches for low abundant tags. Singleton transcripts that are common to both tissues were collated to generate a summary of low abundant transcripts that are expressed constitutively in rice tissues. Finally and most surprisingly, a significant number of tags were found to code for antisense transcripts, a finding that suggests a novel mechanism of gene regulation, and may have implications for the use of antisense constructs in transgenic technology.

P10.4–Pericarp-Specific Gene Expression in Cereals

R.J. Henry, A Furtado and P Bundock

The Centre for Plant Conservation Genetics, Cooperative Research Centre for Molecular Plant Breeding, Southern Cross University, PO Box 157, Lismore, NSW, 2480 AUSTRALIA

Promoters of seed-specific gene expression are required to direct expression of novel traits in grain. We have compared a wide range of promoters in transgenic rice, barley and wheat using green fluorescent protein (GFP) as a reporter. The promoter of the *asi* gene was found to give GFP expression in the pericarp, vascular tissue, nucellar projection and endosperm transfer cells of developing barley seeds. The protein encoded by this gene, the bifunctional alpha-amylase/subtilisin inhibitor has been implicated in plant defence (inhibition of subtilisin) and in the regulation of endogenous alpha-amylase. The presence of an abundant and highly specific barley alpha-amylase inhibitor in barley seeds has been difficult to reconcile with efficient starch mobilisation during germination. Our results suggest that this inhibitor is expressed specifically in the maternal tissues of the seed. Specific inhibition of endogenous enzymes in the peripheral tissues of the seed may have a role in plant defence denying pathogens access to plant carbohydrate reserves. Polymorphisms (SNP) in a 2 kb genomic sequence including this gene have been analysed for wild and cultivated barley suggesting that recombination had occurred in the *as*i gene. Evolution of the *amy*1 gene family in the Triticeae has apparently been paralleled by the evolution of the *asi* gene to specifically inhibit the members of this unique gene family.

P10.5–Microarray Analysis of Wheat Endosperm Transcriptome –the Effect of Nitrogen Supply

C. Lu, K. Edwards, I. Wilson, G. Barker, J. Coghill, University of Bristol; M.J. Hawkesford, Rothamsted Research

To determine the influence of nitrogen supply on gene expression during wheat endosperm development, we used the classical Broadbalk winter wheat experiment as the source for materials at two stages of grain development. 10,000 wheat unigenes generated from 26 wheat different EST libraries were double spotted on to the Super Microarray Substrates glass slides and hybridised in a series of experiments with probes derived from samples grown with different nitrogen fertiliser regimes including long-established farmyard manure (FYM) treatment, no N treatment (N0) and a wide range of N-treatments.

Preliminary analysis of the microarray data has suggested that for the FYM and N0, ~60 genes are significantly up regulated in the whole grain at 7 days post-anthesis, while ~400 genes are up-regulated in the endosperm at 14 days post-anthesis. Approximately 20% of the up-regulated genes were expressed at ratios =2 fold higher in FYM when compared to the no nitrogen treatment. These included the alpha gliadin storage proteins, a grain softness protein, 1 Gsp-1, LMW and HMW glutenins, glutamine synthesis, various endosperm-specific proteins and CM3-2-amylase terrameric inhibitor. In addition there were many up-regulated genes of unknown function plus several potential regulatory factors. Further analysis, now underway, of the results will lead to a better understanding of the effects of nitrogen source on the developing grain transcriptome.

P10.6–New Insights into the Structure and Function of Transaldolase in Plants

Maxime Caillau, Sachiko Shimizu, W. Paul Quick; University of Sheffield

The oxidative pentose phosphate pathway or OPPP provides plants with important substrates for both primary and secondary metabolism *via* the oxidation of glucose-6-phosphate including large amounts of reducing power to drive various anabolic processes. To date, the cellular compartmentation of OPPP enzymes remains unclear.

Whereas several enzymes of the OPPP are believed to have both cytosolic and plastidic counterparts, there is yet no evidence for the existence of two complete sets of OPPP enzymes for both compartments.
Transaldolase is an enzyme of the OPPP catalysing the reversible conversion of sedoheptutulose-7-phosphate and glyceraldehyde-3-phosphate to fructose-6-phosphate and erythrose-4-phosphate. Erythrose-4-phosphate condenses with phosphoenolpyruvate (from glycolysis) to form the first substrate of the shikimic acid pathway.
In this study, two full-length cDNA clones from tomato (*Lycopersicon esculentum* L.) which putatively encode distinct transaldolase isoforms were isolated. Unexpectedly, these two clones were found to share a low degree of identity, and further phylogenetic analysis revealed that plant transaldolase genes may have diverged early in the evolution from different prokaryotic ancestors. Both genes encode for a putative leader sequence, which suggest a targeting of the proteins to plastids. Despite several reports of a cytosolic transaldolase isoform, extensive screening of plant genomic databases tends to eliminate such a possibility.
Both isoforms appear to be differentially regulated at the level of gene expression in tomato tissues, and immunolocalisation studies indicates a further cellular control of expression. Several environmental treatments give rise to distinct changes in transaldolase abundance. Finally, preliminary data will be presented concerning transgenic *Arabidopsis thaliana* and tobacco plants that have altered transaldolase activity.

P10.7–Searching for root QTLs in maize: present status and perspectives for positional cloning using rice as a model

S. Salvi, S. Giuliani, M. Bellotti, M. Maccaferri, S. Stefanelli, P. Landi, M.C. Sanguineti and R. Tuberosa

Department of Agroenvironmental Science and Technology, Via F. Re 6, 40126-Bologna, Italy

Until recently, little progress has been achieved selecting root traits to improve yield. The identification of major root QTLs allows us to apply marker-assisted selection and, eventually, to clone the gene/s underlying such QTLs. The massive genetic information generated for rice and its extensive synteny with the other cereals will facilitate positional cloning in these species. Our long-term goal is to identify major QTLs controlling root traits in maize and clone the genes underlying such QTLs exploiting rice as a model. As a preliminary step, we have analysed QTL data for root traits in maize and rice to identify the syntenic regions more valuable for a cloning approach. In maize, we considered the QTL data of four mapping populations previously investigated for root traits (Tuberosa et al., 2002, Annals of Botany 89: 941–963). Exploiting syntenic information, the maize QTL data were compared with those available for 439 root QTLs in rice. The most interesting correspondence involved maize bin 2.04, where we have identified a major QTL affecting root traits. Extensive testing of BDLs for this QTL confirmed its important effects on root traits. Fine mapping of the QTL is underway. Subsequently, the rice sequence and synteny relationships will allow us to use the markers closest to the QTL to cross-reference the genetic map to the rice genome sequence. The rice genome will then serve as source of new markers to increase the mapping resolution and will provide an interesting source of candidate genes, reducing the use of contiged genomic libraries.

P10.8–Using nutritional genomics to identify key loci that regulate ascorbate pool size

L.C., Garratt, K., Swarup, G.A. Tucker, and M.J., Bennett, University of Nottingham

Increasing the accumulation of ascorbate (ASC) will enhance antioxidant capacities of crop plants, thereby improving their pre- and post-harvest performance by delaying rates of senescence and improving shelf-life, appearance and nutritional content. Any enhancement in the antioxidant status of human nutrition would also be of direct benefit to the consumer since the accumulation of reactive oxygen species have been associated with over 100 medical disorders, ranging from rheumatoid arthritis to cardio-myopathy. Nevertheless, the molecular genetic factors influencing ASC pool size in plants are largely unknown. We have adopted a quantitative genetic approach to identify Quantitative Trait Loci (QTL) that regulate ASC accumulation in the model plant Arabidopsis. Recombinant inbred lines (RILs) arising from crosses between *Arabidopsis* accessions Landsberg (Ler) and Cape Verde Island (Cvi) were used to identify several QTL determining ASC accumulation. Candidate genes co-segregating with 2 ASC QTL are being characterised at the molecular level whilst a third ASC QTL has recently been confirmed using several near isogenic lines. Changes at the genome level, brought about by these QTL(s), their effect on cellular function and ultimately, plant physiology, will be defined using a selection of genomic and metabolomic profiling (LC-MS/MS) tools that have recently been acquired in the BBSRC-Wellcome JIF funded Nutritional Genomics programme.

P10.9–Alteration of Light Illumination during Microbial Growth, An Enhancement Effort of Biomass Production and Carbon dioxide Fixation of Psychrophilic Cyanobacterium *Anabaena cylindrica* IAM M1

A. Wijanarko, Chemical Engineering, University of Indonesia; K. Ohtaguchi, Chemical Engineering, Tokyo Institute of Technology

The photosynthetic of cyanobacterium *Anabaena cylindrica* strain IAM M1 in aerated liquid was preferred for high CO_2 fixation at low temperature environmental condition. Cell growth successfully in single reactor at low temperature and light illumination of 288 K and 1000 lx, however, in accordance with Arhenius prediction, biomass production and average carbon dioxide removal relatively small. Current works was carried to improve the performance of photosynthetic in this low temperature condition and consequently we proposed alteration of light illumination during microbial growth. The overall average value of carbon dioxide transfer rate (CTR_{av}) of cyanobacterial growth at alteration of light illumination was around 1.71 g/[dm^3.h], which was about eleven times of result constant light illumination of 1000 lx. Refer to average value of final cell concentration and light energy utility efficiency of cyanobacterial growth production (η), alteration of light illumination also increased biomass production and actually, produced more efficient than in constant light illumination, which were approximately, three times. Additionally, kinetic studies of this microbial growth at alteration of light illumination also concluded that both of relationship between specific carbon dioxide transfer rate q_{CO2} and X as essential factor concentration of carbon dioxide fixation and relationship between incident specific growth rate ($\mu\phi$) and [HCO_3^-] as essential compound concentration of cyanobacterial growth, followed a substrate inhibition model kinetic equation, that was proposed by *Andrew*.

P10.10–Pathogenicity determinants in plant pathogenic oomycetes

J. Beynon, R. Allen, P. Bittner-Eddy, A. Gordon, L. Grenville, S. Hall and A. Rehmany, Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, UK

Peronospora parasitica is the causal agent of downy mildew on *Arabidopsis* and *Brassica* crops. We have cloned the *Arabidopsis* resistance genes *RPP13* and *RPP1* that recognise the downy mildew isolates Maks9 and Emoy2, respectively. These two interactions result in very different resistance phenotypes. *RPP13* elicits a localised necrotic lesion whereas *RPP1* results in a spreading necrotic lesion. In order to study the basis of these different interaction phenotypes we are cloning the corresponding avirulence genes, *ATR13* and *ATR1*. We have used a map based cloning approach and located the genes to overlapping BAC contigs. The mapping cross has been shown to be segregating for up to 15 different avirulence genes. Using Suppression Subtractive Hybridisation we have identified a range of genes that are specifically up-regulated on infection of Arabidopsis. An analysis of their structure and their relationship to the avirulence genes will be presented.

P10.11–A genomics approach to plant breeding: The combinatorial application of *Arabidopsis* transcription factors to alter tomato traits

F.D. Hempel, L. Adam, J.Z. Zhang, Mendel Biotechnology, Inc.; J. Palys and S. King, Seminis Vegetable Seeds, Inc.

We are currently developing a genetic engineering ‘tool kit’ that can be easily incorporated into standard plant breeding programs to rapidly and systematically screen and select desired traits in tomatoes. In this program, a complete set of *Arabidopsis* transcription factors will be ectopically expressed under the control of 10 different promoters in the context of a two-component gene expression system. To facilitate transcription factor expression, ‘activator’ plants (containing a promoter sequence fused to an intermediate transcription factor gene) and ‘target’ plants (containing a intermediate transcription factor binding site fused to an *Arabidopsis* transcription factor) are genetically crossed. In the F1 progeny transcription factors are expressed under the control of the promoter, just as if each plant had been directly engineered with the specific gene–promoter combination. The F1 population can thus be phenotypically screened for the effects of tissue-specific ectopic expression. This technology will make it easier to rapidly enhance desirable traits in tomato, as it will create a large genetic engineering toolkit that can be used to combinatorially express genes in precisely defined tissues.

P10.12–Manipulating the expression of cytosolic glutamine synthetase in wheat of four different genetic backgrounds

By S.M. Bernard, J. West, C.H. Foyer and D. Habash
Crop Performance and Improvement Division, Rothamsted Research, Harpenden

Worldwide Nitrogen Use Efficiency (NUE) for cereal production is approximately 33%, a slight improvement in NUE would reduce N fertiliser cost as well as nitrate pollution. NUE is a complex trait that includes N uptake efficiency and N utilisation efficiency; the latter relies on N transport, primary N assimilation and N remobilization. Glutamine synthetase (GS: EC 6.3.1.2) which catalyses the fixation of ammonium to amino acid is involved in some aspects of N utilisation efficiency. Manipulating GS expression would therefore be a possibility to enhance NUE. Previous studies in wheat have shown that over-expressing GS in the shoot resulted in an increase in grain N content in two independent lines (Habash, et al., 2001). The aim of this project was to transform wheat from various genetic backgrounds differing in their assimilation and use of N, with the bean cytosolic GS under the control of the rice *rbcS* promoter. The four wheat genotypes selected for this study are Cadenza (a commercial variety routinely used in cereal transformation) and three doubled haploid (DH) lines which are part of a mapping population of 95 lines derived from the cross 'Chinese Spring' (CS) x 'SQ1'. This is the first attempt to over-express GS in three DH lines of varying genetic backgrounds and N use.

Habash, D. Z., A. J. Massiah, et al. (2001). 'The role of cytosolic glutamine synthetase in wheat.' Annals of Applied Biology **138**(1): 83–89.

P10.13–Diamagnetic levitation: novel applications in biological systems

P. Anthony, N.W. Blackhall, A. Catherall, M.R. Davey, L. Eaves, P.J. King, K.C. Lowe and J.B Power, Biosciences, Life & Environmental Sciences and Physics, University of Nottingham

Gravity is a ubiquitous, uniform and inescapable force on the Earth's surface that is central to the development of living organisms. However, its mechanism of action and the consequences of its removal are still largely unknown. Previous approaches for studying microgravitational effects on living systems and biological processes have relied on the use of clinostats, and expensive and non-routine use of space orbiting and/or free fall (usually of a few seconds duration) conditions, all of which have severe limitations. A high-field, closed-cycle, liquid helium-cooled, super-conducting magnet system, that is unique in the UK, is being used at Nottingham to investigate the real-time effects and potential applications of (1) zero ($0 \times g$; diamagnetic levitation), normal ($1 \times g$), or enhanced ($2 \times g$) gravity, and (2) intense magnetic fields on gene expression, physiology and the development of seedlings and cultured cells and tissues of higher plants. Preliminary experiments indicate that seedlings of cress (*Lapidium sativum* L.) held somewhat crudely under these conditions in the magnet bore exhibit significant differences in both root and hypocotyl development. The longer-term objectives of the research are to identify how these physical variables can be used to manipulate cellular processes of relevance to plant biotechnology.

P10.14–Progress in Transforming the Mushroom *Agaricus bisporus*: Agrobacterium Methodologies and the Development of Novel Marker Genes

K.A. Leach, M.P. Challen, T.J. Elliott (HRI Wellesbourne) and J. Henderson (Coventry University)

Successful transformation of the button mushroom *Agaricus bisporus* has recently become possible due to the application of *Agrobacterium*-mediated transformation. Factors such as the strain of *Agrobacterium tumefaciens,* co-cultivation conditions, host strain, type of tissue, and the use of various promoters are known to affect rates of transformation. Using gill tissue from a number of commercial *A. bisporus* strains we have shown that different *Agrobacterium* strains and the use of vacuum infiltration and sonication treatments can dramatically alter the recovery of transformants. The effect of the virulence inducing phenolic, acetosyringone, on the number of transgene copies integrated into the *A. bisporus* genome has also been investigated. The rate of gill tissue transformation to hygromycin resistance was highest using *A. tumefaciens* strain AGL-1 and a sonication treatment (ca. 70–99% efficient).

There is a need for alternative selectable markers in mushroom transformation. Homobasidiomycete *para*-aminobenzoic acid genes can confer sulfonamide resistance and have potential as positively selectable markers. The *Coprinus cinereus* auxotroph, PG78, was transformed to PAB+ prototrophy using PEG-mediated protoplast transformation and the *C. cinereus pab*A gene. PAB+ prototrophs were also generated using heterologous *C. bilanatus* and *A. bitorquis pab* genes. Most PAB+ transformants have proved resistant to sulphanilamide and sulfamethoxazole. Southern blotting is normally used to confirm transgene copy number. With PAB+ transformants we have demonstrated the use of real-time quantitative PCR to determine copy number.

P10.15–Manipulation of gibberellin biosynthesis to control plant stature

L. Sedighi, M. R. Davey, J. B. Power, Biosciences, University of Nottingham; A. Phillips, P. Hedden, Long Ashton Research Station, University of Bristol

Gibberellins (GAs) are the main group of plant growth regulators responsible for stem elongation. GA 20-oxidase and GA 2-oxidase (2β-hydroxylase) are two key enzymes regulating concentrations of active GAs by catalysing GA-biosynthetic and catabolic steps, respectively. Unlike most GA 20-oxidases, an enzyme (CmGA20ox1) from immature seeds of pumpkin (*Cucurbita maxima*) produces less active GAs by diverting substrates away from the GA biosynthetic pathway, converting GA_{24} and GA_{19} to the inactive GA_{25} and GA_{17}, respectively. The GA 2-oxidase (PcGA2ox1) from runner bean (*Phaseolus coccineus*) introduces a 2β-hydroxyl group in the final step of GA biosynthesis, to produce inactive GAs. The effect of overexpressing CmGA20ox1 and PcGA2ox1 were assessed in *Solanum dulcamara* and *Nicotiana plumbaginifolia* following *Agrobacterium tumefaciens*-mediated transformation of leaf explants. Transformed plants exhibited a range of phenotypes. Some were severely dwarfed with smaller, deep-green leaves, while others were intermediate in height between dwarfed and wild-type plants or, in some cases, taller than wild-type plants. Dwarfed plants of *Solanum* flowered earlier than wild-type plants and produced more fruit and more seeds per fruit. Taller transformants of *Nicotiana* flowered before dwarfed and wild-type plants. PCR and RT-PCR confirmed the presence and expression of the transgenes in *Solanum* and *Nicotiana*. Genetic manipulation may provide an alternative approach to the use of chemicals to modify plant stature.

P10.16–Expression of the lol1 gene in chicory (*Cichorium intybus L.*) inhibits ice recrystallisation

F.M.K. Williams, M.R. Davey, J.B. Power, Biosciences, University of Nottingham; C. Sidebottom, Unilever Research Colworth, Bedford

Chicory (*Cichorium intybus* L.) is an important vegetable exploited for leaves, roots and forced shoots (chicons). Increased tolerance to low temperatures would be beneficial for the growth and storage of chicory. A gene, *lol1*, isolated from over-wintering rye grass (*Lolium perenne* L.), encodes an apoplastic protein. The latter binds to ice crystals, inhibiting their growth, which may reduce freezing damage to cells. Cotyledons, excised from 17-day-old seedlings of chicory cv. Brussels Witloof, were scored and immersed for 3 sec in an overnight culture of *Agrobacterium tumefaciens* strain LBA4404 habouring pTOK47, which conferred supervirulence, and the binary vector pGPT. The latter carried *lol1* and the neomycin phosphotransferase (*npt*II) gene conferring kanamycin resistance on transformed plant cells. Shoots were regenerated on Murashige and Skoog-based medium with 75mgl^{-1} kanamycin sulphate. The transgenic status of regenerated kanamycin-resistant plants was confirmed by PCR and RT-PCR, targeting the *lol1* gene. Inhibition of ice-recrystallisation was observed in crude protein extracts from young leaves of 3-month-old transgenic plants grown at 24 °C, but not in extracts from non-transformed plants. Cryo-SEM images were taken of young leaves excised from transgenic and non-transformed plants cooled to –6 °C overnight. Ice crystals were observed in the apoplastic space but not in intercellular spaces; ice formation was less extensive in leaves of transgenic plants.

P10.17–Modification of floral characteristics in ornamental species

V. Watson, M.R. Davey, J.B. Power, Biosciences, University of Nottingham; Q. Cronk, Botanical Garden, University of British Colombia

Studies of *CENTRORADIALIS* (*CEN*) and *CYCLOIDEA* (*CYC*) gene expression in *Antirrhinum majus* have led to a common molecular model for the development and evolution of floral characteristics, the latter being important horticultural traits, especially in ornamental species. The transition to flowering in a shoot marks the end of its vegetative phase and the beginning of reproductive development. This transition involves the reprogramming of the shoot meristem. Two transitions occur; one that forms inflorescences followed by another that results in flowers. Inflorescences, of which there are several types, are either determinate or indeterminate. *CEN* and *CYC* genes have been shown to confer floral characteristics on the shoot meristem. *CEN* is necessary to confer floral positioning and the production of the inflorescence, whereas *CYC* establishes floral shape. The introduction of *CEN* and *CYC* genes into ornamental plants may facilitate studies/modifications of floral development in target species. The possible modification of floral characteristics is being investigated by introducing *CEN* and *CYC* genes into *Petunia* and *Saintpaulia*, using *Agrobacterium* and microprojectile-mediated transformation. Since flowers of both *Petunia* and *Saintpaulia* are zygomorphic, changes in floral development associated with over-expression of *CEN* and *CYC* genes should be readily identified in transgenic plants.

P10.18–Virulence gene expression enhances Agrobacterium-mediated transformation of rice

M. Goddard, L. Kannaiyan, S.C. Andras, M.R. Davey, J.B. Power, Biosciences, University of Nottingham; J. Pickett, Rothamsted Research, Harpenden

Several factors have been shown to influence the efficiency of *Agrobacterium*-mediated gene delivery to rice, including the target plant cells, exposure of bacterial cells to the phenolic wound signal molecule acetysyringone and the use of supervirulent bacterial strains possessing extra copies of virulence (*vir*) genes. *A. tumefaciens* strain LBA4404 carrying pBBR1MCS-5 with a constitutive mutant *vir* GN54D gene, and the binary vector pMOGB22 (with *gus* and *bar* genes), has been used by other workers to transform cells of *Catharanthus roseus* in a 'ternary transformation system'. In the present investigation, scutellum-derived callus of rice (*Oryza sativa* cv. Taipei 309) was inoculated with *A. tumefaciens* LBA4404 carrying the modified binary vector pSLJ7321 (*npt*II, *hpt*, *nahG*), with and without pBBR1MCS-5. Calli were also inoculated with *A. tumefaciens* LBA4404 carrying pBBR1MCS-5 and the two binary vectors, pMOGB22 and pSLJ7321. Transformation frequency was enhanced by the use of the extra *vir* gene. Transformation of rice calli was also successful with the two binary vectors used simultaneously. T-DNA was stably integrated into regenerated plants and transcription confirmed by RT-PCR; enzyme assays detected increased protein activity. This system may enhance the transformation efficiency of recalcitrant cereal species. It could facilitate transfer of selectable marker genes on a separate plasmid to the genes of interest, enabling antibiotic resistance genes to be removed subsequently by segregation, producing marker-free plants.

P10.19–Micropropagation and Germplasm Conservation of Spontaneous Plant Species (*Ramonda serbica* and *Ramonda nathaliae*)

E. Kongjika, E. Caushi, B. Preci, Zh. Zeka, A. Mullaj, Institute of Biological Research,Tirana, Albania

Many tropical species of decorative plants of the family *Gesneriaceae* can be propagated using the methods of tissue culture. The European Balkanic Gesneriad species are Tertiary relicts. *Ramonda serbica* Panç. is a typical Albanian species; while *Ramonda nathaliae* Panç. & Petrov. is characteristic for FYROM territory.
Two nutrient media were used to germinate the seeds of *Ramonda serbica* and the seeds of *Ramonda nathaliae*. The phytohormone-free nutrient basal medium JG-B, poorer in salts comparing to MS medium, proved to be the most successful for seed germination. The use of the cytokinin 6-benzylaminopurine (BAP) affects the organogenesis of ramonda plants. Rapid promotion of axillary bud formation and the development of shoot bundles observed in the variants with JG-B medium supplemented with BAP at high level (1 mg l^{-1}) and especially in the medium with the combination of BAP and auxin IAA (0.5 mg l^{-1} each). Indirect organogenesis with the involvement of callogenesis stage are observed especially in the variant with NAA 10^{-4} M and BAP^{-5} 10 M. The formation of meristemoids derived from cell proliferation and differentiation of callus surface is followed by the regeneration of organs. The initiation of callus, formation of meristemoids and adventitious organs for two Ramonda species are very similar models. The method of minimal growth storage of the plants from different stands is the bases of the creation of a gene bank 'in vitro' for two ramonda species. A simple phytohormone-free nitrate medium JG-B, normal temperature and light levels are used successfully for germplasm conservation of ramonda plants. The data from our experiments will permit us to evaluate the possibility for the improvement of decorative values by intergeneric hybridization within European Gesneriads.

P10.20–Controlling stature in apple trees by genetic modification of gibberellin biosynthesis

S.M.W. Bulley, D.J. James, HRI, East Malling; A.L. Phillips, P. Hedden, Rothamsted Research

We are examining the feasibility of developing dwarf apple cultivars that could be grafted onto invigorating rootstocks without needing supplementary applications of chemical growth retardants. To this end, we are attempting to reduce the gibberellin (GA) content of apple trees by genetic modification of GA biosynthesis. We used RACE to obtain two very similar (presumably homoeologous) GA 20-oxidase cDNAs, *MdGA20ox1A* and *MdGA20ox1B*, sharing 94% nucleotide identity, from shoot tips of the cultivar Greensleeves. The corresponding genes are highly expressed in developing embryos, at lower levels in shoot tips and young leaves, and at very low levels in ovaries 5 days after pollination. Both cDNAs were shown to encode enzymes with GA 20-oxidase activity by heterologous expression in *Escherichia coli* and incubation with [^{14}C]GA_{12}. A separate apple GA 20-oxidase cDNA sharing 67% and 68% amino acid identity, respectively, with *MdGA20ox1A* and *MdGA20ox1B*, was shown to be expressed in stamens and, to a lesser extent, in sepals and unpollinated ovaries. Transformation of Greensleeves with constructs containing a *MdGA20ox1A* cDNA fragment in sense or antisense orientation behind the CaMV 35S promoter

resulted in dwarf plants with reduced concentration of GA_1 in shoot tissues compared with the untransformed control. The appearance of some of the dwarf lines suggested that they may have enhanced drought tolerance.

P10.21–Production and characterization of transgenic wheat with altered gibberellin catabolism

D.J. Evans, M. Stone, N.E.J. Appleford, M.D. Wilkinson, H.D. Jones, Q. Ma, S.J. Croker, A.L. Phillips, P. Hedden and J.R. Lenton, Rothamsted Research

The gibberellin (GA) plant hormones control a number of developmental processes, including the growth of the stem. Restriction of GA biosynthesis by chemical or genetic methods is used routinely in agriculture to control plant stature. We are evaluating ectopic expression of a gene encoding the GA-catabolic enzyme, GA 2-oxidase (GA2ox) as a non-chemical approach to reducing GA content in crop species. We have produced homozygous lines of wheat, cv. Canon, expressing a bean *GA2ox* cDNA under control of the maize ubiquitin promoter. Transformation with the *ubi::PcGA2ox* construct produced severely dwarfed plants with reduced shoot negative gravitropism and increased tillering, as well as moderately dwarfed (semi-dwarf) plants. We will present a comparison of the phenotypes of dwarf and semi-dwarf lines with a null segregant (tall) control in terms of seed germination, a-amylase production, leaf and stem growth, time to anthesis and grain development. We are also examining GA content and the expression of a number of endogenous GA-biosynthetic genes in these lines. In future work, we will seek to target the modification of GA content to specific tissues and developmental processes. To this end we are transforming wheat with *PcGA2ox* controlled by seed-specific promoters.

P10.22–Use of *Arabidopsis* mutants to evaluate the role of ethylene in pod development

T. Yang, JA Roberts, Biosciences, University of Nottingham; M. Maunders, ATC Cambridge, UK

Senescence is a sequence of biochemical and physiological events that lead to the eventual death of a cell, organ or the whole organism. The time course of the phenomenon is genetically determined, however, in spite of its biological and practical importance, the events that regulate senescence are unclear.

To increase our understanding of the process we have chosen to focus our research on pod development in *Arabidopsis*. Pod and leaf senescence share a number of features in common, however, the yellowing of the former organ occurs over a much more predictable time frame and as a consequence it may be easier to identify the molecular events that are critical for the process to take place. We have undertaken and analysis of chlorophyll content in silique tissues and seeds in *Arabidopsis* mutants to evaluate the role of ethylene during pod development. Our data show that an ability to respond to ethylene may be important for the regulation of seed maturation and pod dehiscence. In contrast, the time course of silique senescence remains unaffected in mutants either insensitive (Etr & Ein4) to the gas, or that display a constitutive ethylene response (ctr). These observations are discussed in relation to how the terminal events of pod development may be regulated.