Frontiers of plant biology research*

The Adelaide Congress was a watershed event highlighting the transgression of molecular techniques to automated dimensions for understanding gene expression and regulation through microarrays into a systems biology approach. An exciting curtain-raiser to the crisp and precise inaugural session was the aboriginal dance, setting the pitch for technical sessions. Broadly, the sessions stressed upon developmental biology, metabolomics and cellular trafficking, interactive mechanisms (including symbiosis, plant—microbe interactions, molecular ecology, abiotic and biotic stresses), and the genetic machinery and its control acting through small RNAs/RNAs.

Developmental biology

Environmental cues such as photoperiod, temperature and endogenous signals regulate flowering. Genetic analyses of mutants in Arabidopsis have identified several genes involved in multiple genetic pathways that control flowering. Flowering Locus T (FT) mRNA/protein was important for long-distance signal generated in photoperiodically induced leaves to initiate floral development, affirming the results of classical experiments done fifty years ago, whereby the inductive photoperiodic signal perceived by a single leaf could induce floral transition. The identification, mapping and expression of the photoperiod genes under variable day length and vernalization regimes were presented in a long day crop plant, i.e. ryegrass. The epigenetic regulation of flowering by MSI1 and VRN2 genes were also topics of interest.

Photomorphogenetic responses at various wavelengths of light are mediated by photoreceptors such as phytochromes, cryptochromes and phototropins. Peter Quail discussed genomic approaches for identifying components of phytochrome signalling to establish transcriptional networks. Two novel phytochrome regulated genes, PAR1 and PAR2, control shade avoidance responses in Arabidopsis. Mutant studies showed the potential role of ethylene in the phytochrome-mediated control of vegetative development. M. Wada overviewed the physiological functions of phototropins and their signal transduction pathways. A novel phytochrome-related phosphatase type 2C was responsible for phytochrome signalling and sugar sensing.

Studies on pattern formation in ovule development propound that alteration in the activity of orthologues of ovule expressed YABBY and KANADI genes are responsible for the evolution of novel ovule forms during diversification of angiosperms. In addition, polyclomb group proteins are involved in regulating the initiation of endosperm development and imprinting. Genes for female gametophyte development have been isolated using T-DNA mutagenesis and transposon mutagenesis in Arabidopsis where seed development is also under epigenetic control. Mutants with reduced endosperm cell number enabled isolation of WRKY class transcription factors and a LRR kinase regulated by the FIS complex. Function and regulation of MEDEA provided an insight into epigenetic controls.

In the context of anther and pollen development, the DYT1 gene has been found to express early and specify anther cell fate. Male-specific gametophyte mutants were characterized using Ds – insertion lines in Arabidopsis. Nearly 20 genes have been identified from 230 mutants using TAIL-PCR and classified under genes responsible for signal transduction, transcription regulation, metabolism and for some unknown functions. Becker reported on the pollen and sperm cell transcription analyses performed on the Affymetrix Array. Of the 6587 genes selectively expressed in these cell types, 11% display a functional bias towards signalling, vesicle transport and cytoskeleton formation. Abnormal pollen development, due to activity of the MSI1 chromatin remodelling factor, could also contribute to embryo abortion. The S-locus for self-incompatibility interacts between the SRNases and the F-box proteins in Antirrhinum, while in Brassica the stigma-specific S-receptor kinase functions in regulating pollen rejection. Activity of the Generative Cell Specific 1 gene (GCS1) from Lilium has been identified as crucial for fertilization in angiosperms. In a cell cycle mutant cdc2a, only a single gamete exclusively fertilized the egg cell and the unfertilized central cell nucleus divided to form a rudimentary endosperm leading to abnormal embryo development.

The subfamily of transcription factors, the Tower of PISA genes, plays an important role in shoot development of Arabidopsis. The Auxin Response Factor (ARF) uncouples fruit development from pollination/fertilization and is the key for development of seedless or parthenocarpic fruits. Studies have shown that suppressing RAB gene expression in tomato fruit inhibits softening of the fruit.

Using the genomics approach, early stages of embryo development were resolved employing giant embryos of Phaseolus coccineus. The role of genes such as DEK1, CR4 and SAL1 during cell fate specification and differentiation of aleurone cells was also explained in cereals. A novel B3 domain protein functioned as an active transcriptional repressor during seed development in Arabidopsis.

Segregation analysis of genes for apomixis in dandelion revealed that diplospory was under female specific control by a single dominant locus, DIP localized on one of the NOR chromosomes. Attempts were also made to produce viable diploid apomictic maize through de novo engineering. Elongate, a mutant coded for a putative chromatin remodelling protein mimicked a diplosporous type.

Plants are characterized by a modular and iterative growth pattern. This iteration of the single unit is dependent on tight regulation of hormonal and environmental cues. Shoot architecture is governed by the controlled expression of genes at the shoot apex as well as in the lateral organs. Members from three gene families, YABBY, KANADI, class III HD-ZIPs were found to influence the patterning of lateral organs derived from apical and vascular meristems. The differentiation of the leaf blade into distinct adaxial and abaxial surfaces is the resultant of YABBY (promotes adaxial cell
identity) and LEUNIG which represses YABBY to allow abaxial-adaxial patterning instead of needle-like growth. The hormone interactions particularly of auxin and cytokinin balance, auxin polar transport have now found a new meaning in the lateral branching pattern. By study of mutants’ axr1-axr4, Ottoline Leyser’s group showed the regulation of shoot branching by a novel hormone and a known auxin. Interestingly, both the auxin and the novel hormone signalling involve targeted protein degradation. Furthermore, a novel peptide hormone, ATPNP-A, was shown to be a key regulator of cellular homeostasis and growth.

Patterning of root stem cells is determined by the polar auxin transport route. Root architecture and root hair morphology are important agronomic traits and studies in barley show that the CesA and Csl genes are involved. Studies on genetic control of vascular tissue morphogenesis has provided convincing information on the interactive role of cytokinins and transcription factors in the differentiation of the protoxylem, protophloem and procambium cells from the root tip stem cells. The cell type specific biosynthesis of IAA in root tips has revealed that indeed meristematic cells of the root apex also synthesize IAA possibly through an alternative pathway. Microarray analysis of gene expression during root tip regeneration revealed that cell-specific identities are laid down within 24 h of excision and cell fates are restored. Factors affecting root growth under normal and mechanical stress were also addressed.

Several enlightening lectures and posters portrayed the beauty and the brains behind the development of the cell wall, cuticle and the cytoskeleton. The cell wall is remodelled during rapid growth phase. The microtubule cortical array directs deposition of cellulose in the cell wall. Co-visualization of cellulose synthase complexes and the microtubules has shown that as the cortical array reorients, so do the complexes. Several Arabidopsis mutants displaying variable cell wall compositions and architecture have been identified using Fourier-transformed Infra-red Spectroscopy. Monoclonal antibodies enable tissue-specific localization and identification of cell wall composition. Modern microcopy resolves immuno-localization of microtubules-associated proteins and microtubule dynamics.

In evolution, cell cycle components are highly conserved but some plant-specific genes have evolved. The complexity of the cell cycle and the cytoskeleton involving endocyto control, chromatin dynamics and the relation between cell cycle and cell fate was discussed with its regulatory mechanisms in the coupling of cell division potential with cell fate specification. Current models suggest that the mammalian tumour suppressor retino-blasta (RB) and plant RB-related protein have dual roles in regulating cell cycle progression and differentiation. Using conditional loss and gain of function alleles, the function of RBR in plant stem cell maintenance and in connecting cell cycle activity to cell differentiation was explored. Similarly, the role of the D type cyclins and the pathway in which they operate during plant cell cycle control and growth and development was elucidated.

Metabolomics and cellular trafficking

Interestingly, Natasha Raikhel in her lecture on ‘Exploring chemical space in the plant world’ described the use of organic chemicals for studying gene function and analysis of regulatory networks thereby improving crop value. Chemical genomics allow the instantaneous, reversible, tunable and conditional control of a phenotype, providing many advantages over the traditional genetic approaches.

Multilevel phenotyping including expression profiling with concomitant robotized enzyme assays and metabolic profiling enabled deciphering of upstream profiles of sugar signalling pathways. Trehalose-6-phosphate (Tre6P) regulates carbon allocation in response to dynamic changes of carbon supply. Accumulation of sucrose in leaves shows increased levels of Tre6P that could lead to redox activation of ADP-6-glucose pyrophosphorylase diverting photo-assimilate towards starch and not sucrose. To avoid the complexity of polyplody in sugarcane, downregulation of key steps of carbohydrate metabolism by RNAi or antisense RNA were used to repress the partitioning of carbon towards respiration so as to optimize partitioning of carbon between cell wall polysaccharides and sucrose. In wheat, the study of the reproductive phases under normal and stress conditions reveals that fructan accumulation and nitrogen remobilization are regulated in parallel as a part of a programme of monocarpic development. Zeeman reflected data indicating two mechanisms for starch breakdown in chloroplasts of Arabidopsis, i.e. the loss of a glucose binding chloroplast protein phosphatase, SEX4 causes starch accumulation and is regulated by reverse phosphorylation. Interestingly, SEX4 in animals is related to Laforin, a protein that controls glycogen metabolism. This raises issues of evolutionary significance in separation of pathways in animals and plants. For a better quality of fibre in cotton, sucrose synthesis plays an important role in fibre initiation. Fibre-specific β-1,3-glucanase gene controls the closure of the plasmodesmata and this phenomenon is directly related to the final fibre length.

Plasmodesmata serve as a ‘cytoplasmic highway’ for traffic between cells. This exchange led to the concept of higher plants functioning as supramolecular organisms. Immunodetection studies show tropomyosin (an actin-binding protein) association with plasmodesmata and that plasmodesmata are regulated by myosin and not actin function. Using mutants that abnormally accumulate seed storage proteins, the molecular mechanism underlying vacuolar targeting have been defined. Using various truncated VSR-AI derivatives it has been shown that AtVSR1 undergoes homo-oligomerization and plays a role in lyticvacuolar protein trafficking. Analysis of 385 mitochondrial and 567 chloroplastic signal sequences in Arabidopsis thaliana has shown that differences at the N-terminal portion of transit peptides are essential not only for transport to chloroplasts but also for mis-targeting of chloroplast proteins to mitochondria. The role of basic amino acid motifs for efficient localization to golgi apparatus has also been elucidated. Raising transgenic plants with commercially valuable products and their efficient targeting into suitable sites such as vacuoles and protein bodies (PB) is of prime importance. KDEL an ER retention signal directs to PB1.

Water uptake in plant roots is mediated by water channels known as aquaporins. Using gene-specific tags, immunodetection and aquaporin-GFP transgenics, changes in the aquaporin expression levels have been reported in Arabidopsis roots in response to salt stress. The variation in diurnal hydraulic conductance of the different plant systems is associated with
different response in aquaporin gene expression and root anatomy. HKT transporters are important channels playing central role in Na/K co-transport, and Na or K transport. Non-selective cation channels (NSCCs) are other Na⁺ channels which cause rapid uptake of Na⁺ under salt stress. Functional yeast screens utilizing yeast mutants are being utilized in order to elucidate the molecular identity of NSCCs in yeast and plants and specify amino acids conferring ion specificity to cation transporting ATPases. An NaCl inducible CLC-type chloride channel has been identified in soybean. Fluorescence dye quenching experiments indicate that CLC-type channels are involved in the sequestration of chloride ions into vacuoles thus enhancing salt tolerance. Transgenes with inducible gene expression systems and Na⁺ transporter genes such as *PpENA1* and *AhHKT1* have been developed for tissue-specific expression of genes. To decipher the underlying pathways and mechanisms governing distribution of cell-specific compartmentation of Ca²⁺ and Mg²⁺ ions, X-ray microanalysis and tracer studies have been employed in leaves of 37 plant species. Ammonium as a nitrogen source is key for efficient biomass production in plants and its uptake is regulated by specific transporters of high (HATS) and low (LATS) affinity transport systems. In *Citrus*, HATS is governed by a feedback mechanism depending on the N-status and the gene is regulated by light and sucrose. Recently, an amino acid transporter was characterized as Lysine and Histidine Transporter 1 (LHT1) from *Arabidopsis*. Peptide transporters such as HvPTR1, localized at the plasma membrane in plant cells have also been reported in barley. These play a central role in the mobilization of organic nitrogen in the form of peptides from the endosperm to the embryo during germination. *BOR1* from *A. thaliana* was the first boron efflux transporter identified in living systems. In addition, six genes (BOR2–7) highly similar to *BOR1* have been identified in the *Arabidopsis* genome and post-transcriptional processes are critical for expression of boron transporters. Sucrose transport across cotyledon plasma membrane is mediated by H⁺/sucrose symporters (SUTs). Fluorescent dye tracer studies, in situ hybridization, immunolocalization, SUT1-promoter–GUS fusions were employed in cereal seeds to unravel such pathways. In addition to H⁺-coupled transporters, three novel pH- and energy-independent sucrose transporters have been identified in pea serving as sucrose facilitators. For phosphorus, PHT1 transporters are involved in transport and homeostasis of Pi.

**Interactive mechanisms**

Two plenary lectures by Jens Stougaard and Jeff Dangl addressed two important mechanisms of interaction between plants and their symbiotic partners and microbial pathogenic invaders. Stougaard presented the current state of research in nodulation and symbiosis in the model legume *Lotus japonicus*. The differentiation of a nodule requires complex interaction between nodulin genes, phytohormones, particularly GA and brassinosteroids as well as their ratios with other compounds. Two types of nodules have been identified, determinate which lack meristems and are formed on legumes of non-galegoid clade (*Lotus*, bean) while those with persistent meristems formed on legumes of the galegoid clade (*Medicago*). In *Medicago* the host-plant controls the differentiation of the bacteroids as well as the bacterial cell cycle, while it is not so in determinate nodules. Interestingly, a study has shown that the signalling pathway crucial for symbiotic nodulation has been recruited by the root-knot parasite to infect roots of *Lotus*. Dangl’s talk on plant immune system focused on Pathogen Associated Molecular Patterns (PAMPs) and PAMPs Triggered Immunity (PTI) as pathways mediating pathogen resistance in plants. A fundamentally different conceptual framework for activation of NB-LRR protein (a class of R proteins containing nucleotide-binding sites and leucine-rich repeat domains) activation was provided by the ‘guard hypothesis’. A special section was devoted to cereal pathogenesis, particularly to rust and powdery mildew resistance in wheat. The molecular basis of non-host resistance or immunity of an entire plant species against non-adapted pathogens involving PEN secretory machinery was also presented.

Under molecular ecology, evolution of new species and perceived difficulties for neospecies establishment have been conducted on *Senecio eboracensis* using artificially resynthesized hybrid plants and a genetic mapping population. The speciation process in *S. squaridus* by polyploidy and hybridization using cDNA microarray analysis of changes in the pattern of floral gene expression indicate that hybridization and polyploidy have immediate and distinct effects on gene expression. Genetic diversity of Canadian forest trees was analysed using expressed sequence tag polymorphisms (ESTPs), microsatellite markers (SSRs), RAPD and allozymes providing genetic benchmarks and indicators for framing guidelines for conservation and sustainable management in forests. In *Salix cinerea*, AFLPs and microsatellites have been utilized to ascertain the geographic source of seedlings and scale and frequency of seed or pollen dispersal, so as to develop eradication strategies. Various genes for resistance to heavy metals and drought have been mapped using QTls, SSR and AFLP markers. In *A. halleri*, QTL regions for Zn tolerance and in *Hordeum spontaneum* QTL controlling drought resistance have been identified.

**Genetic machinery and its control**

Flowering plants are remarkable for their degree of genomic fluidity, as evidenced by enormous variation in genome size, chromosome number and genome arrangement. Jeff Bennetzen described the structure and evolution of flowering plant genomes and Takaji Sasaki summarized the work on sequencing, structure and evolution of the rice genome. He presented the work on comparison of the genome sequence of the orthologous regions of wild rice species and cultivated rice harbouring domestication-related genes to study the course of evolution of agriculturally and biologically important genes. Several resources such as whole-genome, chromosome-specific libraries, extensive EST collection, transformation systems, wild germplasm and mutant collection, as well as DNA chips were introduced to establish genetic programs in wheat. Several other advances in genomics such as evolution of DNA sequence diversity in maize, comparative analysis of transcription factors in rice and *Arabidopsis* and a set of genes, such as evolutionary history of pentatricopeptide repeat proteins, were also presented. The use of Affymetrix whole genome tiling array containing the entire *Arabidopsis* genome on a single array (6.4 million oligos) to identify the whole complement of transcription units (coding and non-coding) and their associated regulatory
elements (for example, transcription factors binding sites, and DNA methylation sites) was described by Joseph R. Ecker.

Kazuo Shinozaki described the involvement of at least four (two ABA-dependent and two ABA-independent) independent regulatory systems in stress-responsive gene expression. Data on the functional analysis of several miRNAs and siRNAs supporting the crucial regulatory roles of small RNAs in plant adaptation to abiotic stresses was presented by J. K. Zhu. The use of map-based cloning approach to identify and isolate the abiotic stress tolerance genes in cereals was also highlighted.

D. C. Baulcombe and his group are characterizing miRNAs and siRNAs using high throughput sequencing technology in wild type and mutant Arabidopsis plants and by tracking their association with Argonaute proteins. The results indicate that there are thousands of loci in Arabidopsis genome producing silencing-related RNAs with a potential to regulate gene expression through genetic and epigenetic mechanisms. Use of short RNAs to silence individual genes to study their cell biology, particularly in context with viral resistance and other therapeutic targets was reflected in a wide array of diseases. The progress in design and testing of an Arabidopsis small RNA microarray was also presented. Importantly, growth in the understanding of gene silencing has prompted a complete symposium on 'The biology of small RNAs and the epigenome' held during January 2007 at Riverside, University of California.

Advances in the identification and functional analysis of the components of the proteolytic machinery were presented. The complexity of the ubiquitin/26S proteasome pathway, which targets the proteins for degradation by covalent attachment of multiple ubiquitins, was illustrated by estimate that >10% of plant proteins are the targets of the pathway and over 1500 Arabidopsis genes encode this pathway components. J. Callis has identified >450 RING domain containing E3 ligases in the Arabidopsis genome, which mediate diverse biological processes. The role of regulated protein degradation in ethylene signalling and disease responses was highlighted.

Plants are model systems for the genetic analysis of DNA damage response. Using transgenic Arabidopsis and tobacco, frequency of homologous recombination was elevated upon treatment with UV, gamma radiation and after infection with the fungal pathogens (Peronospora parasitica and TMV). It was observed that the rates of somatic recombination between repeats that diverge by one out of 617 identical nucleotides, reduces by nearly three-fold while rates of meiotic recombination are higher in an isogenic background, than in crosses with divergent ecotypes.

The epigenetics session addressed the role of methylation in various processes. Heterochromatin formation in Arabidopsis is guided by double-stranded RNA which triggers methylation of histone H3 at lysine 9 (H3K9) and CG/non-CG methylation on identical DNA sequences. In Arabidopsis, resetting of the Flowering Locus C expression level occurs early in the development of progeny and a wider chromatin domain is targeted for repression by vernalization.

Wide applications emerging from current biotechnology research were also addressed. Studies on efficacy and immunogenicity of lupin containing alpha amylase inhibitor gene suppressed experimental asthma indicating the usefulness of GM plants for oral immunization against allergens. However, weevil-resistant cowpeas evinced allergic response in mice raising safety concerns of transgenic foods. Prospects of allergen specific immunotherapy for pollinosis using seed-based edible peptide vaccine and oral administration of transgenic plants consisting of cytokine interleukin and glutamic acid decarboxylase fusion protein to suppress the development of autoimmune diabetes were discussed.

Concurrent workshops were held on apomixis, rice functional genomics, forest tree molecular biology and genomics and the CAMBIA BiOS initiative. Kolunow informed of a new apomixis website (www.pi.csiro.au/apomixis/index.htm) that will form a platform for free interaction among apomixis researchers. Om Rajora and fellow speakers highlighted the genomics work on Eucalyptus and Pinus trees and the importance of sequencing of the Populus genome for wood quality improvement. Jeckson explained the BiOS initiative-biological open source where they are developing new open source enabling technologies as examples of a dynamic and useable protected common shared resource.

The technique of Laser Capture Micro-Dissection (LCMD) has been used extensively for cell-type specific transcriptional profiling and is currently being done for rice. Fluorescence Correlation Spectroscopy (FCS/FFCS) is being used to unravel transcriptional networks of MADS-box factors. Sacco De Vries described the novel technique of Biomolecular Fluorescence Complementation (BIFC) to study molecular interactions. Magnetic resonance imaging (MRI) has been used for the first time in plants to study DNA repair in plant tissues.

In posters from India, Mukesh Jain reported a comprehensive genome-wide analysis of early auxin-responsive SAUR gene family in rice. The work involved the identification of SAUR gene family members, their chromosomal distribution, phylogenetic relationship, upstream and downstream elements, and expression. Jyoti Vora presented her work on biochemical and environmental perspectives of Aloe vera and internalization of various metal toxicants and pollutants which could interfere with its projected curative properties. Atika Chandra presented her work (done in collaboration with Palacky University, Czech Republic) on the unique property of an Agrobacterium strain that secretes high levels of cytokinins and has high infectivity for pigeonpea tissues. This could be a promising strain for developing high frequency transformation systems for recalcitrant legume plants. P. S. Abuja reported the isolation of 11 full-length cDNA clones along with their expression levels with environmental cues in the catechinate synthesis pathway of tea. Sanjay Gawhna showed the identification and characterization of low-temperature responsive full-length cDNAs with promoter analysis showing cis-acting elements important in regulation of gene expression at low temperatures.

As a satellite event, resident and non-resident Indians came together to discuss the Genome India International Initiative. This is a registered society working for the mutual growth of resident and expatriate Indian scientists and seeks to build collaborative research networks. Led by Chittaranjan Kole (based in US) and P. K. Ranjekar (Pune), the consortium has a website (http://www.genome-india.intl.org/).

Overall, worthy choice of speakers of the plenary sessions must be complemented for the requisite foundation that they laid to the proceedings in some absorbing concurrent sessions and the en-
Apiculture in India*

Honey bees are one of the important primitive social insects as well as a rich source of honey. Honey has been traditionally used in various diet preparations, medicines, cosmetics, ointments, candles and house-held bee-wax items2, besides Ayurvedic drug preparations. The propolis of the bee hive is used in lip balms and tonics, whereas royal jelly is used to strengthen the human body, for improving appetite, preventing aging of skin, leukaemia and for the treatment of other cancers. On an estimate, about 80% of honey is used directly in medicines and 10% is used in Ayurvedic and pharmaceutical production. Honey bees during foraging for pollen and nectar from flowers of different plant species, enhance agricultural productivity to the tune of 30–80% annually through cross-pollination3. Five species of honey bees are found all over the world, namely Apis florea, *A. cerana*, A. *dorsata*, A. *mellifera* and *Trigona iridipennis*. However, *A. cerana* and *A. mellifera* are reared in hives in India.

Currently, China captures 40% of the world market and the biggest importers of honey are Germany, Japan and the United States. Germany imports about 90 thousand tonnes of honey annually. India produces about 70,000 tonnes of honey every year of which 25–27,000 tonnes is being exported to more than 42 countries, including the European Union, Middle East and the United States (2002–03). The major honey-producing states are Punjab, Haryana, Uttar Pradesh, Bihar and West Bengal4. Due to the economic importance of honey bees and their products, the biotechnological interventions need to upgrade the qualitative and quantitative production taking into account further investigation on genetic stock of Indian bees that may lead to new biotypes.

To evolve an effective network programme by identifying key issues on apiculture in India, a brainstorming session was held under the chairmanship of Raghavendra Gadagkar (Indian Institute of Science, Bangalore) along with senior DBT personnel, and 20 scientists/academicians of our country to formulate future viable lines of research work.

In the opening session of the meeting, Gadagkar spoke about the uniqueness in honey-bee science and its application regarding flavour management to lure worker bees towards their specific pasture, giving the example of orange spray in bee colonies. Appreciating the lead efforts of DBT, he mentioned about the needs of various biotechnological interventions in different areas of honey bee ecological science. He focused on genomic studies of our native honey bee species, study on sex determination, role of bees in pollination along with their selective behaviour towards specific crops, and development of molecular markers to study diversity of native species. The honey bee genome has been already sequenced and our country is believed to have the largest bee diversity (out of eight species found in the world, six occur in India). S. Natesh (DBT) emphasized the need for biotechnological research in view of economic importance of bee colonies for pollination as well as molecular analysis of bees for disease resistance and other traits for native bee species, *A. cerana*. Other participants presented their views on biotech-