

PHYTOGEN

A NEWSLETTER FOR AUSTRALIAN PLANT SCIENTISTS

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Thanks to all the contributors for being prepared to give their time to provide informative articles for this issue of Phytogen



AUSTRALIAN SOCIETY OF PLANT SCIENTISTS

News from the President

August 2011

Dear Members,

Science and Scientists are under pressure. Never before has science and technology been so fundamental to our way of life and to provide solutions to meet the needs of a growing population. Technological solutions to problems always have their origins in basic scientific research. The destruction of the CSIRO field trials by Green Peace was reprehensible. Field trials are essential if we are to understand the risks and benefits of GM crops. We have to work out how to feed 9 billion people using fewer resources, and this is one option to be explored. No matter what your personal views are on GM crops, without rigorous research we will always just be guessing. Our sympathy goes out to those who have lost years of research in this one reckless act.

At the other end of the political spectrum, researchers in the field of climate science continue to come under pressure. One recent strategy by those who like to ignore the weight of scientific evidence for climate change, GM, vaccines or whatever, is to attack the scientific community as elitist. Anyone who has seen how much pleasure young scientists get from disproving the status quo knows this is not true. Another more insidious strategy is to try to undermine the peer review process. We all know peer review is not perfect, but those of us who publish in peer-reviewed journals, and spend hours reviewing papers (for free), know just how rigorous the process is. Occasionally a poor paper slips through, and very rarely a fraudulent one, but they are almost always quickly forgotten or sidelined when the data are not able to be corroborated by subsequent experimentation. This is what happened with the Piltdown hoax: the 'fossils' were put aside for decades before they were discovered as fakes because they just didn't fit with all the new research coming out of Africa showing that humans walked upright before, not after, they got big brains. It is accumulated knowledge that makes science convincing, or as I tell my students Yoda-style: 'One paper does not the science make'. So, next time you get asked to review a paper, regard it as a privilege and not a chore.

An important part of science is the frank and free discussion of experiments and ideas. Conferences play an important role here. The recent International Botanical Congress in Melbourne was a great event with many excellent symposia organised by ASPS members (and see the report on IBC, pp 20-26). It was a great opportunity to slip in and learn something from a companion discipline. Special thanks to Anna Koltunow (CSIRO Plant Industry), who came on as co-chair of the science committee earlier this year, and did so much to ensure its success. Thanks are also due to Rana Munns (immediate past president, ASPS) for her work behind the scenes.

Our national meeting ComBio is an important meeting place for Australian plant scientists. Being in Cairns, it should be attractive to those of us from the southern states. The Society AGM is held during the conference and I would urge you to come and get involved. It is the last week in September, during the AVCC common week. I hope to have the low-key ASPS mentoring program up and running by then, so that mentors and mentees can meet up over a meal at the ASPS dinner.

See you in Cairns!

Ros Aglindon

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A message from the editor

Dear Fellow ASPS Members,

Well I am very excited about this issue of Phytogen. It is full of interesting and thought stimulating articles starting with "*News from the President*" in which Ros puts a number of issues on the agenda for us as a society of plant scientists. Well worth reading - if you have reached this page without doing so then please go back.

Under the *State of Affairs* banner, Victoria has come forward to provide an easy-to-read, well illustrated account of what's happening in Plant Development research. Thank you to the contributors and to Prem Bhalla for coordinating this effort. One of our young scientists, Elizabeth Neilson (University of Melbourne) reports on her PhD research on cyanogenic glucoside synthesis in *Eucalyptus* in "*From Our New PhDs*". I am particularly delighted to have this report (thanks Elizabeth) and hope that other new PhDs will be inspired to contribute in subsequent issues. "*Education in Plant Science*" is also featured with John Harper and Geoff Burrows (Charles Sturt University) compiling "A Hitchhikers Guide to Botanical Teaching Websites" which will no doubt prove very useful for many members (thanks guys!!). There are an unprecedented variety of reports in this issue – all interesting reading. Among these are: The Global Plant Council; Science meets Parliament; International Botanical Congress; International Conference on Plant Metabolism and updates on Functional Plant Biology and Plants in Action. Many people contributed to these reports. Thank you for your efforts. Also in this issue is an article on "*The Plant Accelerator*" in which Mark Crowe outlines the capabilities of this facility and available research opportunities. Well worth reading and considering research options. And remember ComBio in Cairns!!

Queenslanders please note that it is your turn next for "*State of Affairs*". Any other suggestions, and of course contributions – book reviews, reports, significant issues for plant science, education issues, are most welcome.

Keep those contributions coming in and I hope you all enjoy reading this issue.

Tina Offler

State of Affairs – Victoria

Plant Development

The University of Melbourne

Melbourne School of Land and Environment

Professor Prem L Bhalla and Professor Mohan B Singh (Email premlb@unimelb.edu.au; mohan@unimelb.edu.au)



Work at the Plant Molecular Biology and Biotechnology group of Melbourne School of Land and Environment is focused on understanding molecular control of male gamete development in flowering plants. In addition, the group is also aims to understand molecular control of meristem development and floral transition in a major crop legume, soybean.

Male gamete Development

Plant reproduction is vital for our food production. Most of our staple food such as wheat, rice, and corn are the result of successful sexual plant reproduction. Sexual reproduction is one of the important events in the life cycle of flowering plants involving development and functioning of male and female gametes. Despite its importance our understanding of gamete formation and fertilisation processes in flowering plants is limited.

In animals the germ line cells are established at early embryo development and remain as a distinct stem cell population throughout the animal's life. On the contrary, plants have distinct vegetative and reproductive stages, and the male germ line in plants originates in flowers from the cells of a previous somatic lineage. Work in our laboratory is focused on unravelling the molecular controls of male germ line development and plant sperm cell development and function. We have obtained novel insights into the molecular basis of male germ-line initiation and male gamete development in flowering plants. Transcriptional repression of male germ-line genes in somatic cells has been identified as a key mechanism for spatial and temporal control of male germ-line development. We are now building on this discovery to understand the mechanism of transcriptional repression and the discovery of novel gene products particularly transcription factors associated with plant reproductive development.

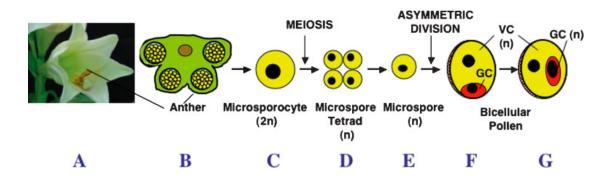


Figure: Male germline development. A: Lily flower. B: Developing anthers showing microsporocytes. C: Meiosis resulting in the formation of a tetrad of haploid microspores (D). E: A microspore. F. Early bicellular pollen formation after asymmetric division. VC, vegetative cell; GC, generative cell. G. Mature pollen (Source: Singh and Bhalla, BioEssays 29, 1124-1132)

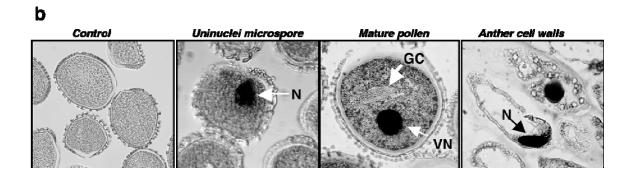


Figure: Immuno-detection and nuclear localization of a repressor, GRSF in the nuclei of uninucleate microspores (N) and the vegetative-cell nucleus (VN) of mature bi-cellular pollen while no such localization was observed in generative cell (GC) nucleus. Anther-wall cells also exhibit nuclear localization (N) of GRSF (Source: Haerizadeh, Singh, Bhalla, Science 313, 496 – 499)

Unravelling gene networks controlling stem cell identity in Legume Shoot Apical Meristem

Legumes, such as pea, chickpea, lupin and soybean, are of fundamental importance for agricultural systems providing sustainable pasture production and cereal rotation capabilities together with high quality products such as vegetable oils, protein and nutriceuticals (anti-oxidants, phytoestrogens and folate).

Plant shoot apical meristem contains pools of undifferentiated stem cells that are responsible for the continuous formation of all above-ground organs such as stems, leaves, and flowers. However, our understanding of the shoot apical meristem at the molecular level is limited. Legume plants such as soybean and pea have unique aspects of plant development. Our aim is to understand molecular control of legume meristem differentiation. Our approach is to use high-resolution transcriptomics to unravel complex gene regulatory mechanisms that control dynamic cell organization and architecture of shoot apical meristem in legumes. Our study unravelled transcriptional features distinguishing the shoot apical meristem and non-meristematic plant tissue and showed that plant meristems display a unique transcriptional profile highlighting gene programs with roles in shoot apical meristem generates of organ polarity. We also identified a gene that could potentially serve as a novel marker for differentiating cellular domains in the meristem. Further, our data also showed that plant meristem possess shared 'molecular signatures' in apical and axillary meristems; hence providing a rich source of novel target genes for further investigations into a fundamental process that impacts plant growth and productivity.



Figure: Pea seedling and shoot apical meristem (Source: Liang et al 2009, Journal of Experimental Botany, Vol. 60, No. 14, pp. 4201–4213)

MicroRNAs in shoot apical meristem of soybean

The small RNA-based silencing system has revolutionized our understanding of gene regulatory pathways. Plant microRNAs (miRNAs) play crucial regulatory roles in various developmental processes. We have characterized the miRNA profile of the shoot apical meristem of an important legume crop, soybean, by integrating high-throughput sequencing data with miRNA microarray analysis with the objective of discovering miRNAs that potentially play important roles in regulating the two functions of vegetative shoot apical meristem: maintenance of pluripotent stem cells and the initiation of leaf primordia. Our study led to the identification of 32 conserved miRNAs and 8 novel miRNAs. The target genes for conserved miRNAs expressed in the shoot apical meristem include those with roles in the establishment of organ polarity and organ boundaries, as well as roles in the biogenesis of miRNAs. This project was performed in collaboration with A/Prof Bernie Carroll, (UQ) and Dr Xiujie Wang, Institute of Genetics and Developmental Biology, The Chinese Academy of Sciences, Beijing. Work is now underway to investigate the role of the novel miRNAs identified during our study in shoot apical meristem maintenance and function.



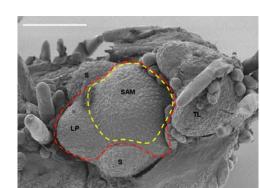


Figure: Soybean plants and scanning EM of a soybean shoot apex from a 10 day old soybean plant. LP, leaf primordial; TL, trifoliolate leaflet; S, stipule. Scale bar 100 um (Source Wong et al 2011 Journal of Experimental Botany 62(8): 2495-2506.

Floral Transition in soybean

Soybean is a major crop representing over 55% of annual oilseed production worldwide. Though flowering is central to seed production, molecular control of this vital process in soybean remains to be determined. The translation of knowledge gained from the model plant Arabidopsis to corresponding processes in legume crop plants remains a challenge due to unique vegetative and floral developmental complexities of legume plants.

Floral transition is described as a shift of the shoot apical meristem from leaf production to the initiation of a floral meristem. In order to uncover gene networks involved during this process we are using a system level approach. Study on gene networks associated with the floral transition in soybean, and their comparison with the existing knowledge in the model plant Arabidopsis, will allow for the identification of evolutionarily conserved processes controlling the floral transition, and identification of the processes that have undergone independent variation and selection during 92 million years of divergent speciation as both Arabidopsis and soybean have diverged from a common ancestor. Furthermore, the availability of individual soybean genotypes that show variability in the photoperiod (and/or temperature) stimulus requirements for the initiation of flowering offers a good system to dissect floral transition pathways in crop legume plants.

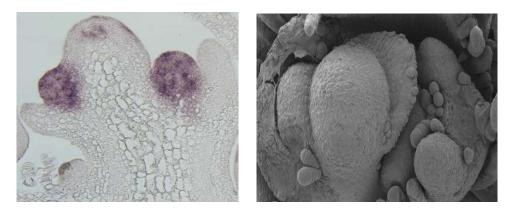


Figure: Flowering soybean shoot apical meristem (Source: Wong, Singh, Bhalla The Plant Journal 57, 832-845 (2009))

The University of Melbourne Department of Genetics

Professor Chris Cobbett (Email: ccobbett@unimelb.edu.au)



Heavy metal homeostasis and detoxification in plants

Our work has been focused on the use of *Arabidopsis thaliana* as a model to investigate heavy metal homeostasis and detoxification in plants. Through this we have identified the zinc-transporting HMA ATPases as essential for root-to-shoot zinc translocation and have genetically characterized the biosynthesis of phytochelatins, the major detoxification mechanism for a range of heavy metals including cadmium. Arising out of this work has been our work on the tripeptide glutathione (g-glutamyl-cysteinyl-glycine; GSH) which is the substrate for phytochelatin biosynthesis.

GSH has a broad spectrum of functions in plants. In addition to heavy metal detoxification it is important in other stress responses, is a key element of the intracellular redox buffer that protects cells from

oxidation and has roles in development, particularly via effects on the action of auxin.

In Arabidopsis GSH is synthesized in two enzymatic steps, first by GSH1, found exclusively in plastids, forming the pathway intermediate γ -glutamylcysteine (γ -EC) and then by GSH2 which is located in both plastids and cytosol suggesting that GSH biosynthesis can occur in both compartments. In Arabidopsis GSH1 and GSH2 are encoded by single-copy nuclear genes. Our work has been focused on the characterization of mutants in this pathway, on the requirement for compartment-specific expression of the enzymes and on the recent identification of plastid GSH transporters.

Mutations in GSH1 have variable effects ranging from cadmium sensitivity (the *cad2* mutant), to failure of root growth (*rml1* mutant), to late embryonic lethality (*gsh1* null mutant). *gsh2* null mutations cause early seedling lethality. With the availability of Arabidopsis *gsh1* and *gsh2* null mutants we have manipulated the pathway of GSH biosynthesis by introducing compartment-specific GSH biosynthetic enzymes. Redirecting GSH1 activity or GSH biosynthesis exclusively to the cytosol by complementing the Arabidopsis *gsh1* and *gsh2* mutants with *E. coli* GSHA and GSHB, respectively, had no significant impact on phenotypes or stress resistance. Also, restricting GSH biosynthesis within the plastid is sufficient for normal plant development. These observations suggest efficient exchange of γ -EC and GSH between the plastid and cytosol compartments.

We have recently identified three genes, *CLT1*, *CLT2*, and *CLT3* encoding plastid-localised transporters that transport GSH or γ -EC from the plastid to the cytosol (Maughan et al, 2010). Interestingly, the CLTs are similar in sequence to the major chloroquine resistance gene of the malaria parasite, *P. falciparum*. A *clt1clt2clt3* triple mutant is GSH-deficient and, like *cad2*, is cadmium sensitive. In collaboration with Prof Andreas Meyer (University of Bonn) we have been using the thiol reactive agent, monochlorobimane (MCB), which forms a fluorescent conjugate with GSH, to visualize GSH *in situ*. This is shown in Figure 1 where various GSH-deficient mutants are stained with MCB. In addition, our collaboration has used a redox-sensitive derivative of GFP, roGFP2, to measure the levels of GSH in different sub-cellular compartments. For example, in the *clt1clt2clt3* mutant roGFP2 is more oxidized when in the cytosol indicating a low level of cytosolic GSH while in the plastid there is no difference from the wildtype (Maughan et al, 2010). We are currently using these mutants to examine further effects on gene expression and development.

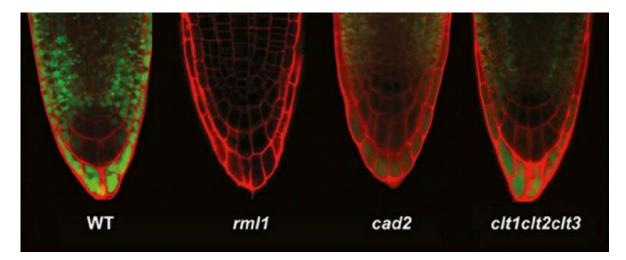


Figure 1. Monochlorobimane staining of GSH in roots of wildtype and various GSH-deficient mutants of Arabidopsis (acknowledgement from Maughan et al, 2010)

Maughan SC, Pasternak M, Cairns N, Kiddle G, Brach T, Jarvis R, Haas F, Nieuwland J, Lim B, Müller C, Salcedo-Sora E, Kruse C, Orsel M, Hell R, Miller AJ, Bray P, Foyer CH, Murray JAH, Meyer AJ, Cobbett CS (2010) Plant homologs of the *Plasmodium falciparum* chloroquine-resistance transporter, *Pf*CRT, are required for glutathione homeostasis and stress responses. Proceedings National Academy Sciences USA, 107: 2331-2336.

The University of Melbourne Department of Genetics

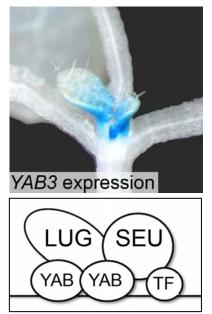
Dr John Golz (Email: jgolz@unimelb.edu.au)



John Golz heads the plant development group in the Genetics Department at the University of Melbourne. Since coming to Melbourne University in 2006, his group has been studying the genetic basis of patterning in the model plant *Arabidopsis*. In particular, the group is interested in understanding how the YABBY (YAB) class of transcriptional regulators promotes cell identity specification during leaf development.

Leaves of higher plants typically display an asymmetric distribution of cell types along their dorsoventral axis, with those specialized for light-capture being present in the upper part of the leaf blade and those for gaseous exchange located more ventrally. Differences are also apparent between leaf surfaces, with the lower epidermis tending to have smaller and in some cases more irregular-shaped

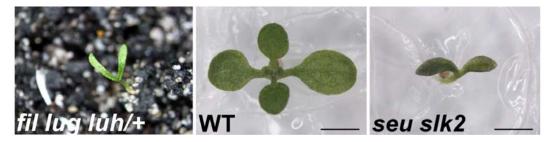
cells than those of the upper epidermis, as well as having a greater density of stomata. Classical observations have suggested that dorsoventral patterning is established during the early stages of leaf development. Molecular genetic studies have since confirmed this by showing that several families of transcription factors involved in dorsoventral patterning are expressed in either dorsal or ventral domains of the leaf primordium as it emerges on the flank of the shoot apical meristem [1]. Characterization of the YABs in Arabidopsis and Antirrhinum has shown that they promote ventral cell identity [2, 3]. Indeed this function appears conserved in a range of other dicotyledonous plants and may well be the ancestral function of this gene family [4]. Intriguingly, the Golz group has also shown that YABs promote dorsal (topside) cell identity, a function that is only apparent when multiple yab mutants are combined [2, 5]. Another surprising YAB function is their role in promoting shoot apical meristem (SAM) activity. YABs are expressed in the bottom side of leaves (see opposite panel; [3, 6]) yet their activity extends into the dorsal domain of the leaf and



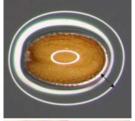
adjacent shoot. This implies that YABs function non-cell-autonomously, possibly acting through novel signaling pathways [2, 5, 7]. More recently the Golz lab have shown that YABs physically interact with two related transcriptional co-repressors (LEUNIG (LUG) and LEUNIG-HOMOLOG (LUH)) and their associated co-regulators (SEUSS (SEU) and SEU-LIKE1-3 (SLK1-3)) [5]. Thus YABs are part of a large regulatory complex (see opposite panel) that promotes leaf patterning and SAM activity. Current studies are aimed at understanding the role of YABs within the complex and identifying the components of the signaling pathway that are regulated by this complex.

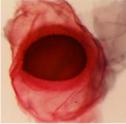
In addition, the Golz group has now turned its attention to embryonic patterning. The basis for this interest stems from the observation that when mutations in the YAB gene FILAMENTOUS FLOWER (FIL) are combined with co-repressor mutants, the resulting seedlings initially lack a functional SAM (see figure next page). As this defect occurs during embryogenesis, it implies that the LUG regulatory complex controls an embryonic patterning process required for SAM formation. The Golz group subsequently confirmed this by showing that combined mutations in two co-regulators

(SEU and SLK2) leads to a complete loss of embryonic SAM formation (see figure below). Furthermore, as the complex is presumably localized to the developing embryonic leaves (cotyledons) through its interactions with YABs, the role in SAM formation presumably reflects the activity of yet another signaling pathway.



One surprising aspect of the group's work has been the recent discovery that the co-repressors regulate a polysaccharide biosynthesis pathway in the developing seed [8]. In myxospermous species such as







Arabidopsis, cells in the outer layer of the seed coat (testa) produce and secrete large quantities of pectinaceous mucilage into a region between the radial and outer tangential cell walls. Here it remains trapped as the testa dehydrates during seed maturation. When the mature seeds are next exposed to water following dispersal, the mucilage swells rapidly and ruptures the wall of the dead testa cells. Following its release, the mucilage envelops the seed in a gel-like capsule (upper panel), which is thought to protect the seed from dehydration, aid soil adhesion and provide a nutritive environment for the developing seedling.

Mucilage is composed of the acidic polysaccharide pectin, which appears as a red halo surrounding the seed when stained with the dye ruthenium red (see middle panel). However a similar treatment applied to *lub* mutant seeds produces no halo, indicating a failure to release mucilage (lower panel). Working in collaboration with Prof Tony Bacic's group in the School of Botany at University of Melbourne, members of the Golz group showed that this defect was associated with changes in the mucilage hydration properties brought about by an increase in the number of galactose residues attached to pectin. This change in pectin structure was caused by decrease expression of the β -galactosidase *MUM2* in the *lub* seed coat, indicating that LUH is a positive regulator of *MUM2*. This study raises the intriguing possibility that some of the patterning defects observed in co-repressor mutants may be caused by modifications of the cell wall, an exciting possibility that the group is now exploring.

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Professor John L. Bowman (Email: john.bowman@monash.edu)



Our laboratory studies pattern formation in plants:

How does a single cell develop into a multicellular organism with specific tissue and organ patterns?

We are studying pattern formation in land plants, which represent one of the several independent evolutions of multicellular organisms. We are using *Arabidopsis*, a diminutive flowering plant and *Marchantia polymorpha*, a liverwort and member of the bwsal lineage of land plants. Both plants are models for studying many aspects of plant biology as they are amenable to genetic and genomics approaches.

In *Arabidopsis*, we are particularly interested in the genetic control of pattern formation, focusing on the roles of three families of transcription factors. The first, Class III HD-Zip genes are required for the proper development of the shoot apical meristem, from which all above ground plant organs are derived, the top sides of leaves, and the central portion of vascular bundles, the xylem. The second, the KANADI gene family, has a complementary role in that these genes are required for the proper differentiation of the bottom parts of leaves and the peripheral portion of the vascular bundles, the phloem. Members of the first two gene families are found in all land plants suggesting they are part of an ancient genetic system directing pattern formation along the central-peripheral axis of plants. The third gene family, the YABBY gene family, is found only in seed plants and plays an important role in the development of leaves. In the long term, the ability to manipulate the size and shape of leaves and the production of vasculature could lead to increased productivity of many crop and tree species.

We are also investigating gene function in *Marchantia* as an approach to elucidating the molecular basis of morphological evolution in land plants. The origin of land plants was one of the most important events in the earth's evolutionary history, allowing metazoans to colonize land. Land plants evolved from a freshwater charophycean green algal ancestor and that ancestor likely possessed certain developmental features that were inherited by land plants and are shared with extant charophytes. In particular, land plants share with the Charales growth by an apical cell and retention of the zygote. However, the origin and diversification of embryophytes involved dramatic evolutionary changes in life history and body plan that allowed for more complex forms. Key features associated with the retained zygote), three dimensional tissue patterning and differentiation in both haploid and diploid phases, sporophytic shoot apical meristem (SAM) with the capacity for branching, lignified vascular tissues; production of lateral organs from the SAM, and the origin of roots. We use both genetic and genomic approaches to elucidate the evolutionary history of genes and to illuminate the ancestral functions of genes, to hypothesize a general view of how genes evolve and become co-opted to pattern novel organs.

From Our New PhDs

Characterisation of cyanogenic glucoside synthesis in Eucalyptus

Elizabeth H. Neilson

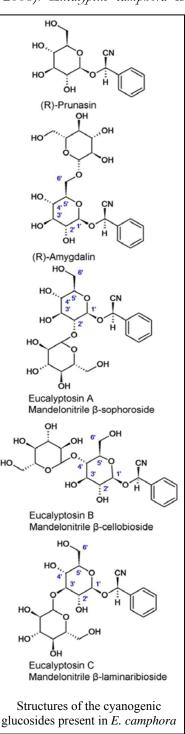
Cyanogenic glucosides are defensive metabolites, releasing toxic cyanide following tissue disruption and contact with catabolic enzymes. Approximately 4% of *Eucalyptus* species are cyanogenic, possessing the phenylalanine-derived cyanogenic glucoside prunasin (Gleadow et al., 2008). *Eucalyptus camphora* is

unique as it possesses multiple foliar cyanogenic glucosides. In addition to prunasin, *E. camphora* possesses the cyanogenic diglucoside amygdalin and further unknown cyanogens (Neilson et al., 2006). Furthermore, *E. camphora* displays unique ontogenetic influence over cyanogenic glycoside synthesis. Six-month old *E. camphora* saplings are significantly lower in cyanogenic glycoside concentration than their adult counterparts, with some saplings acyanogenic. The presence of acyanogenic saplings is noteworthy as no acyanogenic adults were identified in population screens; including the mother trees (Neilson et al., 2006). This suggests that the initiation of cyanogenic glycoside synthesis is ontogenetically controlled, so even though synthesis is delayed, all *E. camphora* saplings are hypothesised to produce cyanogenic glycosides by reproductive maturity.

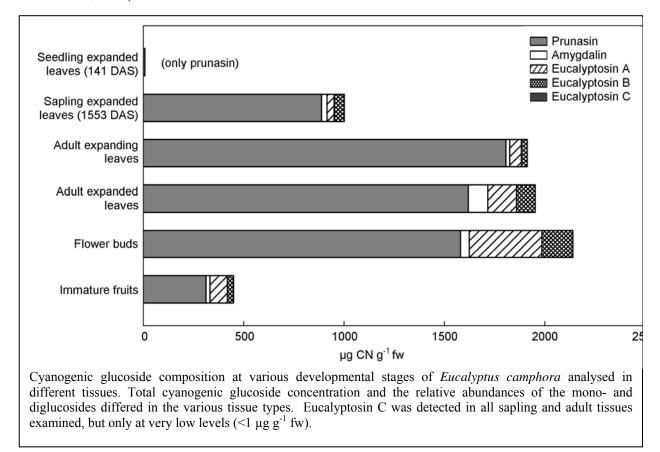
The overall aim of my PhD research was to investigate how cyanogenic glucoside synthesis is controlled throughout *Eucalyptus* ontogeny. In particular I aimed to identify the unknown cyanogenic glucosides in *E. camphora* and examine the cyanogenic glucoside profile throughout plant ontogeny. In addition, I investigated how prunasin synthesis was regulated in a related species, *E. yarraensis* (synthesises only prunasin), by identifying the first step in the biosynthetic pathway and by measuring changes in mRNA expression levels throughout ontogeny.

Using different analytical methods (LC-MS/MS, GC-MS and NMR) the unknown cyanogenic glycosides were found to be three phenylalanine-derived cyanogenic diglucosides characterised by unique linkage positions between the two glucose moieties. This is the first time that multiple cyanogenic diglucosides have been shown to co-occur in any plant species. In addition, two of these cyanogenic glucosides have not previously been reported and were named eucalyptosin B and eucalyptosin C. Interestingly, the different cyanogenic diglycosides could be distinguished by unique MS/MS fragmentation patterns. This knowledge should facilitate analysis and structural elucidation of cyanogenic diglucosides from other plant species, especially when they are present in low abundance.

Quantitative and qualitative differences in total cyanogenic glucoside content were observed across different stages of whole plant and tissue ontogeny, as well as within different tissue types. Seedlings of *E. camphora* produce only the cyanogenic monoglucoside prunasin, but "switch on" prunasin biosynthesis at different times. Once initiated,



total cyanogenic glucoside concentration increases throughout plant ontogeny with cyanogenic diglucoside production initiated in saplings and reaching a maximum in flower buds of adult trees. The role of multiple cyanogenic glucosides in *E. camphora* is unknown, but may include enhanced plant defense, nitrogen storage and transport, pollinator attraction and/or a role in seedling germination (see Neilson et al., 2011).



To further investigate how cyanogenic glucoside synthesis was regulated, I aimed to identify the first step of prunasin synthesis. Using various PCR techniques, four cytochrome P-450 genes encoding CYP79 enzymes were detected and isolated from *E. yarraensis*: CYP79A34, CYP79A35, CYP79A36 and CYP79A37. The CYP79A34 gene was functionally cloned and expressed in *Saccharomyces cerevisiae* using the USER TM cloning technique and a modified pYeDP60 vector, respectively. The recombinantly expressed CYP79A34 was shown to catalyze the conversion of L- phenylalanine into phenylacetaldoxime, verifying its involvement in the first step of prunasin biosynthesis. The cytochrome P-450 shows narrow substrate specificity, as the structurally related amino acid – L-tyrosine – was not metabolised. This is the first demonstration of a member of the CYP79 family catalyzing the conversion of L- phenylalanine into a phenylacetaldoxime in cyanogenic glucoside biosynthesis.

Using quantitative real-time PCR, CYP79A34 mRNA levels were found to be positively correlated with an increase in prunasin concentration during *E. yarraensis* ontogeny, suggesting that prunasin synthesis is regulated at the transcript level. Interestingly, CYP79A34 transcript levels significantly increased in leaves subject to cold temperatures and nitrogen application. Overall, my PhD uncovered many novel aspects of cyanogenic glucoside synthesis in *E. camphora* and *E. yarraensis*, particularly in terms of ontogenetic regulation. Cyanogenic *Eucalyptus* species are emerging to be an excellent experimental system to study cyanogenesis as a polymorphic trait (see Møller, 2010) and my PhD research provides a solid platform to further investigate these species and their unique control over cyanogenic glucoside synthesis.

My PhD research was undertaken with Prof. Ian Woodrow and Dr Jason Goodger in the Plant Physiology Laboratory, School of Botany, The University of Melbourne. In addition, I had the privilege of spending several months with Prof. Birger Møller and his lab at Copenhagen University, Denmark. My work in Denmark was generously funded by the Holsworth Wildlife Research Fund and the ARC-NZ Research Network for Vegetation Function. My future endeavours will continue in the field of cyanogenic plants, working in collaboration with Monash University and the University of Copenhagen on cyanogenic *Sorghum bicolor*.

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Visiting the Copenhagen port with Birger Møller, Denmark

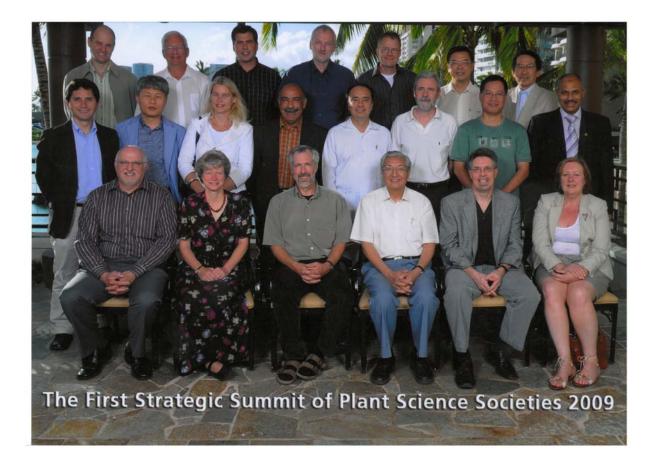
Our recently completed PhDs who are the future of plant science and our society are encouraged to provide highlights of the research that earned them their new degree. This article is an excellent example of the achievements of our young plant scientists. Supervisors please encourage your graduating PhDs to contribute to this section of Phytogen.

Tina Offler

Global Plant Council Report

The Global Plant Council Forges Ahead

On June 28 – 29 this year, the Global Plant Council (GPC) met in the beautiful city of Qingdao on the coast of eastern <u>Shandong province</u> in <u>China</u>. The GPC meeting was generously hosted by the Chinese Society of Plant Biology (CSPB) and expertly and smoothly organized by Professor Zuhua He, the Secretary General of CSPB, and his team of capable assistants. Fourteen of the 20 GPC member societies were represented at the meeting, either by serving presidents or by the society's chosen representative.



The main focus of the meeting was to identify and discuss global challenges that human society is facing and for which a concerted action is needed from plant scientists around the world. The goal was to develop focused topic areas and a deployment strategy that would allow GPC to move forward into active participation in the global debates that can be informed and impacted by the work and talents of the plant science community: world hunger, human health and well-being, climate change, energy and biomaterials, and sustainability and environmental protection. During the meeting, we decided that the best way forward was to generate two-page proposals to hold workshops on key issues related to global challenges and to seek funding for them. These workshops would bring together plant scientists, breeders and other specialists from all over the globe with the necessary expertise to generate a road map as to how plant science can address, mitigate, or offer solutions for the issues that GPC plans to address.

The Council identified nine key issues that GPC feels must be discussed and facilitated in the global plant community in greater depth. These nine key issues, in order of perceived priority for GPC action, are:

1. Digital Seed Bank – to provide a stable perpetuation of crop genetic diversity for future generations, initially focusing on those crops that provide most of the calories for the world food supply, by creating a digital database of genome sequences, phenotypic information, expression data, proteome and metabolome assessments etc., that would be open to all breeders and scientists and would supplement physical long-term seed storage.

2. Local-level Diversity and Yield Stability – to provide a means by which breeding efforts targeted to specific environmental conditions, utilizing local germplasm that is being characterized around the world, can be facilitated and enhanced in the public sector.

3. Increasing/Enriching Agricultural Diversity - to promote the deployment of underutilized seed and root crops and cropping systems that might have nutritional and environmental benefits, as well as a return to the farmers that are growing them.

4. Biofortification - to advocate for development of new and existing crops that are more nutritious so that people receive the daily-required nutrients directly from unprocessed foods. Articulate what can be accomplished by conventional breeding and what might require alternative approaches and advocate for open sharing of data and information regarding biofortification efforts.

5. The Plant Environment Metagenome – to facilitate our understanding of the "whole plant" with a view toward crop improvement and sustainability. The "whole plant" includes not only the plant itself, but the entire microecology of interacting microorganisms within and upon its surfaces, both within an agricultural cropping system and in a natural environment.

6. Development of Medicinal Plant-based Products – to advocate ethnobotanical and natural product research and development of useful plant compounds for human health, as well as to establish the means by which new products can be efficiently tested and brought to market.

7. Species Information for Sustainable Adaptation Capability to Climate Change - to explore/develop an approach toward facilitating natural and managed ecosystem adaptation (or ameliorating the effects resulting from) to changes in climate that are already taking place and to integrate existing plant interaction information into an ecosystem perspective for development of effective and predictive models.

8. Developing Perennial Rice/Wheat/Maize - to promote a vision where possible for the conversion of current mega-crops to perennial forms to stabilize land use and inputs and to promote sustainability along with yield maintenance.

9. Sharing Information and Resources – to develop a position statement for facilitating the global free exchange of information, phenotype and genotype data, and resources (including germplasm) that are in the public domain for approval by the GPC membership.

As you can imagine, each one of these topics and their perceived priority generated much spirited discussion within the group present at the meeting. GPC will now solicit input from plant scientists among all of the represented societies both during the development of the proposals and also as participants in the workshops once they are funded. Each member society will be asked to provide suggestions as to whom amongst their membership can provide expert and relevant input to these activities. GPC will also identify experts from other disciplines as needed for input and active participation to assure that GPC proposals and workshops will provide the best informed advice to the scientific community, breeders and agricultural stakeholders, policy makers and global organizations.

In addition to the development of these focused issue statements and action items, each member society reported on their own activities within the purview of the GPC mission. The list of activities is substantial, and the exchange opened up areas in which societies may choose to collaborate and maximise the impact of their individual programs. This is something that GPC will continue to promote and facilitate as a distinct value to our member societies.

GPC is well along in the process of registering as a not-for-profit organization, with our registration being implemented in Switzerland as a neutral base of operation. Our website, <u>www.globalplantcouncil.org</u>, is up and running and we will continue to improve on its content and its utility. Suggestions are always welcome and should be directed to the Executive Director. We will also continue to offer membership to plant science societies and other interested agricultural organizations around the world who have not yet signed up with GPC, and an active recruitment effort is underway.

GPC member the European Plant Science Organisation (EPSO) has kindly volunteered to host the next Annual Meeting of the Global Plant Council in Freiburg, Germany either before or after the joint EPSO/FESPB meeting that runs from July 29th to August 4th, 2012

I hope that this report on our activities will offer some insight into what the GPC is about, but should you require any further information please visit our website or feel free to contact me.

Mel Oliver Executive Director Global Plant Council olivermj@missouri.edu

Science Meets Parliament 2011

Prem Bhalla and John Rathjen attended this year's Science Meets Parliament 2011 event held from 20 - 21 June 2011. This event provides an opportunity to the Australian science community to understand how scientists can be involved in the political process and learn how to effectively communicate science to politicians.

Day 1 of the program focused on sharpening the communication skills of scientists. The highlight of the day was the dinner speech given by The Hon John Brumby who highlighted the Australian discoveries and the importance of science in our daily lives. During the evening, the "Respect the Science" campaign was also launched. The campaign website is: <u>www.respectthescience.org.au</u> By clicking on 'what makes science so credible' it is possible to watch and download the Respect the Science presentation.

Science Meets Parliament 2011 is organised by the Federation of Australian Scientific and Technological Societies and its new name, "Science & Technology Australia" was also launched during the conference dinner. The S&TA new website is www.sta.org.au.

Day 2 of the event focused on meetings with parliamentarians, a speech by Professor Margaret Sheil, CEO of ARC on ERA process and a meeting with Senator the Hon Kim Carr. We also had an opportunity to meet members of the Greens. The highlight of this day was the address of Professor Ian Chubb, the Chief Scientist for Australia, during lunch at the National Press Club. Scientists also experienced the drama of Question Time at the parliament!

In brief, our involvement helped us gain a better understanding of the political process, how policy is developed, and sharpened our communication skills with the press. We recommend this event to all scientists.

Prem Bhalla

Conference Report:

International Botanical Congress, Melbourne, Australia



Generic comments and summaries of selected plenary lectures and symposia

MELBOURNE AUSTRALIA I 23-30 JULY 2011

Generic Comments

"A conference of 2000 odd delegates with topics ranging from global climate change, food security, genomics and evolution to plant systematics and international agreement on the taxonomy of Acacias presents unique challenges to those of us with a general interest in plant biology! Finding unifying themes across such diverse topics is obviously not easy but across a number of plenary presentations and symposia, photosynthesis and agricultural crop productivity received considerable attention" ------ **Bob Furbank** (CSIRO, Plant Industry, Canberra).

"The conference was in a fantastic venue by the river close to the centre of Melbourne. It ran smoothly from most aspects, thanks to all organisers but especially to Anna Koltunow. Some innovative experiments by the hired congress management were clearly still in an experimental phase. The rapid-fire poster sessions consisting of 5-minute poster presentations were poorly attended and could have benefited from broadcast to TV monitors outside the auditoriums. The on-line posters were difficult to access at the venue and it was even harder to discuss with presenters. Should this be repeated there would need to be a huge number of monitors (e.g., 1 per 4 posters) and session times to meet presenters" *----- Christine Beveridge (University of Queensland)*

"The plant cell wall community was well represented at the IBC conference. Many of us enjoyed the great diversity of subjects covered over the course of the conference with the juxtaposition of plant biotechnology with conservation and taxonomy providing a particularly interesting dynamic. The Congress Gala-Gardens of the World must also surely rank as one of the most visually sumptuous conference dinners and was thoroughly enjoyed by all attendees" ------ Rachel Burton (University of Adelaide).

Plenary Lectures and Symposia

IBC2011: Photosynthesis and Global Food Security

Bob Furbank (CSIRO, Plant Industry, Canberra)

The challenge we face in feeding 10 billion people by the year 2050 was introduced by David Fischoff of Monsanto and broadened by Ken Cassman to include auditing yield potential across the globe. The importance of photosynthesis in the "yield equation" of crops under both water limited and high yielding environments was the focus of a presentation by Richard Richards who indicated that we had to substantially increase photosynthesis in wheat to improve yields. Jeff Amthor elaborated on this theme with a review of strategies to improve photosynthesis in crop plants.

There could not have been a better introduction to the session I was co-organising with Rowan Sage "Novel approaches to engineering C4 photosynthesis into C3 crops". In fact, Jeff Amthor virtually invited his audience to come and have a friendly heckle of the ambitious approach of C4 engineering! This session served as a report to the plant science community on progress in the Bill and Melinda Gates Foundation funded C4 Rice Consortium. This consortium represents a \$12M project, now in its third year, to provide proof of concept for the introduction of the C4 pathway into rice by building a "parts list" of essential genes and a toolbox of these genes responsible for C4 anatomy and biochemistry. Rowan Sage introduced the session by outlining the scope of the challenge of engineering C4 photosynthesis into C3 crops, using evolution to guide our efforts. He showed a rather poignant slide comparing the cost of one F18 fighter jet to the cost of the project over 20 years (the jet cost Paul Quick, coordinator of the consortium reported on the large body of work at the more!). International Rice Research Institute where mutants in vein spacing, believed to be a crucial step in C4 evolution, and mutants in C4 function have been isolated and partially characterised in sorghum and rice. The work required to generate a million or more mutants, screen for vein spacing phenotypes then back cross and analyse physiological phenotypes is prodigious. Erik Murchie from Nottingham described the challenges of characterising vein spacing mutants in rice and achieving stable phenotypes. The generation of gene constructs to install the biochemical elements of C4 photosynthesis in transgenic rice was covered by Sarah Covshoff of Cambridge University who indicated that virtually all of the genes encoding the key enzymes and many of the transporters had been cloned and transformed. Susanne von Caemmerer and I both focussed on which parts of the "Ferrari of Photosynthesis" are essential for the pathway to function. It appears that lignification of the bundle sheath compartment may be crossed off the essential list as only small effects on photosynthesis were observed in low lignin mutants of sorghum. In maize husk, which has widely spaced veins akin to a C3 leaf, C4 photosynthesis also appear to function, reducing fears that rice mesophyll cell numbers would need to be reduced from 10 to 2 between vascular bundles in order to achieve any kind of C4 function. Richard Leegood, from Sheffield, reminded us that many of the C4 photosynthetic enzymes are expressed in rice and their function is far from clear in C3 plants. Xinguang Zhu from Shanghai showed us that systems biology is essential to guide our engineering efforts and understand the implications on metabolism and photosynthetic performance of the genetic modifications we have made in rice.

There is no doubt that this multinational consortium faces a great challenge but given the mild nature of the heckling and the rate of progress so far, perhaps there are grounds to be a little optimistic of the chances of success.

IBC2011: Plant Cell Walls at the IBC

Rachel Burton (School of Agriculture, Food and Wine, University of Adelaide)

The plant cell wall symposium took place on Friday with Chris Somerville presenting one of the morning's two plenaries entitled "Cellulose synthesis". Even though cellulose is such an abundant biomolecule Chris pointed out that there are still many fundamental features of its synthesis and form to be elucidated; how many microfibrils wind together to make a cellulosic strand- 30, 36 or 24? What does a functional complex look like and how come some subunits and not others are interchangeable? How do these complexes stick to the microtubules? He then described an approach that he referred to as an "underdeveloped area in plant science" that entails screening a battery of drugs to disrupt the synthesis process, allowing it to be teased apart. A compound that has successfully provided illumination is morlin. This drug shortens the microtubules and leaves them flapping in the cytoplasmic stream, tethered at one end only, whilst along their length the cellulose synthase complexes have ground to a halt. Such observations have been complemented by the identification of the first new complex component in ten years, the cellulose synthase interacting protein that binds to tubulin. Here too CESA complexes move more slowly along the microtubules in plants where this gene is mutated providing strong evidence that complex propulsion requires some type of physical anchoring to, and

guidance along, the microtubules. In a further twist, some phosphorylation mutants of *cesa1* can also slow complex movement, but this can depend on which side of the microtubule the complex is tracking along. It seems that the sides of each microtubule are not the same and that phosphorylation of the CESA proteins may be required to provide a better fit. In summing up, Chris suggested that the cellulose synthase complex may be akin to a ribosome, the complexity of which will take many years and some clever biology to clearly define.

The "Biosynthesis of plant cell walls" symposium, sponsored by the new ARC Centre of Excellence in Plant Cell Walls took place in the afternoon of the same day. Charlie Anderson led off by describing the uptake of fucose labelled by click chemistry methods via the salvage pathway in plants. The fucose becomes integrated into a range of polysaccharides and Charlie described the distribution patterns in root sections after a pulse of labelled fucose had been given. This approach revealed fibres containing labelled fucose oriented in a longitudinal pattern similar to cellulose microfibrils. Since other sugars can be labelled using the click chemistry method this promises to provide an elegant way to follow the synthesis of a range of cell wall polysacchardies in living cells. Ken Keegstra next described progress made in defining xyloglucan and glucomannan biosynthesis. Some of the genes involved in this process have been defined, as either a backbone or a sidechain player. The focus is now to begin exploring the regulation of these genes and the behaviour of the proteins they encode. The enzymes involved in the biosynthesis of both these polysaccharides are targeted to the Golgi where the number of transmembrane domains they possess dictates their orientation. A difference of one will place the active site either "outwards" for synthesis to occur in the cytoplasm or "inwards" where the action takes place in the lumen. These differences in orientation were confirmed by expressing epitope-tagged versions of the proteins in yeast followed by protease treatment to remove the tags, or not, according to whether they poke out into the cytoplasm or the lumen. This clearly highlighted the differing orientation between members of the CslC and CslA families and given the large number of other cellulose synthase-like genes present in plants, such an approach will be an excellent way of defining their location and orientation. Yihua Zhou presented a summary of the many brittle culm mutants that have been identified in rice. Although these mutants share a common feature in their brittleness the lesions they carry are in a bewildering array of genes, emphasising yet again what a complicated business it is to build a cell wall. We went from bc1, a cobra-like gene, to bc3 a gene involved in membrane trafficking, through to *bc14*, a putative glycosyltransferase containing DUF266 and *bc15*, a chitinase-like protein. This last mutant is a new addition to the collection that, although it displays brittleness, actually has an abnormally thickened wall, with less cellulose and more lignin. Such mutant collections will surely provide invaluable information about monocot wall biosynthesis whilst equally well giving clues about wall deconstruction, a key goal in the quest to make use of plant biomass in the biofuels arena.

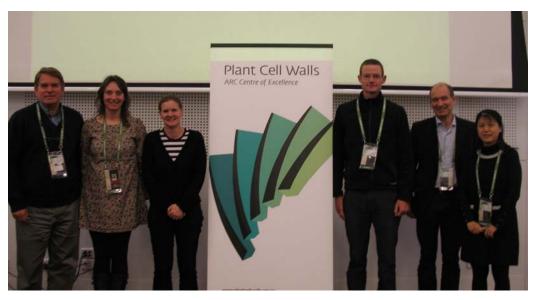
The final three talks were focused on (1,3;1,4)- β -glucan. Monika Doblin did a great job of introducing this polysaccharide and explaining why we are so interested in it, particularly from a human health perspective. Although it is a linear polymer with no side chains, we still understand very little about how and where it is assembled. In a bid to develop tools to help pin down the location of the proteins Monika described the development of a set of antibodies specific to either CSLF or CSLH. These will be instrumental in tracing these proteins in a range of cereals where their use will need to be combined with orientation studies such as those described by Ken. In the next presentation I returned to the rice theme and presented our exploration of the amount, distribution and regulation of (1,3;1,4)- β -glucan synthesis in this plant. Rice grain contains only very low levels of this polysaccharide, as confirmed by TEM and Megazyme analysis, which seems to be counterintuitive when the size of the Cs/F family is taken into account. However, there is now evidence that the transcript of the key Cs/F gene, which appears to be the main player in the cereals we have examined so far, is subject to post-translational effects in the form of miss-splicing. This appears to be triggered by the presence of an antisense transcript, evidence of which we have now also found in other cereals with equally low levels of (1,3;1,4)- β -glucan. Geoff Fincher finished the session with a thorough overview of all the genetic factors that must be considered in the study of these polysaccharide synthase families. These range from the clues that published genome sequences can provide in the clustering of these genes, which may sometimes correlate to quantitative trait loci, through to the power of using large germplasm

collections for association mapping. The reasons for the arrangement of these genes in clusters still remains obscure but by monitoring their transcription under conditions of stress or pathogen attack we may glean some valuable insights.



Staff from the ARC Centre of Excellence in Plant Cell Walls enjoying the Melbourne sunshine on the steps of the Convention Centre.

L to R; Sie Chuong Wong, Riksfardini Ermawar, Helen Collins, Rachel Burton, George Dimitroff, Monika Doblin and Alan Little.



Speakers in the Plant Cell Wall Symposium. L to R; Ken Keegstra, Monika Doblin, Rachel Burton, Charles Anderson, Geoff Fincher and Yihua Zhou.

IBC2011: Cellular Dynamics -- Symposium 061

Chair: David McCurdy (University of Newcastle); Co-Chair: Chris Staiger (Purdue University)

The advent of fluorescence-based imaging and advanced microscopy techniques has enabled real-time visualization and quantification of plant cellular dynamics in unprecedented detail. This Symposium brought together six outstanding researchers to present their latest findings in this field.

Prof Hiroo Fukuda (University of Tokyo) described his recent discovery of MIDD1, a novel membrane-associated MAP regulating patterns of secondary wall deposition in metaxylem vessels. MIDD1 is a microtubule (MT)-end tracking protein that promotes local MT disassembly resulting in pits on walls of metaxylem vessel cells. Depletion of MIDD1 by RNAi or overexpression caused formation of secondary walls without pits, and reduced MT density, respectively. MIDD1 therefore integrates spatial information in the plasma membrane with cortical MT dynamics to determine xylem cell wall patterning.

Prof Chris Staiger (Purdue U) described detailed tracking of the amazingly rapid dynamics of the cortical actin cytoskeleton using variable-angle epifluorescence microscopy. Single actin filaments grow at rates of 1.7 um/sec, display incessant remodelling featuring filament buckling and straightening events, and are depolymerised by prominent severing activity involving ADF4. Mutants defective in myosin XI also show disrupted cortical actin dynamics. Surprisingly, actin dynamics were essentially unchanged in elongating versus elongated cells, leading to the proposition that the energy intensive rapid remodelling of cortical actin may be a survey mechanism enabling rapid cellular signalling in response to stress.

Dr Haruko Ueda (Kyoto University) presented her studies identifying myosin XI-K, a member of the plant-specific myosin class XI, as the primary motor protein regulating ER dynamics in plant cells and involved in regulating ER organization as well as the orientation of actin filament bundles. A model was proposed whereby dynamic three-way interactions between ER, F-actin and myosins determine the architecture and movement patterns of the ER strands, and cause "cytosol hauling" traditionally defined as cytoplasmic streaming.

Prof Zhenbiao Yang (UC, Riverside) talked about his recent studies published in *Cell* detailing the role of auxin signalling in coordinating cytoskeletal organization and endomembrane trafficking required for cellular interdigitating in epidermal pavement cells of Arabidopsis. Auxin is a signal that activates pavement cell polarization to form lobes and coordinates lobe formation with indentation formation by activating ROP GTPase signalling through an Auxin-Binding Protein 1 (ABP1)-dependent and cell surface-based auxin perception system. This new auxin signalling mechanism regulates the interdigitated cell pattern through modulation of PIN1 localization to lobe tips, possibly affecting PIN1 localization by affecting PIN1 endocytosis and recycling. Actin was proposed to play a role in inhibiting PIN1-specific endocytosis.

Prof Haiyun Ren (Beijing Normal University) talked about her recent work describing an Arabidopsis formin (AtFH14) that tracks microtubule dynamics and is involved in cell division. AtFH14 expressed in BY-2 cells decorated MT-specific arrays such as the preprophase band, spindle and phragmoplast, in addition to inducing the co-alignment of MTs with actin filaments. Knockdown of AtFH14 indicated that these interactions were required for normal organization of the mitotic apparatus. Expression of truncated domains of AtFH14 revealed the somewhat surprising discovery that both the Pten and FH1FH2 domains were required for localization with these MT arrays. AtFH14 therefore appears to be a unique plant formin that tracks MT dynamics.

Dr Michael Sheahan (University of Newcastle) presented his latest findings on the role of specific actin isoforms in chloroplast positioning. Induction of cell division through culture in a number of species causes chloroplasts to cluster around the nucleus in an actin-dependent manner, ensuring unbiased chloroplast inheritance. Chloroplast repositioning is, however, defective in Arabidopsis plants carrying mutations in the vegetative actin *ACT*7 but not in plants with mutations in the other

vegetative actins, *ACT2* or *ACT8*. Intriguingly, the actin cytoskeleton appeared normal in *act7* mutants and overexpression of *ACT2* or *ACT8* under *ACT7* regulatory sequences suppressed the *act7* phenotype. However, analysis of actin transcript and protein levels in vegetative *act* mutants revealed no compensation by the non-mutated vegetative actins, even though total actin levels increased as culture proceeded, indicating the possible involvement of reproductive actins in chloroplast re-positioning.

Apart from the success of the Symposium itself, hopefully a highlight for the visiting international scientists was our day trip on the Wednesday to the 12 Apostles on the Great Ocean Road. The scenery was magnificent and only matched by the beautiful Victorian weather that day!

David McCurdy - Newcastle



Group photo after the Symposium. (L-R) Haruko Ueda, Hiroo Fukuda, Chris Staiger, Michael Sheahan, Haiyun Ren, Zhenbiao Yang, David McCurdy

IBC2011: Plant Modelling and New Signalling Molecules

Christine Beveridge (University of Queensland)

Plenary speaker Professor Przemyslaw Prusinkiewicz, as usual, gave a dynamic and enjoyable presentation on plant modelling. He presented published work on how auxin transport controls phyllotaxis, through to unpublished work showing how just a few simple rules can provide the full range of dynamic tree structures seen in the environment today. All this along with beautiful visualisations made an attractive topic of conversation and appreciation.

A common theme of the Keynote Symposium on new signals (KNS03), which covered the new plant hormone, strigolactone (Christine Beveridge), peptides (Yoshikatsu Matsubayashi) and FT/florigen (Markus Schmid) was that plant hormones should be considered as a larger set of signalling molecules including the classical chemical structures as well as signalling peptides and proteins. A related symposium (SYM153) showed how strigolactones, introduced again by Koichi Yoneyama, have likely affected plant development and signalling at least as early as moss (Catherine Rameau) and involve signal transduction cross-talk with the structurally related karrikins which are derived from smoke and which are also ancient compounds (Steve Smith). Adding more complexity to signalling, Helen Irving presented new data suggesting hormone cross-talk may occur even within a single receptor protein.

IBC2011: Plenary Lecture (Chanyarat Paungfoo-Lonhienne)

Professor Tetsuya Higashiyama (Nagoya University)

Susanne Schmidt (University of Queensland)

Live cell analysis of plant fertilisation

A quest of plant biologists over 140 years has ended: Prof. Higashiyama's research has discovered how pollen tubes are guided. His group identified the chemical attractant of pollen growth, speciesspecific polypeptides named LUREs that are related to defensin-like proteins. His talk was a highlight at the IBC, presenting a series of elegant experiments that unravelled the mechanisms of angiosperm fertilisation and novel imaging techniques.

Studies of pollen tube guidance and target ovules have a long history. In 1860, pollen tube growth was observed in vitro but the chemo-attractant derived from the ovule was only successfully identified in 2009 by Prof. Higashiyama's group. *Torenia fournieri, a* unique plant species with protruding embryo sac enables in vitro studies, and he showed a fabulous movie of pollen tube attraction by the embryo cell; fascinating because pollen tubes keep growing towards the ovule, which is micro-manipulated away from the pollen tube.

Using laser cell ablation, the group unequivocally demonstrated that two synergid cells adjacent to the egg cell attract the pollen tubes, and that one synergid cell was sufficient to generate a diffusible signal required for attraction. As the attraction signal is species-specific, it was hypothesised that the attractant molecules rapidly evolve. Performing EST analysis of synergid cells from the protruding embryo sac of *T. fournieri*, cysteine-rich small peptides (CRPs) genes were most abundantly expressed. The study focussed on the three largest CRP contigs (TfCRP1-3). Among them, TfCRP1 and 3 were expressed specifically in the synergid cells and are related to defensin-like peptides.

The activity of these molecules in attracting pollen tubes was determined by using recombinant CRPs. When TfCRP3 was injected in front of a pollen tube, the tube turned sharply towards the micropipette. To quantify the activity of pollen tube attraction, they coated gelatin beads with the peptides and placed them in front of pollen tubes. TfCRP3 showed significant activity to attract pollen tubes depending on concentration. Both TfCRP1 and 3 showed the ability to attract pollen tubes and are species-specific. Their role in pollen tube attraction was confirmed by the decrease of frequency of attraction when injecting antisense oligomers in to protruding embryo sac cells in order to down regulate LUREs.

The entire double fertilisation process was imaged with a live-cell imaging system and an Arabidopsis marker line for sperm cell nuclei. He showed us the amazing images of the process which undoubtedly proved that the two sperm cells at the front and back of the male gametophyte unit are equal in their function of fertilisation to the two female gametes. Finally, he presented the new Live Imaging Centre of Nagoya University. An example of the facility is a two-photon microscopy for *in vivo* imaging which can provide 3D construction from time-lapse imaging, a very useful tool for deciphering processes in living tissues.

Conference Report:

2nd International Conference on Plant Metabolism

Over 400 delegates from all continents attended the 2^{nd} International Conference on Plant *Metabolism* (2nd ICPM) held from 30th of June to 3rd of July, 2011 in Qingdao, a beautiful coast city by the Yellow Sea in Eastern China. The meeting was themed as *Plant Metabolism and Modern Agriculture* and sponsored by the Chinese Academy of Sciences (CAS). The organising committee invited about 40 leading scientists in the area to give plenary presentations and selected some short talks from submitted abstracts. Oral presentations were blended with an exhibition of about 200 posters throughout the four-day sessions. The program covered a wide range of topics including primary and secondary metabolism, metabolic signalling and gene regulation, biotic and abiotic stresses, biotechnology and crop productivity and human nutrition. Below are a few snapshots of the scientific 'show':

The meeting was kicked off by Wilhelm Gruissem (ETH, Zurich) and Xiao-Ya Chen (CAS), who, respectively, discussed modelling Arabidopsis metabolism for crop improvement such as increasing vitamin A in rice and engineering gossypol biosynthesis in cotton for improving pest resistance. Lothar Willmitzer and Alisdair Fernie (Max Plank Institute) presented new tools and ideas in metabolomics using isotopes and networking for diagnostics and system biology, while Daniel Kliebenstein (UC, Davis) devoted his talk to natural variation in plant metabolism and genetics.

Regulation of development and plant-microbe interaction through carbon and nitrogen flux and signalling was another focus of the meeting. Here, Yong-Ling Ruan (Uni Newcastle) reported progress towards unravelling sugar-mediated molecular pathways responsible for early fruit and seed development (or their abortion) and Wolf Frommer (Carnegie, Stanford) described the isolation of a novel glucose effluxer that is induced by pathogen infection. Yi-Fang Tsay (Academia Sinica, Taipei) showed that a previously characterized nitrate transporter also functions independently as a nitrate sensor. Peter Gresshoff (Uni Queensland) dissected molecular components regulating nodulation in legumes.

For secondary metabolism, Barry Pogson (ANU) outlined effort in identifying regulatory proteins controlling carotenoid biosynthesis in Arabidposis and wheat. This was synergized with talks given by Cathie Martin (John Innes Centre) and Harry Klee (Uni Florida) on engineering phenylpropanoid for healthy food and identifying volatiles for better flavour in tomato, respectively. Cell wall biology and modification was also highlighted at the meeting. To this end, Richard Dixon (Noble Foundation) described new strategies to reduce lignin content for biofuel production; Laigeng Li (CAS) reported that genes involving in lignin biosynthesis also play roles in reproductive development and Vincent Bulone (KTH Biotech) provided an update on mechanisms underlying cellulose biosynthesis. Linking metabolism with cellular signalling, Sheng Luan (UC Berkeley) illustrated how a Ca⁺⁺ sensor, (CBL) regulates global scale signal transduction through interaction with protein kinases (CIPK).

The scientific program was followed by a post-conference tour to two magnificent cultural and tourist destinations: QiFu, Confucius's home town, and Tai Mountain, one of the five holy mountains in Buddhism. The meeting was hailed as a great success by most attendees. To capture the exciting

advances highlighted at the meeting, it has been decided that a cohort of selected presentations will be published as full papers in *MOLECULAR PLANT*, a new journal published by OXFORD PRESS with current IF 4.3, ranked in the top 8% among 187 plant science journals. The 3rd ICPM will be held in 2014 at a site yet to be announced.

Yong-Ling Ruan



ComBio 2011 Cairns

Cairns Convention Centre 25 - 29 September 2011

Enticement to Attend and Participate

Dear ASPS members,

The preparations for ComBio in Cairns from 25-29 September are nearly completed and it is shaping up to be a great meeting in surroundings conducive for some relaxed but robust discussion.

- The full program is now available and can be downloaded from: <u>http://www.asbmb.org.au/combio2011/program.html</u>.
 - The final timetable is also available and can be found at: <u>http://www.asbmb.org.au/combio2011/timetable.html</u>.

The program is diverse enough to be of interest to all members and so *if you haven't registered and want to then please do so.*

As an added incentive, on-site posters will be allowed.

As well as many plenary speakers there are 11 symposia and an additional session showcasing younger scientists directed towards plant science as well as the numerous other sessions many of which will appeal to ASPS members.

In addition to the scientific program, there is the Society dinner this year to be held at Adelfia on Tuesday the 27th. It is a Greek restaurant and there are plans beyond eating. I will forward more details to those registered soon.

So see you in Cairns for both a stimulating and social conference in a few weeks time.

Best Wishes Graham Bonnett

Education in Plant Science

The Good, the Bad and the Ugly: a hitchhikers guide to botanical teaching websites

John Harper & Geoff Burrows

School of Agricultural & Wine Sciences, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678

As teachers of first year Botany we try to use our own images and short videos where possible or surf Google when we need to quickly find a picture or additional information to illustrate some point.

We were thinking that it would be good if *Phytogen* readers could share their favourite teaching sites. What follows are a few that we have found with some comments on each. We have also looked at apps. While searches of the iTunes store using 'botany' or 'plant' find lots of apps we have found that there's little to date that can be integrated into university teaching. 'Leafsnap', 'Plant Histology' and 'Virtual Cell' are worth a look. 'Plants versus Zombies' although a popular game may mean future botany students have strange expectations!

As mentioned in past issues of *Phytogen*, we have created some web resources, designed to help our on-campus and our Distance Education students grasp some tricky concepts and to inspire them to learn more about plants. See the leaf morphology, gynoecium, floral symmetry, virtual floral formula and plant family recognition applications.

http://www.csu.edu.au/faculty/science/herbarium/index.htm

We will leave you to decide whether these resources are G, B or U!

Aussie educator

http://www.aussieeducator.org.au/tertiary/subjects/botany.html#tbt

This is a web portal we didn't know about until we were putting together this article. It looks like a good starting point when looking for teaching resources, not only for Botany!

Plants in Action

http://plantsinaction.science.uq.edu.au/edition1/?q=content/home-page

A free on-line resource from our own society! Well worth a look. Perhaps we should have a collection of our best teaching links on the ASPS website?

Khan Academy

Our Botany students told us about Sal Khan last year. He was working as a Hedge Fund analyst but started tutoring his cousin, Nadia, in mathematics in 2004 over the internet. Other relatives and friends asked for his help and he eventually set up a not-for-profit organisation *Khan Academy* (Bill Gates is a sponsor) teaching on-line with over 2400 free videos on Youtube. His photosynthesis lectures are great. No fancy Powerpoint slides – just an online 'black board' and 'digital chalk'. A couple of years

ago we started to go back to using the whiteboard in classes with lots of Q&A (with the Powerpoint resources/files as the backup to the live lecturing). Yes, the students love it!

http://en.wikipedia.org/wiki/Salman_Khan_(educator) http://www.khanacademy.org

Sydney Uni biology learning resources

http://sydney.edu.au/science/biology/learning/

There are a number of different pages here covering e.g. plant anatomy and plant pathology. Some beautiful images and on-line questions to help students learn.

Plants in Motion

http://plantsinmotion.bio.indiana.edu/

This is a great site. Students are amazed to see plants 'strut their stuff' and it transforms student perceptions of plants.

Virtual Cell

http://vcell.ndsu.edu/animations/photosynthesis/movie-flash.htm

Some quality animations from a group at North Dakota State University.

We were disappointed though that the first animation we looked at (on photosynthesis) only talked about ATP production and the names of the electron acceptors and missed so much of this important process.

Botanical Society of America

http://www.botany.org/plantimages/ http://www.botany.org/outreach/ http://www.botany.org/outreach/Broader_Impacts_Resources.pdf

Some beautiful plant images for downloading and outreach programs to encourage school students to think seriously about Botany careers and the public to appreciate plants.

American Society of Plant Biologists

http://my.aspb.org/?page=Education

Plant Cell

http://www.plantcell.org/site/teachingtools/teaching.xhtml

Some really nice general Powerpoints and a lot of information on plant hormones! Worth keeping an eye on this website.

http://www.youtube.com/user/PlantTeachingTools

YouTube portal for botany teaching movies from Plant Cell.

Virtual Crops

http://www-plb.ucdavis.edu/labs/Rost/Virtual%20crops.htm

Produced by Undergraduate UC Davis students. We have used the Virtual grape site for our Wine and Viticulture students and GB has contributed some images to it.

The Roots of Plant Intelligence

http://www.ted.com/talks/lang/eng/stefano mancuso the roots of plant intelligence.html

Always interesting things from the TED network. This one is good for starting a discussion, if not a fight!

Update on:

Functional Plant Biology

Editor-in-Chief: Dr Rana Munns Assistant Editor: Dr Yvonne Cheng

Functional Plant Biology is healthy and successful, and has recovered from the low impact factor for 2009. The impact factor for 2011 is running at about 2.8. This is due to the high quality of papers accepted, despite the fact that we cannot pay authors for them. Impact factors for most journals come from reviews and special issues, but FPB is a not-for-profit journal, run by CSIRO Publishing on behalf of the Australian Academy of Science, and cannot fund authors to travel to conferences etc.

FPB is finding its niche in the environmental biology arena, and on cross-discipline studies that integrate across different levels of organisation such as cellular and whole plant.

Special issues provide interest for the journal, and research fronts act to highlight specific areas and draw attention to papers published as a group that may not get the same recognition if published separately. A special issue on Actinorhizal plants has just been published, and papers focusing on biotechnology for breeding beans (*Phaseolus vulgaris*) for abiotic stress will come soon. Next year there will be three special issues:

plant phenomics crops for a changing climate from genome to phenome in cereals.

Editor-in-Chief, Rana Munns

Update on:

Plants in Action

As announced last September, the first edition of the plant science text book, '*Plants in Action*', published in 1999 by the Australian Society of Plant Scientists, along with the New Zealand counterparts, is now on-line and free. http://plantsinaction.science.uq.edu.au/edition1/

Open access web resources are transforming education, and *Plants in Action* is the first Plant Science textbook contributing to this unrestricted sharing of scientific knowledge. It is attracting about 500 hits a day, most from Australia, but many from India, UK and USA. We hope to increase the usage in developing countries with the new edition.

The major sponsor is the Australian Centre for International Agricultural Research (ACIAR), along with the University of Western Australia, University of Queensland, and University of Western Sydney.

The revised second edition of *Plants in Action* will also be an open access publication. The overall structure of the first edition will remain. Additional chapters on pathogen resistance mechanisms and emerging issues of global resources and climate change will complement the *Plants in Action*2.

Each chapter has an editor, who is inviting co-authors to write different sections. Authorships of each chapter will be attributed. An editorial assistant (Jen Price) is providing help in downloading text and images from the first edition, and formatting text for the revised edition. An IT expert will place the text and the images on the web. A password will apply until authors are happy with the final chapter, which will then be lifted so it becomes open access. There are some funds available for a graphic artist to draw complex diagrams.

The home page for the new edition is: <u>http://plantsinaction.science.uq.edu.au/</u>

We anticipate that most chapters will be completed by December 2011 and will be available for open access then.

Rana Munns



The Plant Accelerator® - a facility for Australian plant scientists

Over the last decade, biological science has been dominated by DNA sequencing and the advent of genomics, transcriptomics, and a host of other "Omics" technologies. While these developments have revolutionised many fields of research, sheer volume of sequence data alone cannot replace observation of real biological outcomes in the form of phenotyping; rather than removing the bottleneck of gene discovery, it has just moved it downstream to the characterisation of the physical effects of those genes.

This new bottleneck, combined with a desire to develop tools to dissect genetically and phenotypically complex traits into (hopefully!) simpler ones, was the motivation behind the construction of The Plant Accelerator at the Waite campus of the University of Adelaide. This state-of-the-art plant growth and analysis facility, established with the support of NCRIS, the South Australian government and the University of Adelaide, was officially opened for business last year, and is already attracting interest from researchers and companies around the world.

This international interest notwithstanding, the main role of The Plant Accelerator remains to provide cutting-edge phenotyping resources to our core customers - Australian plant researchers. Along with our partner institute, the High Resolution Plant Phenomics Centre in Canberra, The Plant Accelerator is a dedicated service facility focussed on providing high-quality research infrastructure. The services that we can provide include full consultation on project design, development of pilot trials, full staffing support throughout the project and customised image processing; we also make no claim on any IP developed during a project. As a NCRIS facility, this service is available to Australian publicly-funded researchers at marginal cost only, but is also open to commercial organisations and academic institutions worldwide.

The core of The Plant Accelerator's phenotyping capacity, which is built around the LemnaTec Scanalyzer 3D platform, consists of four SmarthousesTM; these are fully climate-controlled greenhouses equipped with computer-controlled conveyor belts carrying up to 600 plants per room. Plants are carried on this conveyor system in individual carts, each labelled with an electronic tag (an RFID chip) for full traceability of that plant and the data associated with it throughout the course of an experiment. RFID identification also allows single-plant level control of watering and nutrient supplementation. As well as managing plant movement and tracking, the conveyor system allows for plant locations to be rotated throughout an experiment, thus reducing possible positional effects.

Each Smarthouse is linked to one of two imaging halls, within which are five imaging chambers. Cameras in these chambers record images of plants in a range of different wavelengths, from far infrared through to UV-fluorescence, providing a diversity of phenotype information. Visible cameras quantify overall plant morphology, size, colour, shoot mass and other physical characteristics, near infra-red cameras detect water content of the leaves and soil, far infra-red provides information about leaf temperature and transpiration rate, and UV lighting detects chlorophyll and GFP fluorescence. The throughput of each of these imaging halls is sufficient to record data from all the plants in one Smarthouse in a single day, providing a total capacity in the Accelerator for up to 2,400 plants to be phenotyped three times each week.







Top left: Plants in one of the four Smarthouses, showing the conveyor belt system and plant carts Top right: Maize plants passing through the imaging stations Middle left: Maize plants in the Smarthouse Bottom left: One of the 34 conventional glasshouses



for subsequent release of those seeds from quarantine.

Additional facilities at The Plant Accelerator include high-specification standard greenhouses, growth rooms and chambers, and laboratory space, all of which are publicly available for hire. Over half of the plant growth area of the Accelerator operates as PC2 research space, and several of the greenhouses and two Smarthouses are quarantine approved areas, allowing us to carry out projects with both transgenic and imported material. Included in our quarantine permit is the capacity to grow plants to seed set State of the art facilities are all very well, and there is no doubt that The Plant Accelerator can justifiably lay claim to being a world-leading centre. However, the true value of research infrastructure is through the science that it enables, and here too The Plant Accelerator offers many benefits. Primary among these is throughput; with the capacity to record dozens of parameters on up to 1,200 plants per day, it enables the identification of much rarer events than can be studied without automation technology. Moreover, because the measurement is fully objective and the images are taken under very controlled conditions, a high degree of accuracy and reproducibility are achieved in combination with this throughput.

A further major benefit of the Accelerator is the elimination of inter-plant biological variation achieved by substituting imaging for destructive testing in a time course experiment. When combined with the accuracy and reproducibility mentioned above, this can result in greater than 80% reduction in measurement standard deviation, and as a result, far fewer plants may be needed to detect a subtle phenotype than would be the case with conventional approaches. In fact, such phenotypes may only be measurable with a facility such as The Plant Accelerator, with several projects already having achieved new, exciting, and often unexpected insights into plant growth responses.

Finally, by measuring plants at repeated one or two day intervals, responses of those plants to stimuli, be it salt exposure, chemical treatment or nutrient supplementation, can often be separated into individual components for more detailed analysis. For example, work on salt stress has shown that The Plant Accelerator can differentiate between osmotic tolerance (a rapid response to the changed osmotic potential of the soil after salt exposure) and the longer-term sodium exclusion or tissue tolerance phenotypes (Rajendran et al, 2009); similarly, we are able to distinguish between different drought response mechanisms such as escape and dehydration avoidance (Berger et al, 2010). As a result of this phenotypic dissection of these types of complex traits, genetic characterisation of the components contributing to them now becomes feasible.

Of course, the benefits of The Plant Accelerator are not limited to its physical resources and capabilities. In fact, we hope that the true value of carrying out experiments with the Accelerator comes from the experience and knowledge of our staff, and the contributions that they can make to a project. We have a broad cross-disciplinary range of skills, spanning plant breeding, genetics, horticulture, bioinformatics and genomics and, as one of the first and largest facilities in the world running the LemnaTec Scanalyzer 3D phenotyping system, have developed a detailed knowledge of this system and its capabilities. Our team are keen to actively contribute not only to the running of a project, but also its inception and design; we are even happy to help with the preparation of grant applications for projects to be run at the Accelerator.

Phenotyping at The Plant Accelerator is, with the exception of weighing, entirely imaging based. Initially this may seem a limiting factor, but because of the range of wavelengths used and the advanced image processing of the resulting images, many aspects of the plant can be accurately inferred from these data; in principle, it is possible to use imaging to study any physical or biochemical change in the plant that is correlated with a difference in the reflectance or absorbance across any of the measured light spectra. A case in point is the consistent and reliable predictor of plant biomass from imaged leaf area, as described in a paper that we published earlier this year (Golzarian et al, 2011). Further calibration of the system for a wide range of commercially and experimentally valuable species is currently underway, and other experiments are also in progress to evaluate image-based testing approaches for traits as diverse as root water uptake efficiency to chlorophyll content and health. The Plant Accelerator team are always happy to discuss possible ways to infer any other phenotypic trait from imaging data, and to look at pilot projects to evaluate the success of these options.

Being a centre with a strong focus on agriculturally important crop species, an essential extension to this 'inference by imaging' approach is to confirm that phenotypes observed under controlled growth conditions translate into measurable changes under field conditions. While this need is equally true for greenhouses anywhere as it is for The Plant Accelerator, the throughput and resolution of projects in the Accelerator mean that, once it is validated, it may be possible to replace years of field experimentation with a few months of detailed and environmentally-controlled phenotyping. Such validation is the subject of a major project to grow a range of wheat lines in both the Accelerator and field trials, and to correlate the controlled-environment phenotyping results with commercially important features such as yield. Unfortunately though, the nature of such a project necessitates that it too requires several years of field data, so watch this space for progress!

Despite being based around a commercial-installed phenotyping platform, The Plant Accelerator is still, and intends to remain, a work in progress. Our team includes systems development, bioinformatics and mechatronics experts, and we are always looking to improve existing services and develop new ones. An example of this is our recent success in obtaining ARC Linkage grant funding, with the Australian Centre for Visual Technologies and LemnaTec (the commercial provider of the phenotyping system) to further develop the image analysis capabilities of the system. However, it is crucial to our ability to fully and successfully meet our customers' needs that we obtain input on future directions for The Plant Accelerator from our main intended users: the Australian plant research community. We are actively seeking this feedback, and would encourage any readers who would like to provide comments, or even who just have questions about the Accelerator, to please get in touch using the contact details below.

The Plant Accelerator provides an exciting new facility for Australian researchers; its facilities are currently unrivalled in the world outside of major multinational corporations, it is located in the Waite Campus, the Southern hemisphere's largest plant science research centre, and it is openly available to all researchers. We encourage you to talk to us about how we can support your research, and look forward to working with you on your next phenotyping project.

Mark Crowe Scientific Marketing Manager, The Plant Accelerator (<u>mark.crowe@plantaccelerator.org.au</u> / 0434 331588 / http://www.plantaccelerator.org.au)

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Were you aware that....?

- **ASPS Website.** The ASPS website has been thoroughly revamped and is being continuously upgraded.
 - Membership dues can now be paid on line.
 - You can advertise jobs, PhD scholarships, conferences, books by contacting Kiran Sarfaraz via advertise@asps.org.au. To cover the costs involved, the society has introduced a small charge of \$30 for members and \$70 for non-members FOR EMPLOYMENT ADS ONLY. Advertising conferences and books (edited by society members or containing chapters written by society members) are FREE OF CHARGE.
- **RN Robertson Travelling Fellowship.** This named Fellowship recognises and celebrates the sustained contribution made by RN Robertson (Sir Bob) in nurturing young plant scientists in Australia spanning across four decades from the 1950's. The Australian Society of Plant Scientists is indebted to Hank Greenway and Joe Wiskich who generated and championed the early development of the RN Roberston Travelling Fellowship.
- **Student Travel Funds.** Funds are set aside each year to sponsor student travel to our annual conference (2011 ComBio, Cairns), and contribute to their professional development in plant science. Support will vary from year to year depending on the Society finances, location of meeting and number of applications. The Treasurer will apply a formula in calculating individual entitlements and takes these factors into account. Applicants must be financial members of ASPS and presenting a paper or poster at the ComBio meeting.
- **4 Postgraduate Section.** We are proud to announce that student members who have recently completed their PhD and had their thesis passed can submit a summary that features in Phytogen. Members of the Council feel that this is an important opportunity for our postgraduate students to showcase their research. Such successful student members are advised that the summary can be accompanied by a key image in suitable format and that they should submit their items to the editor of Phytogen at any time for inclusion in the next issue.
- Society Funding for Workshops and Conferences. The society has a total of \$10,000 available each year to provide seeding money and sponsorship for up to four conferences organised by members. The amount available to assist each conference will be about \$2,500. For more details see the website: <u>http://www.asps.org.au</u> and take the link to conferences.
- Corresponding and Life Memberships. Life Membership recognises an outstanding and sustained contribution to the Society by a long-standing ASPS member who, through their professional activities, has substantially enhanced the international profile of Australian plant science research. Corresponding Members are high profile overseas colleagues who have contributed substantially to plant science research within Australia. If you know of a deserving recipient for Life or Corresponding Membership, please consider putting a nomination forward. The procedure to follow is outlined on the ASPS website (see: http://www.asps.org.au and click on "About ASPS" where there is also a list of Life and Corresponding members).