

## Plant phenome to genome: a mini-review

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**Abstract.** Rapid advances in biotechnologies have provided a template for defining the genome–transcriptome–proteome in many plant species and these advances now highlight a particular challenge to link the molecular biology–based studies to changes in the phenome of plant species. Selected examples are provided to review advances in defining environment–phenotype interactions, the genome–transcriptome–proteome in plants and translating research outputs more broadly to society. The specific examples include computer modelling of plant phenotypes and responses to environmental signals, advances in small molecule signal transduction, visualising macromolecules and defining the complex genomes that are important to society. The need to translate research outputs to society more broadly is also discussed.

### Introduction

Advances in biotechnologies have provided a template for defining the genome–transcriptome–proteome in many plant species and these advances now highlight a particular challenge to link the molecular biology–based studies to changes in the phenome of plant species. The matrix of data, representing the diversity of small molecules through to microscopic and normally visible features of organisms, describes the phenotype or appearance of an organism. The structure of this mini-review is based on the biotechnologies and concepts presented at the 18th International Botanical Congress, 2011 (Melbourne), which provided a basis for linking candidate genes and expression networks, to the overall phenome of an organism. This linkage is facilitated through accurately describing the interactions between genotype, environment and stage of development (Siebner *et al.* 2009; Casey *et al.* 2010) as they apply to plant species.

### Defining the phenotype

The overall phenology of plant species has been described by Else Friis and Mike Crisp, and traditionally, these studies are in the context of determining the evolutionary history of plant species (Crisp and Cook 2009; Friis *et al.* 2010; Crane *et al.* 2010). The detailed descriptions of floral structure and function, as required for making deductions about the evolution of angiosperms, are, however, also important in establishing an accurate description of the phenotype for molecular level studies. The scanning electron microscope images of early Cretaceous fossils indicated that the evolution of angiosperms was not as rapid as previously thought – these early fossils include representatives of monocots. In the context of floral structures, this observation would be consistent with the underpinning molecular networks being just as complex as those involved in any other phenotype, in contrast to a “rapid evolution” model that would suggest a change involving relatively few genes. The detailed phenology of plants is also considered within the trait ontology (TO) consortium. The continued increase in the volume and level of sophistication of

phenotypic information makes it particularly important to describe and classify the plants under study in the tradition carried out for evolutionary studies (Meier 2001; Jaiswal *et al.* 2002). The TO consortium addresses the database structure required to organise and integrate diverse sources of information efficiently, as well as maximising access across databases. The implementation of this philosophy for food crops in GRAMENE (Jaiswal *et al.* 2006) has focussed on the phenotypes captured as traits in quantitative trait loci (QTL) studies and in structured mapping populations, in order to facilitate the identification of the candidate genes underpinning the trait studied. The curation process for establishing a useful database involves systematically aligning QTL to (for example) the rice (*Oryza sativa* L.) genome sequence making use of the molecular markers (of known DNA sequence) originally used to locate the QTL. An essential process was to enter the very heterogeneous descriptions of traits mapped as QTL into code, using the TO consortium guidelines. The quality of the GRAMENE annotation provides a basis for interpreting trait data in crops that have not been as extensively studied as rice. Many features of processes in development are shared between crops (Meier 2001) and details of an aspect of plant phenology such as head development can be important in determining responses to environmental factors such as water stress and frost (Powell *et al.* 2012, this issue). Defining the underpinning molecular mechanisms is important for future approaches to breeding (Webster *et al.* 2012, this issue). The capacity to relate mutation and molecular studies of phenology in rice or *Arabidopsis thaliana* (L.) Heynh., to equivalent features in a complex polyploid such as wheat (*Triticum aestivum* L.) is proving to be extremely valuable in defining research strategies.

### Fundamental studies to define the environmental–phenotype interface

Insights into the overall phenology of plants have been gained by Przemyslaw Prusinkiewicz through computer modelling and

then testing predictions in model systems such as *A. thaliana* (reviewed in detail in Prusinkiewicz and Runions 2012). Prusinkiewicz has suggested that, based on computer modelling, it is evident that self-organisation can be seen at all levels of plant development and is, in fact, a fundamental link between molecular biology and the expression of a phenotype. The whole-of-system summary provided by Prusinkiewicz includes *ecosystem–branching structure–phyllotactic patterns–inhibitory mechanisms–small molecules or protein receptors–molecular changes*, and focuses on the concept that molecular level interactions provide the means but not the final outcome for determining the phenotype. At numerous points, the “normal” processes of cell growth, shape and organisation are modified by restrictions in space, the effects of gravity, polarity factors (Robinson *et al.* 2011) and a wide range of external factors. The diverse effects of a small molecule such as auxin were used to illustrate how important features of plant development can be reduced to relatively few, more general, mechanisms through computer modelling. The competition between subregions of tissues for auxin as well as the interactions of auxin with the auxin-transporter PINFORMED1 (PIN1) protein have been shown to be involved in creating the gradients of auxin, and computer modelling correctly accounted for the effects of modifying the auxin–PIN1 interactions that were carried out experimentally (Reinhardt *et al.* 2003; Bayer *et al.* 2009). The active transport, rather than passive diffusion, of auxin predominated over long distances (Kramer 2004). Refinement of the computer-modelling used further experimental evidence including the interaction of other auxin binding proteins such as ABP1 and LAX1, and mechanical stress, since these variables affected the localisation of PIN1 (Wabnik *et al.* 2010; Heisler *et al.* 2010). In roots, the distribution of PIN1 proteins matched the observed auxin flow and was related to the gravimetric response of root growth. Complementing the work by Prusinkiewicz, Christine Beveridge has described the suite of auxin, cytokinin and stigolactone (including GR24, a synthetic stigolactone) molecules that interact to modulate the growth of adventitious rooting under different environmental conditions (Rasmussen *et al.* 2012).

The environmental influences on plant phenotype, focussing on the effects of light, have been discussed by Peter Quail. The transcription factor family, which is an important early mediator of the effects of light, is comprised of phytochrome-interacting factors (PIFs), 487 amino acid proteins characterised by a G-box CACGTG binding domain and a photoactivated phyB binding domain. These protein entities inhibit the expression of genes involved in a range of plant activities including seed germination, seedling response to growth in darkness and shade-avoidance (Leivar and Quail 2011). Light (red and far-red) activated phytochrome molecules reverse the transcription repression activities of PIFs by inducing their phosphorylation and ubiquitylation, and rapid degradation through the ubiquitin signalling pathway. The PIFs were viewed as a centre point for the transfer of information related to the light or shade conditions in which the plant is located, and modification of the transcription of genes leading to changes in aspects of the phenotype to deal with the environmental conditions. Some of the fundamental aspects affected within the plant included GA synthesis, the circadian clock and response to high temperature.

## Environment–phenotype interface and yield improvement in wheat

Translating the research outputs from studies defining the environment–phenotype interface into improved yield of a major crop such as wheat is addressed by four papers in this issue of *Functional Plant Biology* (Powell *et al.* 2012; Parish *et al.* 2012; Webster *et al.* 2012; McIntyre *et al.* 2012). It is generally accepted that environmental changes will include higher temperatures (estimated to increase by 2°C by 2050), extended periods of water deficit and higher CO<sub>2</sub> levels (Powell *et al.* 2012), in addition to continued pressure from weeds, pests and diseases. The importance of small molecules such as ABA and sucrose for reproductive stage tolerance to a range of abiotic stresses was considered by Powell *et al.* (2012) in light of the need to establish fast screening procedures for the appropriate phenotype. It is evident that increased levels of ABA in developing panicles of rice as a result of water stress relate to the repression of the sucrose metabolism and the subsequent pollen sterility. Transgenic-based reduction in ABA levels in the anther was shown to improve tolerance to water stress (Ji *et al.* 2011). Later in grain development, the supply of sugars to the grain via mobilisation of stem carbohydrates has also been demonstrated to be important in tolerance to water stress. Key enzymes in the mobilisation of stem carbohydrate process in rice, maize (*Zea mays* L.) and wheat are the cell wall invertases and fructosyl exohydrolases (Yue *et al.* 2008; Zhang *et al.* 2009; Joudi *et al.* 2012). In addition to providing a carbon source to sensitive tissues such as developing pollen, sugars including glucose, fructose and sucrose are also considered to be signalling molecules in their own right (Moghaddam and Van den Ende 2012).

Variation among wheat varieties for water-soluble stem carbohydrates (WSCs) in the stem was addressed by McIntyre *et al.* (2012; this issue). WSCs are composed of oligosaccharides of  $\beta$ -2,1- or  $\beta$ -2,6-linked fructosyl residues using sucrose as a starting point. The genetic variation in the levels of WSCs that accumulate in the stem involved at least five chromosomal regions, with each explaining 6–7% of the variation in this phenotype. Preliminary studies analysing the genetic variation in the expression of a suite of candidate genes identified a region on chromosome 7A that accounted for 36% of the variation in *sucrose synthase5* (*SS5*) expression as well as variation in WSC content. Similarly, variation (30%) in the expression of *6-sucrose-fructosyl transferase* (*6-SFT*) was co-located with variation in WSC content on chromosome 6B. Not all variation in candidate gene expression was co-located with variation in WSC, as would be expected if a network-level set of interactions linked carbon and nitrogen metabolism, and moderated the phenotype of WSC content. As discussed by McIntyre *et al.* (2012), translation of these research outputs into breeding for the WSC content phenotype are most likely to involve targeted whole-genome analyses of segregating progeny in a breeding program.

Powell *et al.* (2012) also described the variation in levels of cell wall invertase in anthers as a significant variable in maintaining pollen fertility and hence grain set under water and frost stress conditions. Overall, Powell *et al.* (2012) argued that water and frost stress-responsive phenotypes in the reproductive phase of plants need to be carefully defined in order to achieve the

advances required to maintain grain-set under these stress conditions. The details of reproductive phase physiology and molecular biology, with a focus on pollen development, can be found in Parish *et al.* (2012; this issue). The tapetum is the tissue that is particularly sensitive to abiotic stress and is thus responsible for reductions in grain yield in a range of self-fertilising crops (Ji *et al.* 2011). The timing of programmed cell death (PCD) in tapetal cells is presented by Parish *et al.* (2012) as a critical phase in anther development and pollen viability because PCD provides the precursors for pollen exine, nutrients and callase enzyme to release microspores, as shown by mutation studies in rice (Li *et al.* 2006) and *A. thaliana*. The transition phase, as tapetal PCD starts and release of microspores into the locular fluid occurs, is illustrated in the figure on the front cover of this issue of *Functional Plant Biology*. The extensive studies in *A. thaliana* and rice summarised by Parish *et al.* (2012) describe that inhibiting the expression of transcription factors DYSFUNCTIONAL TAPETUM1, MYB33/MYB65, TAPETAL, DEVELOPMENT AND FUNCTION1, ABORTED MICROSPORES, AtMYB80 (controlling an aspartic protease named UNDEAD as a mutated locus), UDT1 (Undeveloped Tapetum) and TDR (Tapetal Degradation Retardation) generate a phenotype of tapetal vacuolisation and hypertrophy that resembles the phenotype resulting from cold stress in rice anthers. In rice, the cysteine protease gene *OsCPI* has been identified as an important factor in the PCD process. Consistent with tapetal cells being key variables in pollen development, the stage of young microspore development is when tapetal cells are very active metabolically. A blockage in the orderly progression of tapetal cell PCD through mutation, cold stress or water deficit stress disrupts the timely supply of pollen exine, nutrients and callase enzyme to the microspores.

Changes in the supply of sugars to the anther, especially to the tapetal cells, is generally accepted as a fundamental reason for loss of viability leading to male sterility. Key enzymes for converting the primary sugar, sucrose, to glucose and fructose are encoded by the invertase family of genes. As discussed in detail in Webster *et al.* (2012; this issue), the invertase genes comprise a complex gene family. Within the three broad families of genes encoding cell wall, vacuolar and cytoplasmic invertases (Tymowska-Lalanne and Kreis 1998), there exist isoforms. For wheat, Webster *et al.* (2012) characterised all the cell wall invertase genes in the genome that could be identified in the available genome sequence datasets. Genome-level sequencing of a 1.3 Mb DNA region of chromosome 3B provided the initial indication of complexity in the cell wall invertase (*IVR1*) gene family and this was expanded as survey sequences for each of the flow-sorted chromosome arms of wheat became available through the International Wheat Genome Sequencing Consortium (IWGSC). The genes identified were located to chromosome 3A, 3B (two copies of the gene), 4A, 5B and 5D. A sequence for the D-genome progenitor (*Aegilops tauschii*, Coss) *IVR1* gene matched the gene located on chromosome 5D, based on the survey sequences within the IWGSC. The significance of identifying a suite of closely related genes on different chromosomes (= isoforms) is that it reflects the highly buffered set of interactions that can occur in controlling the conversion of

sucrose to glucose and fructose. The cell wall invertases on the different chromosomes were predicted to be expressed to different levels depending on the levels of water availability. In terms of translating observations to breeding, the findings indicated that single nucleotide polymorphism (SNP) chips for whole-genome scanning must feature the entire suite of cell wall invertases if associations in the levels of expressions are to be made with differences in the phenotype of water stress tolerance.

### Small molecules and proteins in relation to microscopic phenotypes

The matrices of data representing the diversity of small molecules through to proteins, and linking these to phenotypes of organisms at the microscopic level, have been elaborated by Tatsya Higashiyama and Chris Somerville. The model system of the plant *Torenia fournieri* E. Fourn, which has a protruding embryo sac, was used to investigate the attraction of pollen tubes to the micropylar end of the embryo sac *in vitro* along a gradient of diffusible molecules (Higashiyama 2010). Similar attractants have been shown to exist in *A. thaliana*, tobacco (*Nicotinia tabacum* L., *Zea mays* L.) and maize, and comprise cysteine-rich peptides and proteins. Studies in *A. thaliana* suggested that additional communication signals were involved in coordinating the double fertilisation of two female gametes (Hamamura *et al.* 2011). Details of the peptides involved in signalling have been described by Yoshikatsu Matsubayashi and indicate that the peptides required to maintain the actively dividing state of cells in the growing tip of roots (Matsuzaki *et al.* 2010), as well as affecting development more generally (Komori *et al.* 2009), are often sulfanated at tyrosine residues. A diffusible molecule that relays flowering signals from leaves to the shoot apex, named florigen (discussed by Markus Schmid), is now understood to be a phosphatidylethanolamine-binding protein (FT protein). An FT-interacting protein (FTIP1) has been shown to be required for the movement of the FT protein (Liu *et al.* 2012).

The macromolecular complexes involved in producing the basic component of cell walls in plants, namely cellulose, have been described by Chris Somerville. Using a fluorescently tagged cellulose synthase (CESA) to complement a mutation for the normal CESA in *A. thaliana* allowed Paredes *et al.* (2006) to observe cellulose synthesis in real time and provided a dynamic analysis of an important component of the plant's phenotype. The predominant colocalisation of CESA and microtubules, as well as the analysis of mutants and documentation of the effects of chemical inhibitors, led to the interpretation that microtubules stabilised arrays of CESAs as they formed cellulose. In the instances where microtubules depolymerised, the CESA complexes were able to self-organise and continue cellulose deposition, indicating that the microtubules were not crucial to the cellulose formation process. Refining the arrangement of cellulose microfibrils in primary cell walls, which is important in cell expansion, was suggested to involve a cellulose synthase-interactive protein 1 (CSI1), which is found to be closely associated with cellulose along its length (Gu *et al.* 2010). The actual catalytic units forming cellulose fibrils have been visualised as rosettes (CESA polypeptides forming a hexagonal structure) associated with plasma membranes. The rosettes include sucrose synthase polypeptides and occur in

the cytoplasm as well as in the plasma membrane (Fujii *et al.* 2010).

### Impact of genome sequencing

Genome sequences are now recognised as vital templates for analysing the more complex features of plants, and Zander Myburg and Jonathan Wendel have provided updates on genome-level analyses in *Eucalyptus* and cotton (*Gossypium hirsutum* L.). The United States' Department of Energy–Joint Genome Initiative supported the *Eucalyptus* Genome Project under its Community Sequencing Program selections for 2008 and updates for the sequencing of the *Eucalyptus grandis* Hill genome are available at <http://www.phytozome.net/eucalyptus.php> (June 4, 2012) and <http://web.up.ac.za/eucagen/> (June 4, 2012). In parallel, the genome from *Eucalyptus camaldulensis* Dehnh was also characterised at the DNA sequence level by Hirakawa *et al.* (2011). The approaches used in sequencing of the *Eucalyptus* genome followed a combination of sequencing bacterial artificial chromosomes (BAC) clones and whole-genome sequencing using the high throughput DNA sequencing technologies that are available. To date, there has been no attempt to anchor the genome sequence to a molecular–genetic map, a process that is considered important in other genome projects such as the IWGSC. In addition to characterising the details of the genome structure, the *Eucalyptus* genome has been of particular interest for defining the genes for cellulose (lignin) and terpenoid biosynthesis, and P450 genes, since these are of specific commercial interest (Paiva *et al.* 2011; Hirakawa *et al.* 2011). Although the data are still new, the value of genome-level information is already evident, as the identification of polymorphic sequences are contributing to associating genome regions with phenotypic traits of commercial interest as well as fundamental work on defining the evolution of the genus.

The cotton genome analysis provided by Jonathan Wendel focuses on the changes in gene expression following polyploidy. The sequence-based analysis of genomes has indicated the common occurrence of ancient polyploidy in plants based on the findings of rearrangements and duplications (Guyot *et al.* 2004; Cui *et al.* 2006). Wendel discusses the consequences of these events using cotton as a model (Rapp *et al.* 2009, 2010; Flagel and Wendel 2010; Hu *et al.* 2011; Bao *et al.* 2011). Two allopolyploids ( $A_2G_1$  and  $A_2D_1$ ) were studied in detail and demonstrated that the expression of genes from the  $A_2$  genome tended to be largely dominant over the equivalent genes in the  $G_1$  genome in the  $A_2G_1$  allopolyploid. In contrast, the  $A_2D_1$  allopolyploid showed that genes from the  $D_1$  genome were preferentially expressed over those from the  $A_2$  genome. Apparently, methylation changes in the genomes do not accompany the process of forming a allopolyploid. The scale of the experiments was very large and since the  $A_2$  genome was the female parent in each cross to form the allopolyploids, the data indicated that inherent features of the DNA structure competing for transcription factors was a key variable rather than any maternal effects. In one example, the expression of the five profilin genes, coding for the actin monomer binding proteins linked to cotton fibre development, from both contributing genomes to the allopolyploids were all shown to be upregulated

(Bao *et al.* 2011), even though they are located on four different chromosomes.

The genomes of wheat (Gabriel Keeble, Katrien Devos), *Brachypodium* (Katrien Devos), maize (Jer-Ming Chia), lotus (*Lotus japonicus* Regel.) (Sushei Sato; <http://www.kazusa.or.jp/lotus/>, June 4, 2012) and fox tail millet (*Panicum italicum* L.) (Jeff Bennetzen; <http://www.phytozome.net/>, June 4, 2012) have been discussed. A particular challenge in sequencing complex genomes was discussed by Xu Xun (Beijing Genome Institute, Shenzhen, China) and Gabriel Keeble (Centre for Comparative Genomics, Murdoch University, Western Australia) where high throughput short sequencing is evidently insufficient to generate extensive linear strings of genomic DNA sequences. Reducing this complexity through focussing on diploid genomes, flow sorting of chromosomes (Dolezel *et al.* 2012) and analysing ordered BAC clones rather than whole-genome DNA were discussed. Keeble has discussed a detailed analysis of a 1.2 Mb DNA segment from the wheat genome (*ctg506*) to show that it is not feasible to produce a valid genome sequence without combining the power of a BAC-based physical map with the new high throughput DNA sequencing technologies. It was particularly noted that the draft rice genome was published together with a letter indicating the importance of finishing the sequence (Leach *et al.* 2002) and that similar arguments can be made for complex genomes such as wheat and barley (*Hordeum vulgare* L.) in the light of the significant advances in genetic improvement required to increase yield in a changing climatic environment.

### Translating research discoveries more broadly to society

The translation of research discoveries and technologies more broadly to society has been presented by David Fischhoff and Gerard Oostermeijer. Alex Johnson has demonstrated that genetically modified rice can be produced for successfully improving the supply of nutrient iron and zinc to consumers of rice (Johnson *et al.* 2011). Fischhoff has suggested that the often-stated need to double crop yields by 2050 (Tilman *et al.* 2011) was solvable through the integration of improvements in biotechnology, breeding and agronomy with reduced chemical inputs (Fedoroff *et al.* 2010). Weed and pest control have remained important research targets. High throughput application of the primary outputs from genome-wide sequencing of agronomically important crops, namely the identification of SNPs for associating with the traits or phenotypes that are important in particular crops is now accelerating commercial research objectives. Large-scale biotechnology to facilitate both the level of screening for desired phenotypes and high throughput genotype analyses on nonembryo sections of the grain (Deppermann *et al.* 2007) have been key steps in utilising the outputs from genome studies in breeding programmes. Several specific proteins were indicated as being important for future development. The *Bbx32* (*At3g21150*) gene from *A. thaliana* (Khanna *et al.* 2009) has been shown to increase yield in soybean (*Glycine max* L.) through a transcription factor activity (Zn-finger, B-box type) affecting the expression of a large number of genes, including those involved in the circadian clock. Deep sequencing of *Bacillus* genomes has provided new variants for the *Bt* gene in order to

investigate novel sources of insect resistance (Roh *et al.* 2007). In the area of drought tolerance, the ABA drought response gene network was also noted and is discussed in Powell *et al.* (2012; this issue). An RNAi-mediated technology to improve (for example) resistance to nematodes (Price and Gatehouse 2008), and RNAi-based genetically modified soybean with lower saturated fatty acids in the oil of the seed were still key research priorities.

The aspect of translating research concepts more broadly to society has been considered by Oostermeijer in the context of testing models for genetic and demographic consequences of management strategies (Volis *et al.* 2005). The engagement of volunteer community groups as “citizen scientists” to provide accurate information for population viability analyses was providing a much needed and more extensive database for refining models, as well as a mechanism for the transfer of new ideas in management of the landscape.

## Conclusions

The very extensive knowledge base now established for plants, as brought together at the 18th International Botanical Congress, provides the basis for the design of specific genotypes and high throughput diagnostics to drive new advances in the selection of biotic and abiotic stress tolerance in our major cereal food crops. Food security requires a consistent supply of high-quality grain even though major changes may be occurring in the environmental conditions in which plants are grown.

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