

replacement or even cause community turnover to a fundamentally different assemblage. It is also crucial that future research addresses how temperature, CO<sub>2</sub>, or edaphic factors may modify plant responses to precipitation variability. As precipitation is coupled with the processes controlling N availability, ecosystem responses to climate change will depend on how the strength of that coupling varies under future precipitation regimes.

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## Meetings

### Towards physiological sculpture of plants

#### 19th New Phytologist Symposium: Physiological sculpture of plants, Mt Hood, Oregon, USA, September 2008

Seventy delegates gathered in an inspirational location at the foot of Oregon's highest mountain, a spectacular volcanic peak, for an intensive meeting on the translation of fundamental discoveries in plant science (<http://www.newphytologist.org/physiological/>). The subject 'physiological sculpture' was intended to connote the intentional, informed and innovative science-based design of plant functions to meet humanitarian, economic and environmental goals.

'... The historically major role of the public sector in the development of new technology for "minor" crops, and for the developing world, is not keeping pace.'

The meeting took an unusual approach. Rather than delving deeply into a single discipline or problem, the meeting brought together a wide variety of scientific leaders and broad thinkers to consider what kinds of breakthrough advances in crop improvement could be on the horizon. The meeting

focused on science and technology, not on social constraints to innovation. It also focused on what we could do with the rapidly growing knowledge of plant genomics and molecular physiology, not on the transfer of genes over long phylogenetic distances, to create new products and traits. The organizing committee consisted of Richard Jorgensen (University of Arizona, USA), Dick Flavell (Ceres Inc., CA, USA), Harry Klee (University of Florida, USA), Richard Amasino (University of Wisconsin-Madison, USA), Steve Strauss (University of Oregon, USA) and Holly Slater (*New Phytologist*). Several important observations and questions emerged from the meeting.

### Where is the physiological beef?

Several delegates commented that despite the name of the conference, the participation by whole-organism crop physiologists was limited, yet much needed. This reflects a lack of connection from genetic variation all the way up to crop performance, something that systems biology approaches hope to remedy in the coming years. Other important causes of the physiology gap are the dearth of trained physiologists who can conduct research that spans molecular to ecological scales; too little open, published research on the physiology of biotech crops (transgenic and otherwise) to help to understand why and how they perform better; and the growing decline of public funding for translational genomics and biotechnology research that goes beyond *Arabidopsis* and beyond the laboratory/glasshouse. This is in no small part a result of the lack of an effective science-to-commercial pipeline; tremendous regulatory costs and marketing issues have impeded public sector scientists from developing and registering transgenic crops for commercial use. As this area develops, high-quality science that bridges the gap between the molecular and the ecophysiological/applied aspects of research will find a home in this journal. Such studies have long been an area of strong interest to *New Phytologist*, evidenced by recent reviews that have explored the scaling-up of functional genomics, the translation of genomic data for tree breeding, the applications of systems biology, and metagenomics (Wullschlegel *et al.*, 2007; Fuller *et al.*, 2008; Grattapaglia & Kirst, 2008; Martin & Selosse, 2008; Sheehy *et al.*, 2008).

### Long live forward genetics

Where the goal is physiological sculpture, the tendency is to tinker with the tools (i.e. plant processes and genes) that one knows well, rather than to explore new processes and to identify new levers to control them. However, forward genetics continues to be invaluable, even in the days of fast sequencing, as we do not know what genes affect what traits, and modification of transcription alone often misses important sources of variation. Scott Tingey (DuPont Corporation, DE, USA) described a case where intensive transgenic efforts to control fatty acid composition from a known regulatory gene

failed until a forward genetics approach was employed, even though it focused on the same gene (Zheng *et al.*, 2008). The problem was that the focus of the transgenic strategy was on the modification of transcription, but the useful genetic variation was found to be manifested by a post-transcriptional mechanism. As pointed out by Tingey, as well as by John Willis (Duke University, NC, USA) and Daniel Rokhsar (Department of Energy/JGI/UC-Berkeley, USA) when discussing studies of phylogenetically novel and wild plants, the decreasing cost of genomic information allowed by rapid sequencing technologies is making forward genetics feasible in virtually any plant species. A greater degree of phylogenetic diversity in forward genetics efforts is certain to identify novel genes and metabolic circuits useful for crop genetic engineering. Some of the species being studied, like the highly outbreeding and ecologically versatile forest trees such as poplar, provide powerful means for gene identification owing to their very low linkage disequilibrium. In species with large genomes such as in polyploids, association approaches appear to be capable of highly precise gene identification because of the strong bias towards recombination in euchromatic areas.

### Combating menaces

Several researchers discussed new options for improving disease resistance, which were based on increasingly detailed knowledge of plant and pest sequences, and were often facilitated by transgenic technologies. Deborah Delmer (Rockefeller Foundation, NY, USA) discussed the many possible uses of RNA interference (RNAi) to confer resistance to pests of the developing world. In addition to conferring resistance to viruses (Waterhouse *et al.*, 1998), RNAi has been recently reported to protect against nematodes and insects (Huang *et al.*, 2006; Baum *et al.*, 2007; Mao *et al.*, 2007). Brian Staskawicz (UC-Berkeley, CA, USA) discussed how our increasing knowledge of plant-pathogen interactions is guiding successful predictive resistance strategies. He illustrated how transfer of resistance (R) genes from pepper to tomato, a close relative, could provide resistance to major diseases such as bacterial spot of tomato (Tai *et al.*, 1999). Similar opportunities exist for gene transfer in other taxa. This includes Solanaceous crops such as potato, in which genes for resistance to late blight and other major diseases are known, as well as in monocots (Zhao *et al.*, 2005). Staskawicz also discussed how the mode of action of various pathogen virulence 'effector' proteins reveals critical pathogen virulence strategies. Effector proteins are secreted into host cells by pathogenic bacteria, modifying host targets to render cells more susceptible. In resistant genotypes, cognate host R genes surveil effector-mediated host target changes and reroute host pathways from a susceptible to a resistant response (for reviews, see Chisholm *et al.*, 2006; Jones & Dangl, 2006). By cataloguing the 'constellation' of pathogen effectors in naturally infected tomato plants in the field, Staskawicz is identifying conserved,

diagnostic effectors that can guide the design of resistance strategies. Based on this expanded catalogue of R-gene  $\times$  effector-gene pairs, appropriate resistance genes could potentially be chosen that confer strong and stable resistance to all major races of a pathogen. Tzvi Tzfira (University of Michigan, MI, USA) described zinc finger nucleases (ZFN), a powerful emerging technology that has a multitude of potential applications (Zeevi *et al.*, 2008). Tzfira described how ZFNs could be used as sources of resistance (e.g. Takenaka *et al.*, 2007) by designing them to target highly conserved regions in DNA plant virus genomes.

### Transcription factors – use them well, use them with care

Because they control many genes and often entire pathways, transcription factor genes have been considered to be ‘low-hanging genomic fruit’, and thus several companies have focused on them in their genomics projects. Oliver Ratcliffe (Mendel Biotechnology, CA, USA) discussed Mendel Biotechnology’s identification of a transcription factor that promotes drought tolerance in *Arabidopsis* and has been demonstrated to provide drought tolerance in maize (Nelson *et al.*, 2007). The *AtNF-YB1* transcription factor gene appears to provide strong improvement of drought tolerance in maize in multiple years and locations. This is a significant advance given that approx. 70% of the world’s fresh water is used in agriculture, and it has been predicted that water will become increasingly limited in the future as a result of population growth, increased living standards and climate change. Tom Adams (Monsanto, MO, USA) described a large evaluation program at Monsanto that validated *YB1* and several other genes, including multiple transcription factors, as being useful for further development. In their ongoing studies of four commodity crops (cotton, corn, canola, soy) and 31 traits, they tested 2500 constructs in 2008. This included 330 000 field plots at 180 locations in 26 states and six countries. Thus, there is an important need to be able to screen for such genes much more efficiently, especially if these approaches are to be used in the developing world and for ‘minor’ crops such as fruits, vegetables and trees. One important consideration is that strong overexpression and suppression often results in extensive pleiotropic effects: Mike Thomashow (Michigan State University, USA) discussed how the promising *CBF* transcription factor (Cook *et al.*, 2004), which provides cold-tolerance and drought-tolerance, also stunted plants when overexpressed. More careful and subtle expression methods will be needed if genes of potential value as sculpting tools are not to be overlooked in large-scale screens and early field evaluations.

### Phenotype, phenotype, phenotype

The growing disparity between the cost and speed of DNA sequencing vs that of obtaining high-quality phenotypes

was continuously highlighted by speakers as being a major impediment to functional genomics and physiological sculpture. In contrast to the extensive field approach discussed above, Pierre Lejeune (CropDesign NV, Ghent, Belgium) described a fully automated glasshouse system for evaluating new genes in transgenic rice. Because the essential unit is a potted plant that can be treated with a variety of stressors and is analyzed from top to bottom via image-analysis software (including imaging of roots through clear-bottomed pots), it could, in theory, be applied to any plant species. He reported that approximately half of the genes identified as causing a useful phenotypic change in the glasshouse evaluation also showed a similar phenotype in the field, suggesting that the great precision of the system is worth the considerable investment. Unfortunately, such systems are largely unavailable to developing-world and public sector crop scientists. Another shortcoming of most phenotyping platforms is that the only phenotypes measured are morphological, creating a huge gap between gene sequence and whole-plant physiology. DNA sequences generally do not allow prediction of protein accumulation or post-translational modifications, or address epigenetic modifications or metabolite pools. In addition to better whole-plant morphology, high-throughput methods for the rapid measurement of these molecular phenotypes would help to tie the effects of genes to crop physiology – an endeavor that is certain to require complex quantitative integration tools, now often referred to as part of ‘systems biology’.

### Drowning in data

Rebecca Doerge (Purdue University, IN, USA), in discussing the growing challenges of reaching statistically sound conclusions from exponentially growing genomic data sets, indicated that the sizes of data sets are at and above the capacity of statistical theory and available software to analyze them adequately. She also emphasized the importance of precise and relevant phenotyping for statistical evaluations. Her comments provided an important contrast to those of Susan McCouch (Cornell University, NY, USA) and others who suggested that we may be in a ‘post-hypothesis’ phase of genetic biology, meaning that we may soon have sufficient data that we can simply mine it to answer any important question, obviating the need to set up specific experiments to test specific hypotheses. As discussed by Daniel Rokhsar (Department of Energy/JGI/UC-Berkeley, CA, USA), Steve Goff (University of Arizona, AZ, USA) and others, the speed, intelligence and broad accessibility of quantitative and informatic tools continue to be major impediments to high-quality genome annotation, cross-referencing of genomic information among species and translation. As explained by Goff, the United States National Science Foundation-funded ‘iPlant Collaborative’ seeks to provide new tools to overcome some of these serious problems of integration and inference ([iplantcollaborative.org](http://iplantcollaborative.org)).

## Be green, really

As the world faces the growing challenges of greenhouse-gas-mediated climate change, reduced availability of fresh water, and trade-offs between food and energy production, there will be a need for expanded and more rigorous life cycle scale evaluations of the uses of plant biotechnologies to improve the environmental footprint from agriculture. Steven Savage (Cirrus Partners, CA, USA) discussed the likelihood of increasing the market incentives to reduce net greenhouse gas emissions in agriculture and forestry, and to sequester additional carbon in agricultural soils. He cited five broad ways that biotechnologies could contribute towards achieving that end result: yield improvement, as appears to have already occurred with pest-resistant transgenic crops; soil carbon sequestration, as has occurred on a large scale using the low-till systems facilitated by herbicide-resistant soy; fertilizer optimization, through the use of crops engineered for more efficient fertilizer use (demonstrated at the research scale); adaptability, for example with crops such as the maize with improved drought tolerance discussed earlier; and sculpting soil microbial populations or root physiology to reduce nitrous oxide production. A theme that re-occurred several other times in the meeting was the inadequate degree of study of the molecular physiology of roots and of the metagenomics of root ecosystems, given their importance for adaptation and environmental mitigation. Rob Horsch (Gates Foundation, WA, USA) described how C4 rice, if achieved, would both increase rice yields and exhibit dramatically improved water-usage efficiency (Hibberd *et al.*, 2008) – suggesting that major breakthroughs of economic and environmental consequence are feasible with sufficient research. However, Steve Savage also cited several risks to society of not investing, not recognizing, or otherwise not allowing these benefits to be achieved.

Chris Somerville (Carnegie Institute of Plant Research, CA, USA) gave a broad view of biological energy systems. He discussed a number of approaches to reduce the net greenhouse gas emissions from the many different bioenergy options being studied in the large Energy Biosciences Institute that he directs ([www.energybiosciencesinstitute.org](http://www.energybiosciencesinstitute.org)). On the list of systems to avoid were those that support consumption of meat over plant sources of protein, and those that promote the use of annual crops for biological energy production over those of perennial lignocellulosic energy crops. For example, GreenWood Resources (OR, USA), a meeting sponsor, has produced very high yields in some of their trials in perennial coppice systems in poplar (Fig. 1). Somerville suggested a broad survey of species, with special emphasis on perennial grasses such as *Miscanthus* that have high water-use efficiency and require little or no nitrogen fertilizer. Some of these species are able to remobilize nutrients into the roots before harvest, and may contain nitrogen-fixing symbionts (Christian *et al.*, 2008). He also discussed the problem of potential invasiveness of new species and varieties of perennials that are grown



**Fig. 1** Rapid growth of a perennial biofuels crop in GreenWood Resources' hybrid poplar nursery at Los Angeles, Chile. Shown is five months coppice growth from 2-yr-old stools (i.e. annual biomass harvest results in a proliferation of sprouts from 25-cm-high stumps, which are then reharvested in repeated 2-yr cycles). Annual yield, averaged across a group of elite clones, approximates 30 dry metric tons per hectare. Hybrid poplar is considered a premier feedstock for Chile's developing renewable biofuels industry. Photo courtesy of Carlos Sierra Silvia, GreenWood Resources, USA.

on a large scale, and stressed that candidate energy species need to be carefully evaluated in an attempt to prevent problems. He noted that nonnative species which are not interfering with native species may be preferable to native species in order to avert gene-flow issues. However, others considered that the hazard caused by the much larger degree of ecogenetic novelty from nonnative species should suggest just the opposite preference.

## Sculpt forward

The large number of senior scientists, many of whom have been directly involved with public and private breeding efforts, had no shortage of examples and ideas for progress in fundamental science and its translation. There was no shortage of direct linkages made between science and the pressing needs of society as a result of population growth, resource depletion and the desire for economic development. However, a dark recurring theme was that – in stark contrast to the extraordinary progress being made by the large companies on the major commodity crops – the public sector is falling further and further behind in translation. The historically major role of the public sector in the development of new technology for 'minor' crops, and for the developing world, is not keeping pace. This is a result of declining investment, increasing intellectual property-right hurdles and the resulting lack of institutional capacity and will to deal with complex genomic technologies in translation, especially transgenic ones. Bob Goldberg (UC-Los Angeles, CA, USA), in an animated

lecture, emphasized the extraordinary diversity of transgenic innovations that have already been produced, but that they are a tiny fraction of those on the market – largely a result of the huge social obstacles that prevent commercialization of any but blockbuster traits in the major commercial species. Organizations such as the Public Intellectual Property Resource for Agriculture ([www.pipra.org](http://www.pipra.org)) are trying to help, but need large increases of funding, strong institutional collaboration to support and release useful products, and much more participation from the scientific community.

Surprisingly, however, the method of sculpture *per se* barely arose as a scientific issue at the meeting. Both marker-informed conventional and transgenic methods could be useful for nearly all trait targets. The choice of which to use would depend on a range of species, trait, environment, economic, humanitarian and efficiency issues that are idiosyncratic to each application. All would have benefits and potential drawbacks. This contrasts with the extreme polarization in society about the use of transgenic methods. There appears to be an almost complete avoidance of most transgenic methods in breeding outside the major commodity crops in a handful of countries. This is a result of the added regulatory and intellectual property costs, as well as the marketplace and brand-risk issues they bring. Although the meeting did not focus on social issues, it would appear, from the lectures and discussions, that the large majority of attendees implicitly agreed that given the pressing challenges facing humanity and the global environment, all tools should be brought to the table. The extreme attention being paid by society to the method vs the ends is at best penny-wise, and most clearly pound-foolish.

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