19th New Phytologist Symposium

Physiological sculpture of plants: new visions and capabilities for crop development

Timberline Lodge, Mount Hood, Oregon, USA
17–20 September 2008

Program, abstracts and participants

New Phytologist

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Organizing committee

Steve Strauss (*Oregon State University, OR, USA*)

Richard Amasino (*University of Wisconsin-Madison, WI, USA*)

Richard Flavell (*Ceres Inc., CA, USA*)

Richard Jorgensen *(University of Arizona, USA)*

Harry Klee (*University of Florida, FL, USA*)

Holly Slater (New Phytologist, *Lancaster, UK*)

Acknowledgements

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New Phytologist Trust

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Program, abstracts and participant list compiled by Jill Brooke.
Physiological sculpture of plants illustration by Gretchen Bracher, College of Forestry, Oregon State University

Table of Contents

Program

Wednesday 17 September	
04:00 pm	Registration opens
06:00 pm	Welcome Reception (Drinks and light buffet)
08:00 pm	**Guest lecture: A botanist's guide to the natural history of the Pacific Northwest** *David Dalton (Reed College, Portland, Oregon)*
Thursday 18 September	
08:00 am	**Symposium goals and overview** *Steve Strauss (Oregon State University, OR, USA)*
Session 1:	**The landscape** *Moderator: R. Flavell*
08:30 am	**1.1. Harnessing the new sciences in support of agriculture in the developing world** *Deborah Delmer (Rockefeller Foundation, retired, NY, USA)*
09:00 am	**1.2. How biotechnology can enable agriculture to mitigate climate change** *Steven D. Savage (Cirrus Partners, CA, USA)*
09:30 am	**1.3. Breeding genomics** *Susan McCouch (Cornell University, NY, USA)*
10:00 am	**1.4. Roots and drought and breeding better crops** *Adam Price (University of Aberdeen, UK)*
10:30 am	**BREAK**
11:00 am	**1.5. The use of genetic principles for crop improvement** *Scott Tingey (Dupont Corporation, DE, USA)*
11:30 am	**1.6. Integrating breeding & biotechnologies** *Tom Adams (Monsanto, MO, USA)*
12:00 pm	**1.7. The iPlant Collaborative** *Steve Goff (University of Arizona, AZ, USA)*
12:30 pm	**LUNCH**

Session 2:	**Science inroads** *Moderator: R. Amasino*
01:30 pm	**2.1. Next generation genomic technologies for gene mining and functional genomics: Issues of scope, scale and service** *Jeff Bennetzen (University of Georgia, GA, USA)*
02:00 pm	**2.2. Creating tools for the control of transgene integration and for genome editing in plant species** *Tzvi Tzfira (University of Michigan, MI, USA)*
02:30 pm	**2.3. New transcription factor based technology & tools for future crop improvement** *Oliver Ratcliffe (Mendel Biotechnology, CA, USA)*
03:00 pm	**BREAK**
03:30 pm	**2.4. Small RNAs** *Rob Martienssen (Cold Spring Harbour Laboratory, NY, USA)*
04:00 pm	**2.5. The pleiotropic hypothesis of heterosis** *Luca Comai (University of California-Davis, CA, USA)*
04:30 pm	**2.6. The importance of statistics in the era of 'omics** *Rebecca Doerge (Purdue University, IN, USA)*
05:00 pm	**2.7. 200 million years of angiosperm genome evolution** *Daniel Rokhsar (US Dept. of Energy, USA)*
06:00 pm	**Poster session / reception**
07:00 pm	**Dinner**
	Evening lecture *Moderator H. Klee*
08:30 pm	**Gates Foundation approaches** *Robert Horsch (Gates Foundation, Washington, WA, USA)*

Friday 19 September

Session 3:	**Traits and physiology** *Moderator: S. Goff*
08:30 am	**3.1. Understanding and modifying flowering** *Richard Amasino (University of Wisconsin-Madison, WI, USA)*
09:00 am	**3.2. Fruit flavor and nutrition: A paradigm for quality improvement** *Harry Klee (University of Florida, FL, USA)*
09:30 am	**3.3. Toward a systems level understanding of abiotic stress tolerance for applications in agriculture** *Michael Thomashow (Michigan State University,MI, USA)*

10:00 am	**3.4. The challenges of phenotyping technologies for plant biotechnology** *Pierre Lejeune (CropDesign NV, Ghent, Belgium)*
10:30 am	**BREAK**
11:00 am	**3.5. Role of pathogen effector proteins in plant innate immunity** *Brian Staskawicz (University of California-Berkeley, CA, USA)*
11:30 am	**3.6. Evolution of adaptive traits** *John Willis (Duke University, NC, USA)*
12:00 pm	**3.7. Breeding realities** *Erik Legg (Syngenta Biotechnology Inc., NC, USA)*
12:30 pm	**LUNCH**
Session 4:	**Putting it together** *Moderator: S. Strauss*
01:30 pm	**3.8. Genetic engineering novel crop plants: unlimited horizons** *Robert Goldberg (University of California-Los Angeles, CA, USA)*
02:00 pm	**3.9. Development of energy crops** *Chris Somerville (Carnergie Institute of Plant Research, CA, USA)*
02:30 pm	**3.10. Human and institutional drivers for realizing crop improvement** *Richard Flavell (Ceres Inc., CA, USA)*
03:00 pm	**BREAK**
03:30 pm	**Panel discussion: Conference themes and messages** *Amasino, Klee, Goff, Flavell, Freedman, Strauss*
06:00 pm	**Reception**
07:00 pm	**Dinner**

Saturday 20 September

Depart

Speaker Abstracts

Session 1: The landscape
Moderator: R. Flavell

1.1. Harnessing the new sciences in support of agriculture in the developing world

DEBORAH DELMER
Professor Emeritus, UC Davis; Rockefeller Foundation (retired), New York City, New York, USA

In many parts of the developing world, yields of major staple crops are less than 20% of those achieved by large-scale agriculture in the developed world. Reasons for this include the use of poor seed quality, little or no use of fertilizer/herbicides/fungicides/insecticides under conditions of poor soil quality and high pressure from pests and diseases, poor agronomic practices coupled with small farm sizes, weak market incentives to overproduce, and the challenges of unpredictable weather that are particularly severe for rainfed agriculture. While better application of existing knowledge and practices can mitigate many of these problems, all forms of agriculture must continually draw upon new discoveries in order to meet future challenges. Some of my favorite opportunities at this point in time include: 1) to clarify the power of genomics for breeders; 2) to transform 'precision breeding' through advances in homologous recombination; 3) to assess the promise of plant-mediated RNAi for control of insects, parasitic plants and fungi; 4) to apply fundamental knowledge of small RNA function to combat the rising development of DNA satellites that break resistance to geminiviruses; 5) to apply synthetic biology to develop microbes that synthesize products of value to the developing world.

Unfortunately, there are many challenges involved in translating fundamental discoveries into applications relevant to the developing world. For academic scientists, the key issues involve lack of 3 key parameters: TIME, OPPORTUNITIES, and INCENTIVES. Academic scientists are already over-committed in terms of their time; they lack opportunities to meet potential collaborators in the developing world and to learn of their challenges; and they lack the incentives in terms of funding and rewards from their institutions for carrying out translational research. This lecture will offer some suggestions for how these limitations might be overcome.

1.2. How biotechnology can enable agriculture to mitigate climate change

STEVEN D. SAVAGE, JOHN S. VENDELAND
Cirrus Partners, LLC, P.O. Box 1335, Evergreen, CO 80437, USA

As society grapples with climate change, agriculture is being called upon not only to reduce its own footprint but also to play a positive role through terrestrial carbon sequestration and fuel production. Ideally agriculture must produce more with less land, with less environmental impact, reduced nitrous oxide emissions and with optimized soil carbon accumulation. This must be accomplished under increasingly variable climatic conditions. To address this challenge it will be necessary to employ the full range of technological innovations, particularly those involving biotechnology. The key biotech traits for climate change mitigation include both current and novel concepts. To rapidly develop climate friendly farming systems will also require a new model for field research. It will also be necessary to find ways to monetize externalities and conversely to compensate growers for the environmental services provided by improved cropping systems. By combining biotechnologies, extensive cooperative research and novel economic incentives, agriculture can become a significant part of the solution to climate change.

1.3. Breeding Genomics

SUSAN McCOUCH
Cornell University, Ithaca, NY 14853-1901, USA

The ability to select for increased yield, quality and fitness under both favorable and unfavorable conditions is key to the success of plant breeding. Over the last decade, we have used a combination of wide crossing, phenotypic evaluation and marker assisted trait dissection to identify genes and QTLs that enhance the performance of high-yielding, elite rice cultivars. These studies enable us to isolate genes underlying the QTLs, identify the functional polymorphisms that distinguish alleles, and begin to characterize the regulatory and biochemical pathways that determine trait performance in real world environments. Molecular evaluation of existing germplasm resources has provided a map of population substructure that helps guide the selection of parents in a crossing program and also provides insight into the evolutionary history of crop species. As increasing numbers of genes are cloned and characterized and their relationship to a broad range of phenotypes is documented, plant breeders can begin to use reverse genetics approaches to examine the genetic architecture of their materials and identify specific allele combinations that confer favorable or unfavorable attributes in environments of interest. While genomics already provides the basis for marker-assisted selection in plant breeding, its deeper impact is to provide plant breeders with a toolkit for predicting how to synthesize new traits and varieties as a creative prelude to investing in applied breeding programs.

1.4. Roots and drought and breeding better crops

ADAM H. PRICE
Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, AB24 3UU, UK

The improvement of resource acquisition in crop plants has proved difficult due to the multitude of environmental and genetic factors which contribute and interact. Research on quantitative trait loci (QTLs) related to biotic and abiotic in rice (*Oryza sativa*) has revealed a number of traits, genomic regions and genetic sources of good value for breeding, but these are not generally major QTLs. For example, a QTL on chromosome 9 which affects rooting depth has been used to improve root growth by marker assisted selection (MAS), but to date experiments to link this with yield under drought have not been sufficiently robust. This highlights the importance of considering soil properties when linking root traits to field performance. Attempts to identify genes underlying these small QTLs without fine mapping by use of meta-QTL analysis, transcriptomics and sequencing are progressing. For example, auxin transporters appear good candidates for root growth QTLs while aquaporins have been identified as promising targets for a drought avoidance QTL. The prospect of proving candidate gene function is, however, challenging for small-effect QTLs. Case-by-case solutions are required, which combine transgenics and/or association genetics with plant physiology. Once identified, these genes can be used for crop improvement via MAS or GM.

1.5. The use of genetic principles for crop improvement

SCOTT V. TINGEY
DuPont Crop Genetics, P.O. Box 80353 Wilmington, DE 19880-0353, USA

Advances in genetics, laboratory automation and information management have led to the collection of vast amounts of information relative to the identification, organization and expression of genes in many plant species. This information is being used to drive many reverse genetics applications that seek to identify gene function and to provide a description of the cellular and developmental expression of these genes, their protein products, and the interaction between proteins products of different genes. However, these activities typically result in the collection of more information and fall short of helping us understand whether the gene products, or the regulation of the gene products are sufficient to improve plant performance. A forward genetic paradigm will be discussed as a model for knowledge creation; one that is focused on crop improvement through the identification of genes sufficient to improve plant performance.

1.6. Integrating Breeding & Biotechnology

TOM ADAMS
Biotech Strategy, Operations & Prospecting, Monsanto Company, 700 Chesterfield Pkwy West, Chesterfield, MO 63017, USA

Between 1960 and 2000, the earth's population doubled from 3 billion to 6 billion people. By about 2040, another 3 billion will be added. This increasing global population and changing diets has lead to greater demand for food and animal feed from agriculture, while arable land is under pressure. To date, increased grain demands have been met through increased productivity resulting from plant breeding and plant biotechnology. These disciplines are becoming increasingly integrated as we struggle to meet growing demands for food and feed. While herbicide tolerance and insect control are now common biotechnology traits in the major row crops, new traits are being developed that are designed to increase yields, decrease dependence on water and nitrogen, and improve the efficiency of production to meet worldwide demand for grain. Development of crops carrying combinations of such complex traits will require novel approaches to discovery, trait integration, trait stacking, and breeding as we align the right traits with the best genetics to develop products that work in varied environments.

1.7. The iPlant Collaborative

STEPHEN A. GOFF
BIO5 Institute, University of Arizona, 1657 E. Helen St., Tucson, AZ 85721, USA

The iPlant Collaborative (iPC) is a five year NSF-funded project designed to build collaborative networks and cyberinfrastructure for the plant science research community. The iPC Project Team is a multidisciplinary group located at University of Arizona, Cold Spring Harbor Laboratory, University of North Carolina at Wilmington, Arizona State University, and Purdue University. The iPC efforts will be driven by Grand Challenge problems in plant biology chosen by the plant science research community. An iPC Board of Directors has been nominated by an independent nominating committee. Nine Grand Challenge workshops have been proposed to identify specific Grand Challenge projects, and four workshops were chosen by the Board of Directors for iPC support. The Board of Directors also recommended initiating efforts on two 'Proto-GC Projects' addressing cyberinfrastructure needs likely to be needed across multiple Grand Challenge projects. The iPC and efforts supported by this project will be described.

Session 2: Science inroads
Moderator: R. Amasino

2.1. Next generation genomic technologies for gene mining and functional genomics: Issues of scope, scale and service

JEFF BENNETZEN
Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

Technologies for genome sequence analysis continue to advance at a breakneck pace, with no obvious plateau in site. Even the largest genomes are now available for routine re-sequencing analysis, and full genome *de novo* assembly of the largest plant genomes at reasonable costs are on the horizon. Our ability to find the genes in these genomes is expanding as well, with better bioinformatic tools and comparable databases providing the most important resources. Functional analyses of the genes within genomes, although much improved over the last decade, still trail gene mining by orders of magnitude. Real time and single cell analyses of gene function remain a largely elusive goal, but dramatic enhancements in reverse genetic approaches are beginning to put a dent in the great mass of 'hypothetical', 'unknown function', and 'looks like something else so it probably does about the same' types of genes.

More important than the technology *per se*, where the momentum is in the right direction and driven by motivations that go well beyond the public research community, are the issues of access to this technology and the focus of its use. Sources of funding in all sectors are abandoning the individual investigator model in favor of mega-consortia, without any scientific analyses that support this shift. Given that the individual investigator and blue-sky research have been the glory of the life sciences over the last century, this imperative to stratify and bureaucratize biological research seems ill-conceived. One of the great values of the genomics approach is supposed to be empowerment of the average researcher, with data sets and bioinformatic tools at their command. However, even a perfect set of tools requires at least one pair of hands to employ them, and at least one mind to plan their use, but project funding at levels less than 10% indicates that most of the scientists we have trained in the last decades have been shunted from the research community.

2.2. Creating tools for the control of transgene integration and for genome editing in plant species

ANDRIY TOVKACH, VARDIT ZEEVI, TZVI TZFIRA
Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109, USA

The repair of double-strand breaks (DSBs) in plant genomes can lead not only to local mutagenesis but also to the insertion of foreign DNA molecules into the repair site. This phenomenon can be harnessed to mutate specific sites in the plant genome and to target foreign DNA molecules into them with zinc finger nucleases (ZFNs). ZFNs are a new type of artificial restriction enzyme that is custom-designed to recognize and cleave specific DNA sequences, producing DSBs in living cells. We are developing ZFN technology for plant research through the development of constructs, cloning procedures, and *in-vitro* and *in-planta* analyses of newly designed ZFNs. Through active collaboration with our partners from the industry and academia, we are evaluating the use of ZFN technology not only for transgene-mediated gene targeting, but also as a tool for non-transgenic genome editing and for battling the propagation of double-stranded viral genomes in infected plants. We are also engaged in introducing ZFNs as novel *in-vitro* rare-cutting restriction enzymes. The latter are extremely useful for the construction of complex and long DNA constructs and we have shown that ZFNs can be useful for assembling multigene transformation vectors. Our research brings us several steps closer to the implementation of ZFN-based technologies for a variety of uses in plant development.

2.3. New transcription factor based technology & tools for future crop improvement

OLIVER J. RATCLIFFE, NEAL GUTTERSON
Mendel Biotechnology, Inc., 3935 Point Eden Way, Hayward, CA 94545, USA

A rapidly changing climate combined with a human population that is set to rise to more than 8 billion will present unparalleled challenges to agricultural production systems over the next half century. Provision of a reliable supply of food, fiber and energy for the world's people will necessitate substantial increases in crop productivity through enhancement of yield potential and stress tolerance. Delivering such improvements in complex crop traits requires biotechnological approaches that focus on the regulation of entire genetic pathways rather than on the activity of single genes. Since transcription factors are well established as powerful control proteins that act as master regulators of regulatory networks, they represent ideal intervention points for pathway regulation. This presentation will first highlight examples of how transcription factor-based approaches are being applied to enable a forthcoming second generation of biotech crops beyond ‘simple’ traits such as herbicide and insect resistance. In particular, we will focus on technologies that target drought response pathways, since this factor represents the primary limitation to plant productivity. Using selected examples, we will then offer a forward-looking view on the potential for systems biology and other novel tools (e.g., chemical approaches to pathway regulation and cell-type specific interrogation of gene expression) to accelerate the pace of crop improvement.

2.4. Small RNAs

ROB MARTIENSSEN
Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York, 11724, USA

Since their discovery in plants, small RNA have found their way into every aspect of biology, from viral resistance, to gene regulation and epigenetic inheritance. Trans-acting small RNA encode genetic information unencumbered by linkage. They are are mobile, acting as patterning signals, and they match their targets with high specificity so constraining the size of the plant gene-space. They have proven to be invaluable genetic tools. ‘Cis-acting’ small RNA, derived from heterochromatin, are largely chromosomal, guide epigenetic modifications and influence chromosome behavior. They silence viruses on the one hand, but also transgenes on the other, impeding GM strategies in crop breeding. New roles for each class of RNA in reproductive isolation, the evolution of imprinting, Lamarckian adaptation and hybrid vigor have been and will be proposed.

2.5. The pleiotropic hypothesis of heterosis

LUCA COMAI
Department of Plant Biology and Genome Center, UC Davis, Davis, CA 95616, USA

Heterosis, or hybrid vigor, defines the superior performance of hybrids compared to the inbred parents. Historically, performance refers to growth although transgression of any hybrid phenotype is often regarded as heterosis. Considering the major impact of heterosis on agricultural productivity, limited molecular information is available on its basis. Heterosis could be determined by a whole genome interaction, or by an interaction at specific loci. Evidence for the latter scenario is now substantial and it is provided by QTL mapping. What is the nature of growth-determining heterotic alleles? Rapidly changing cis-regulatory regions of developmentally important genes underlie many evolutionary events. Because of their prevalent role in evolution of new traits and because of the deleterious effect of dosage changes in many regulatory proteins we suggest that these type of mutations are the most likely to provide the basis for heterosis. We propose that when regulatory changes are favorable they are often pleiotropic. They confer an advantageous dominant effect while, at the same time, inducing a deleterious, dosage-dependent effect. In other words, innovation has a cost. Heterozygosity at these loci would maximize individual vigor and fitness. The model is consistent with several observations on heterosis and provides testable hypotheses.

2.6. The importance of statistics in the era of 'omics

REBECCA W. DOERGE
Department of Statistics, Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA

This is an exciting and influential time for the field of Statistics in science. Technological advances in genetic, genomic, and the other 'omic sciences are providing large amounts of complex data that are presenting a number of challenges for the biological community. Many of these challenges are deeply rooted statistical issues that involve experimental design, replication, and multiple testing. Using microarray technology as an example, important statistical issues, as well as statistical and graphical integration of results will be discussed with an eye on the current (e.g., gene expression) and future uses of microarrays for marker genotyping, expression quantitative trait loci (eQTL) mapping, testing methylation, and assessing interactions between proteins and DNA (e.g., ChIP-chip).

2.7. 200 million years of angiosperm genome evolution

DANIEL ROKHSAR
DOE Joint Genome Institute and University of California, Berkeley, CA 94720-3200, USA

Large-scale DNA sequencing of plants is providing us with an increasingly diverse sampling of angiosperm genomes. By comparing them, we can learn about the ancestral genomic features they share, as well as characterize the unique elements associated with phenotypic diversity. This talk will discuss progress towards reconstructing the genomes of rosid, grass, and angiosperm ancestors in the face of sequence divergence and rampant gene and genome duplication, and the implications for functional analysis of plant genomes.

Session 3: Traits and physiology
Moderator: S. Goff

3.1. Understanding and modifying flowering

RICHARD AMASINO
Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706-1544, USA

Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure during winter to initiate flowering the following spring. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. A similar cold response occurs to release bud dormancy: in many species that grow in temperate climates, bud dormancy is not broken until the plant has 'counted' a sufficient number of days of cold to ensure that any subsequent warm weather actually indicates that spring has arrived.

Our studies of vernalization in Arabidopsis have revealed that meristem competence is a function of the expression level of certain MADS-box genes such as FLOWERING LOCUS C (FLC) that act as repressors of flowering. Exposure to prolonged cold causes epigenetic silencing of these MADS box genes, thus rendering the shoot apical meristem competent to flower. During cold exposure, specific components of chromatin-remodeling complexes are induced, and these chromatin-remodeling complexes catalyze covalent modification of histones of the chromatin of the flowering repressors resulting in silencing of gene expression.

3.2. Fruit flavor and nutrition: A paradigm for quality improvement

HARRY KLEE
University of Florida, Plant Molecular & Cellular Biology Program, Gainesville FL 32606-0690, USA

It is generally accepted that the flavor quality of most commercially produced fruits has significantly declined in the last several decades. There are many diverse reasons for this decline. Chief among them are the demand for year-round availability, the necessity of long postharvest shelf life, and the challenges of breeding for a complex multigenic trait. In recent years there has been a major shift in emphasis in breeding toward value-added quality improvement. Our efforts to understand and improve flavor quality are illustrative of the challenges facing modern agriculture. Flavor is an elusive and ill-defined concept. Much of our effort has been directed to understanding the biochemical components of tomato flavor. We have defined several of the metabolic pathways for synthesis of flavor-related compounds, developed methods for high-throughput screening of sets of metabolites, and identified over 50 genetic loci influencing synthesis of flavor volatiles. The challenges associated with defining the target, screening diverse germplasm and managing the impact of environment on a multigenic trait involving many independent metabolic pathways are informative for efforts to improve all aspects of plant quality.

3.3. Toward a systems level understanding of abiotic stress tolerance for applications in agriculture

MICHAEL F. THOMASHOW
MSU-DOE Plant Research Lab and Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824, USA

Abiotic stresses, including drought, high salinity and freezing temperatures, greatly limit the geographical locations where crops can be grown and cause significant losses in yield on an annual basis. In recent years, many genes have been identified that can enhance plant tolerance to various stresses under lab conditions. However, taking these findings and using them to improve the stress tolerance of crop species growing in the field has been slow to develop. The reasons are many fold. One is the complex nature of stress tolerance mechanisms and the regulatory pathways that control their action. Another is that tolerance mechanisms and associated regulatory pathways overlap with considerable crosstalk. There is also the fact that in nature, plants are often challenged by multiple stresses at a given time. Given these and other complexities, it is not surprising that we do not yet have the necessary knowledge to design from first principles plants that are more stress tolerant. Such knowledge would include a systems level understanding of abiotic stress tolerance pathways. The ever increasing power of 'omic' technologies has the potential to result in such understanding, but is likely to take many more years to be achieved.

3.4. The challenges of phenotyping technologies for plant biotechnology

PIERRE LEJEUNE, F. LEYNS, C. REUZEAU, C, A. SANZ, Y. HATZFELD, F. FRANKARD, W. VANCAMP, J. DEWOLF, R. PEERBOLTE, P. PUZIO
CropDesign NV, Technologiepark 3, 9052 Gent, Belgium. http://www.cropdesign.com

CropDesign, a BASF Plant Science company, has developed TraitMill™, a high-throughput and high-resolution platform for testing the effect of plant-based transgenes on essential crop traits. The focus is currently on rice, a globally important crop, and a good model for other cereals such as corn and wheat. TraitMill™ comprises allele-design, vector construction, plant transformation, seed increase, and plant phenotyping. The trait evaluation process makes use of robotized plant transport, digital imaging tools and semi-automated statistical analysis for data production and interpretation. The standard phenotype evaluation package includes various parameters such as leaf and root biomass across time, seed yield and seed traits, stress tolerance and yield stability. A range of interesting phenotypes has been generated over the last years, including transgenic rice lines showing altered seed production, green biomass, shoot/root ratio, or flowering time. Validation of such phenotypes can be done in several rice genotypes and other cereals. The phenotypes observed in Traitmill™ was often conserved in field-trial evaluations. Thus TraitMill™ can be used as a good proxy for the validation of transgenes, in view of crop improvement. Our results demonstrate the potential of gene engineering to modify quantitative traits.

3.5. Role of pathogen effector proteins in plant innate immunity

BRIAN J. STASKAWICZ
Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720 USA

It is now well established that all classes of pathogens are able to deliver effector proteins directly to host plants often via specialized infection structures. Pathogen effector proteins are involved with the suppression or modulation of plant innate immunity and fundamentally control plant pathogenesis. Interestingly, the same proteins that modulate pathogen virulence are also involved in triggering genotype-specific plant disease resistance. In this presentation, I will highlight the original approaches that led to the discovery of pathogen effectors and how this information has shaped our current understanding of the 'dual' role of effectors in both plant pathogenesis and the activation of disease resistance signalling pathways. Furthermore, I will provide recent data from my laboratory in our attempts to employ pathogen effector proteins as molecular probes to identify host targets controlling plant innate immunity. Finally, I will present our recent data on elucidating the molecular events that are involved in effector recognition and the activation of plant disease resistance.

3.6. Evolution of adaptive traits

JOHN WILLIS
Department of Biology; Box 90338, 3314 French Family Science Center, Science Drive, Duke University, Durham, NC 27708

A pressing challenge in biology is to understand how plants adapt to their complex and often unpredictable biotic and abiotic environments. Natural genetic variation frequently exists between closely related species, populations, and even individuals within populations for traits important for adaptive responses to unique or extreme environments. Establishing the mechanistic, molecular genetic basis of this variation will result in breakthroughs in our understanding of plant physiology, ecology, and evolution, and will help guide efforts to improve the ability of crops to withstand increasing levels of environmental stresses. For over 50 years, *Mimulus guttatus* and its close relatives have been studied by ecologists because they have evolved the ability to thrive in extraordinarily diverse habitats. Now, genomic resources currently under development are making *Mimulus* an excellent system for the determination of the genetic basis of these environmental adaptations. In this talk I will outline our ongoing efforts to identify genes underlying traits conferring adaptation to seasonal water availability, salt stress, and the toxic soils of mine tailings.

3.7. Drowning in data: translating data into genetic gain

ERIK LEGG
Syngenta Biotechnology Inc., Group Leader - Applied Genomics, P.O. Box 12257, 3054 E. Cornwallis Rd., Research Triangle Park, North Carolina, 27709-2257, USA

The last century of agriculture has seen a consistent and dramatic increase in productivity. Advances in plant breeding, including the wide adaptation of hybrids and more recently, marker assisted breeding, as well as improved agricultural practices continue to create agricultural gains across a diverse range of crop plants. The gap between actual and maximum productivity, highlighted by record corn production of 385 bu/ac in 2007, compared to average US production of 153 bu/ac, indicates that significant potential for crop improvement remains, even in the most intensively studied and manipulated species.

Access to broad information in model species (e.g. genome annotation, gene expression, detailed phenotypic data, vast mutant collections, diverse germplasm, biochemical pathways and epigenetic phenomena) continues to increase knowledge of how plants function and interact with the environment. Although similar data is more and more available in crop plants, they are complemented by richer genetic, phenotypic and genetic X environment data. Further, similar findings often yield distinct hypotheses in any given species.

Advances in the translation of model plant knowledge must be complemented by the generation of robust crop-specific data, testable hypotheses and knowledge. The ability to interpret model and crop plant data in parallel will sustain, if not accelerate the rate of progress towards environment-optimized plants, whether it be corn in the mid-west, tomatoes in glass houses or sugarcane in the tropics. This is vital, as continued improvement in crop performance is critical to food quality, agricultural sustainability and energy for a rapidly growing population.

A number of current and future opportunities for translating our growing bank of knowledge into genetic gain will be explored.

3.8. Genetic engineering novel crop plants: unlimited horizons

ROBERT B. GOLDBERG
Department of Cell, Molecular, and Developmental Biology, UCLA, Los Angeles, CA 90095, USA

Approximately 10.5 billion people will inhabit the earth by the year 2050. We will need to grow more food than has been produced in the entire history of mankind in order to feed this population! And we will need to produce this food on a shrinking amount of agricultural land. During the past 10,000 years humankind has ‘engineered’ major crops such as wheat, corn, and soybean from wild plants that are not suitable for cultivation. In order to feed the world's growing population will require a new ‘green revolution’ that can lead to the production of high-yielding crops that can produce an abundance of nutritionally-balanced food using minimal inputs. Progress in plant molecular biology and genetics over the past 20 years has lead to major advances in plant genetic engineering and knowledge of basic plant processes. It is now possible to engineer any plant for agronomically important genes and regenerate transgenic plants both in the lab and field. However, we are still in the ‘embryonic stage’ of understanding the functions of most plant genes and how they are organized into regulatory networks that program the developmental and physiological processes required ‘to make a plant’. Genetic engineering provides an unlimited opportunity to change, alter, optimize, and construct ‘made to order’ new crops. In order to utilize the full potential of genetic engineering it will be necessary to understand on a grand scale the rate-limiting regulatory circuits that control major plant processes. Cracking the plant regulatory network ‘code’ will uncover the ‘secrets’ of how plant form and function evolved, and will enable novel, new approaches to be taken to improve crop plants - following the tradition of our ancestors many thousands of years ago.

3.9. Development of energy crops

CHRIS SOMERVILLE
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One of the ways in which abundant solar energy can be captured in a useful form is by photosynthesis. Combustion of lignocellulose could become a significant component of a future energy supply that does not contribute to greenhouse gas-induced climate change. To exploit this opportunity, we will need to develop plants that are specifically selected for energy production. These plants have very different properties than most of the plants that are used for food, feed and fiber. I will summarize some of the opportunities and challenges associated with the development of new energy crops through both conventional breeding and transgenic approaches.

3.10. Human and institutional drivers for realizing crop improvement

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The efficiency of crop improvement programs in the world needs to be rethought, given the low food stocks, the need to provide more food on less land, urbanization trends and the need to use more land for renewable energy production. Wonderful progress has been made in improving corn, wheat and rice yields through better breeding, agronomy and mechanization. What are the future rates of yield gains going to look like in these and other vital crops? What discoveries will drive these? What do we need to improve crops faster and more cost effectively around the world? What organizational innovations are needed?

Poster Abstracts

Listed alphabetically by first author, presenting author is underlined

1. Fruit cell wall architecture and fungal pathogen susceptibility

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The cell wall (CW) provides important architectural properties of fruit and is the site of the earliest interactions with other organisms. Pathogens, such as the necrotrophic ascomycete, *Botrytis cinerea*, secrete CW degrading proteins (CWDPs) to infect and decompose plant tissues. CW remodeling by fruit CWDPs and increasing susceptibility to *B. cinerea* occur during fruit ripening. While tomato fruit and *B. cinerea* express similar CWDPs during ripening and infection, respectively, results in Cantu et al. (PNAS 105:859-864, 2008) support the conclusion that it is the fruit enzymes that have a crucial role in the ripening-associated susceptibility increase. The hypothesis is that fruit CW self-disassembly during ripening promotes susceptibility by providing *B. cinerea* with accessible nutrients in circumstances where anti-pathogen responses are limited. Prior to ripening, fruit are resistant to *B. cinerea*; histochemical analysis suggests that anti-pathogen responses are active. Simultaneous fruit and fungal proteome and fruit transcriptome have identified the proteins secreted by *B. cinerea* and the plant genes expressed and proteins released by green and red fruit during pathogenesis. Thus, using this plant-pathogen interaction, the architecture of the plant CW can be related to the perception of the pathogen and the consequent resistance or susceptibility.

2. Conservation of the cold-responsive pathway in freezing sensitive and freezing tolerant *Solanum* plants

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In *Arabidopsis* the CBF pathway has an important role in freezing tolerance. The transcriptional activators CBF1, CBF2 and CBF3 are quickly induced by cold followed by expression of the CBF regulon, nearly 100 genes, resulting in an increase in freezing tolerance. The freezing sensitive *Solanum tuberosum* has 5 CBF genes; its freezing tolerant wild relative, *Solanum commersonii* has 4. Two CBFs of both species, CBF1 and CBF4, are cold-induced. We are determining the makeup of the CBF and other cold response pathways of *S. commersonii* and *S. tuberosum*. We have identified that these species share 50 to 70% of their cold transcriptomes. Further, the CBF regulons of these species comprise about 300 clones, with an overlap of only about 80 clones. Identification of putative orthologous groups of genes between *Arabidopsis* and *S. tuberosum* allowed us to compare their cold- and CBF-responsive genes. Preliminary results suggest there is little conservation between the cold-regulated genes of *Arabidopsis* and the two *Solanum* species. However, 15 groups of putative orthologous genes were identified as being cold-regulated in both *S. commersonii* and *Arabidopsis*, but apparently not in *S. tuberosum*. Whether these genes contribute significantly to differences in freezing tolerance between these plants, remains to be determined.

3. Regulatory changes of a transcription factor lead to autogamy in cultivated tomatoes

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Style2.1 was previously identified as the major quantitative trait locus responsible for a key floral attribute associated with the evolution of self-pollination in cultivated tomatoes. The gene encodes a putative transcription factor that regulates cell elongation in developing styles. The transition from cross-pollination to self-pollination was accompanied, not by a change in the *STYLE2.1* protein, but rather by a mutation in the *Style2.1* promoter that results in a down-regulation of *Style2.1* expression during flower development.

4. Relationship between amino acid composition and resistance to soybean aphid

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The soybean aphid (*Aphis glycines*) can produce yield losses higher than 40%. Resistance to soybean aphid has been found within the soybean genome. The *Rag1* gene limits aphid reproduction and increases mortality. The mechanism of this resistance is unknown. The nitrogen content of phloem sap is a limiting factor for aphid growth. Thus, we explored the hypothesis that *Rag1* resistance is related to the nutritional quality of the plant. Specifically, is the amino acid composition of susceptible plants different from that of resistance plants? Also, do changes in amino acid composition affect aphid population growth? To answer these questions two near-isogenic lines (Susceptible vs. *Rag1*) were grown in field conditions, with or without insecticide application, during summer 2007 in Iowa. Leaves were collected from each plot at three different developmental stages of the soybean plant and the amino acids composition of both lines was analyzed. We identified at least one amino acid (GLU) from the pool analyzed that is present in lower concentration in the resistant isoline when aphids are not present. The same amino acid decreases in response to aphids in susceptible lines, to the levels observed in *Rag1* plants (with or without aphids). Our results suggest that *Rag1* plants have a constitutive defense response against aphids.

5. Reverse genomic analysis of ethylene and sorbitol regulation in apple fruit tissues

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A unique attribute of fruit development is the ethylene mediated regulation of fruit quality and nutritional attributes. We have defined the network genes that are regulated by ethylene and/or sorbitol in transgenic apple fruit tissues obtained from plants silenced for ethylene or sorbitol biosynthesis. Using RNA obtained from these fruit tissues and custom microarrays that contain 8,976 apple genes we were able to examine the differential gene expression patterns identifying 3204 genes significantly ($p < 0.05$) regulated by ethylene, 141 by sorbitol and 57 of these were regulated by both ethylene and sorbitol, the latter a key translocated sugar that is a product of photosynthesis synthesized in many Rosaceae crops including apple. We have classified (unsupervised) the expression of these gene to define their tissue specific expression patterns as well as their functional categorization using Arabidopsis gene annotations. Defining the pathways and their regulation is the first step as it will provide a unique insight into how ethylene and translocated sugar regulate complex traits like fruit quality, shelf-life, nutrition, flavor, disease, disorders and fruit safety.

6. Exploiting natural genetic diversity for improvement of *Populus trichocarpa* as biofuels feedstocks for Canada

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Lignocellulosic feedstocks are potential sources of renewable biofuels, but immense challenges must be overcome before this potential can be realized due to the recalcitrance of secondary cell wall deconstruction. In British Columbia and many other parts of Canada, prime agricultural land is at a premium while there is a large reservoir of marginal agricultural and forested lands suitable for production of trees as potential biofuels feedstocks. Among these, poplars such as *Populus trichocarpa* are attractive candidates due to high growth rates, a reference genome sequence, well-developed genomics tools, and high levels of genotypic and phenotypic diversity. We and our collaborators have identified candidate genes in *Populus* that may modulate secondary cell wall structure and composition, and have begun to generate transgenic lines with altered expression in these genes. In an extension of this work, we will use resequencing strategies to identify SNPs in 7000 cell wall-related genes in a set 20-30 wild *P. trichocarpa* trees, use a large population of wild *P. trichocarpa* grown in common gardens to analyze a suite of wood chemistry traits, and determine the SNP genotypes of candidate genes in this population in order to identify genotype-phenotype associations for key biofuels traits. This approach may allow rapid genetic improvement of poplar as an efficient biofuels feedstock.

7. Role of ARBORKNOX2 (popKNAT1) in regulating secondary growth in *Populus*

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Secondary growth is a developmental process supported by division of meristematic cells within the vascular cambium. We have cloned *ARBORKNOX2 (ARK2)*, a *Populus* ortholog of Arabidopsis *BREVIPEDICELLUS* (also known as *KNAT1*). *ARK2* is expressed in both the shoot apical meristem and vascular tissues. Whole mount *in situ* hybridizations of cultured *Populus* plants show that *ARK*2 is expressed in the procambium in the first elongating internode during primary growth. During the transition to secondary growth in internodes 3, 5, and 7, *ARK2* expression is associated with cambium cells, as well as secondary phloem fibers and secondary xylem cells. In the bottom-most internode, where phloem fibers and secondary xylem cells have matured, *ARK2* is only expressed on the cambium region.

To further explore the regulation of *ARK2* in the secondary growth, we transformed *Populus* with *ARK2* RNAi, artificial micro-RNA (amiRNA) and overexpression constructs. Real-Time PCR data shows that *ARK2* is only partially down regulated in recovered RNAi and amiRNA transgenics, and no plants were recovered with severe knockdown of *ARK2* expression. Compared with wild type, *ARK2* knockdown *Populus* precociously form secondary phloem fibers and xylem tissue during the transition to secondary growth, have more secondary xylem tissue in the bottommost internode, and have thicker secondary cell walls in phloem fibers and secondary xylem cells. In *ARK2* overexpressing *Populus*, the shape of procambium in the first internode is altered, and is associated with an early formation of cambium. The cambium cells show more periclinal divisions, but daughter cell differentiation is inhibited, leading to a wider cambium region tissue and less phloem fibers and secondary xylem tissues. The regulation of secondary growth by *ARK2* was further demonstrated by microarray data analysis, which secondary growth related genes are down-regulated in overexpression transgenics, and up-regulated in knockdown transgenics. In summary, our preliminary data indicate that *ARK2*'s acts in regulating both cambium function, as well as differentiation and secondary cell wall formation of secondary phloem fibers and xylem cells.

8. Modification of gibberellic acid signaling for physiological sculpture of trees

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The large increases in cereal yield that were brought about by the Green Revolution demonstrated that genes affecting gibberellic acid (GA) action could have major agronomic impacts. We are examining whether there could be similar benefits of such genes for plantation forestry, but using transgenic approaches to speed breeding progress. For example, shorter and fatter trees may have improved wind-firmness, reduced water stress, and increased distribution of carbon into soil pools, of possible value for carbon accounting. The genes under study in our laboratory include those for stature reduction by inhibition of GA action, mainly via overexpression of *gai*, *rgl2*, and *GA2-oxidase*. But we are also studying the potential for growth enhancement via overexpression of *GA20-oxidase* and inhibition of *GA2-oxidase*. This poster focuses on two-year field results from poplar trees planted at high density. The tested trees have transgenes for mild inhibition of GA signaling, giving rise to substantial changes in tree form, productivity, and carbon allocation to tree roots.

9. Characterization of DTS49, an RNA binding protein involved in the splicing of DET3 mRNA which encodes a vacuolar ATPase necessary for early hypocotyl cellular elongation

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The precise genetic control of plant cellular development is critical for the success and viability of crops. The structural development of the hypocotyl involves cell elongation that enables the shoot apical meristem to reach the light, where the seedling then switches to a photomorphogenetic growth program enabling organ development. Det3 encodes a vacuolar ATPase necessary for early hypocotyl development in *Arabidopsis thaliana.* Here, we describe an RNA binding protein (RBP) involved in Det3 mRNA processing. RBPs are key regulatory proteins shown to be necessary for proper flowering and cellular development in plants. Specifically, RNA splicing, a crucial RNA processing step for many transcripts, is necessary for the proper translation of Det3. A point mutation in the branch point consensus of *det3* results in inefficient Det3 splicing and reduced DET3 protein levels causing reduced hypocotyl cell elongation and a dwarf adult phenotype. An RBP (DTS49) was identified and found to be involved in the splicing of the Det3 transcript. Overexpression of DTS49 in the *det3* mutant restores Det3 mRNA intron splicing and suppresses the dwarf phenotype. In this study, we investigate the precise role of DTS49 in Det3 mRNA splicing.

10. Viruses as targets and tools in plant RNA silencing

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RNA silencing is a natural plant defence mechanism that targets genomes of invading viruses for degradation. RNA silencing uses small RNA (sRNA) molecules to target homologous RNAs for silencing. In response, viruses have evolved to encode silencing suppressors. Alternatively, viruses can encode sRNA silencing effectors that target host anti-viral defence genes, turning host silencing to their advantage. We are developing a model for the characterization of sRNA effectors in plant–virus interactions. This model system will be used to elucidate the principles governing the dynamic role of RNA silencing in viral plant diseases for extrapolation to crop systems. Illumina sequencing by synthesis was used to profile sRNA populations in Turnip crinkle virus-infected Arabidopsis thaliana and Nicotiana benthamiana. The biogenesis mechanisms of viral sRNAs are being determined based on viral sRNA hotspot profiles in wild type infection versus infections involving host and virus mutants. sRNA-mRNA gene regulation networks that may be initiated or perturbed by the virus have been predicted. These networks may reveal how biotic and abiotic stresses direct epimutation, and through subsequent transformation to stable mutation, provide greater genetic diversity for natural selection. These results will be presented in terms of their applications in crop improvement.

11. The proteome and transcriptome of proteins responsive to salt stress in leaf and root of rice seedlings

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Rice (*Oryza sativa* L.) is the major crop in Thailand. Yield increases are required since agricultural land and water supply is becoming limited. Around 20% of the world's cultivated land and nearly half of all irrigated land are affected by salinity. High concentrations of salts cause ion imbalance and hyperosmotic stress in plants. As a consequence of these primary effects, secondary stresses such as oxidative damage often occur. We have investigated the effect of salinity in two contrasting rice varieties. One is a widely-grown, stress-tolerant cultivar (Pokkali). A detailed understanding of the effects of salt on KDML105 would be valuable when selecting for decreased salt-sensitivity while maintaining its superior culinary properties. We recorded the effects on the leaf proteome of exposing 3-week old seedlings to salinity (7 days, 120 mM NaCl). We have used RT-PCR and two-dimensional polyacrylamide gel electrophoresis (2DE) to monitor protein expression patterns in rice seedlings exposed to high salinity compared with unstressed seedling and to determine how the proteomic and transcriptomic responses are related. More than 200 protein spots were resolved in both cultivars and 9 differentially expressed spots were identified using peptide mass fingerprint analysis. These represented proteins involved in fundamental cell processes such as carbon metabolism and photosynthesis as well as several stress-responsive proteins. All were up-regulated by salt stress. We are now investigating the effects of salinity on the transcripts of these proteins to determine how the proteomic and transcriptomic responses are related.

12. Current -omics science and future systems biology: Is 'omni'-omics systems biology?

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The *first wave* in the post-genomics era has caused a flood of -omics-based science, which includes use of transcriptomics, metabolomics, etc, to study plant physiology, morphogenesis and growth. Systems biology is often used synonymously with omics-biology, however, to fully realize the potential of systems biology improvements are needed in measurement, analysis and modeling of system properties to complement the existing sophistication in molecular science. New detection and analysis approaches at the interface of plant, physical and computational sciences hold tremendous promise that needs to be explored and harnessed in order to understand, model and improve plants/crops. This *second wave* in the post-genomics era will truly enable systems biology investigations. Here we present recent results obtained from application of -omics techniques (454 EST sequencing, microarrays, RT-PCR arrays, metabolomics and proteomics) to studies ranging from that of cell wall and wood formation, hormone signaling and root development and drought tolerance. Emerging data from development of new analytical methods, anticipated bottlenecks and needs for standards and, data integration and modeling will be also discussed. Development and adoption of integrated plant systems biology approaches to understand plant properties will immensely improve the effectiveness of breeding- and biotechnology-assisted improvement in plant traits.

13. The Arabidopsis VIT1 controls iron homeostasis in plants

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Iron is an essential nutrient and iron deficiency often limits plant growth. If the mechanisms of iron uptake, distribution and regulation were clearly understood, it might be feasible to engineer plants better able to grow in soils now considered marginal and to increase crop yield in soils now in cultivation. Plants that serve as better sources of dietary iron would also improve iron malnutrition of human. To assess the role of plant vacuoles in iron homeostasis, we have studied the Arabidopsis Vacuolar Iron Transporter (At*VIT1)*. VIT1 mediates the iron accumulation in vacuoles and controls the localization of iron in Arabidopsis seed. In *vit1-1* loss-of-function mutant, iron dissipated from its normal storage while iron content in seeds was not changed appreciably. To further understand the VIT1 role in iron homeostasis, we analyzed transgenic plants that overexpress *VIT1* gene. Overexpression of *VIT1* did not alter iron content nor iron localization in seeds. However, overexpression of *VIT1* increased manganese and zinc levels in seeds; iron, manganese and zinc levels in leaves. These increased metal accumulation in leaves positively correlated with increased Fe(III) chelate reductase activity and levels of IRT1 protein in roots. The results suggest that VIT1 transports iron from the cytosol into the vacuole and controls cellular metal homeostasis by regulating cytosolic iron pools.

14. Dominant negative mutants of *Brassica napus* KRP1 expressed in canola increases yield

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Cell division in plants is positively regulated by complexes composed of a cyclin-dependent kinase (CDK) and its regulatory partner, cyclin. Active cyclin-CDK complexes phosphorylate downstream targets necessary for proper cell division. KRP is a cell cycle inhibitor that binds and inhibits cyclin-CDK complexes. A knock-out of the mammalian *KRP* (p27) in mice results in larger mice due to increased cell proliferation. We tested whether yield could be increased in plants by blocking KRP function.
Targeted Growth has developed a protein-based approach to inhibit endogenous KRP function based on an engineered dominant negative (DN) mutant KRP protein. The *in vitro* development of DN mutants of KRP will be presented.
To demonstrate that a KRP DN protein could affect cell division in a seed crop and potentially increase seed yield, proof-of-concept experiments were performed in *Brassica napus*. The At *KRP1 DN* gene was overexpressed in a seed-specific manner and large scale field trials identified several events with increased total seed yield of 17–21% over controls.
Intrinsic yield has been considered a quantitative trait, wherein many plant genes contribute to overall yield. Targeted Growth has demonstrated significant seed yield increases can be obtained in a commodity crop through manipulation of a single plant gene, namely *KRP1*.

15. Poplar allelic variation promotes long term CO_2 subterranean sequestration

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Subterranean CO_2 sequestration incorporates atmospheric C in to organic tissues such as woody roots for extended periods. Forests are the second largest sink of C; they absorb ~ 120Pg /y, of which about half becomes subterranean tissue. Extensive woody root systems result from long-lived tree species. Woody roots are less susceptible to decomposition and predation; therefore, the C fixed during secondary growth (SG) of roots remains sequestered longer. Extensive knowledge of harvestable wood formation (stem SG) is available, but accessibility limitations have restricted the study of root SG. Obvious differences in function, physiology and chemical composition between stems and roots, predict diversity of genes regulating the development of root and stem SG. This study has investigated the genetic differences between stem and root development by comparing gene expression of their cambia during SG. Total RNA extracted from both tissues and hybridized to Affymetrix whole genome Poplar microarray chips. Analysis of the data will yield candidate genes for a future Population Genetics study that will generate simple tools to enable land managers, breeders, restoration ecologist and modelers to select and measure naturally evolved trades of high CO_2 sequestration yields.

16. The ectomycorrhizal fungus *Laccaria bicolor* shapes the root system of poplar trees through phytohormone signaling

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Growth and health of poplar trees, one of the most important woody crops, is favored by symbiotic interaction with ectomycorrhizal soil fungi. Formation of ectomycorrhizae is accompanied by strong alteration of root development, such as increased root branching and arrest of root elongation. During my PhD project, I investigate the molecular processes involved in the early symbiotic cross-talk that leads to those root modifications, using *Populus tremula x Populus alba* and the ectomycorrhizal fungus *Laccaria bicolor.* I set up an *in vitro* co-culture system, which permits production of poplar/*L. bicolor* ectomycorrhizae under controlled conditions. Transcriptome profiling of poplar roots (NimbleGen oligo array) during early crosstalk with *L. bicolor* (3 days of contact) showed regulation of 891 genes, which comprise transcription factors, genes involved in ethylene signaling and biosynthesis as well as auxin signaling related genes. Further analysis will help to gain insights into the role of ethylene and auxin in poplar root development during ectomycorrhiza formation.

17. Formation of woody biomass regulated by class III HD Zip genes

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Secondary growth and wood formation in trees is a key process in the formation of woody biomass as a source of biofuels and for carbon sequestration.

The gene family of Class III HDZip genes has been shown to play a central role in regulating polarity and vascular development in Arabidopsis - a plant which is a poor object for the study of secondary growth.

In this project all poplar Class III HDZip genes were cloned and expressed in hybrid aspen. To circumvent an endogenous regulation mechanism involving microRNAs the sequences were also mutated to render them microRNA resistant.

Lines expressing the mutated Poplar ortholog of the Arabidopsis Revoluta gene (Populus Revolverblatt) show a spectacular phenotype with stunted growth, radialized and rolled leaves, and a double and at times triplication or quadruple layer of the xylem, suggesting the formation of multiple layers of cambium. ClassIII HDZip genes have thus been shown to be crucial for the formation of lignified tissue in trees.

18. Identification of elemental processes controlling genetic variation in soybean seed composition

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Complex traits governed by many genes can be dissected into more elemental processes that may be under simpler genetic control. For example, final protein concentration (SPC) in soybean seeds is determined predominately by accumulation (mg seed^{-1}) of protein, oil and carbohydrate components. Increases in SPC can arise from increases in protein synthesis or by reductions in oil and carbohydrate accumulation. Likewise, component accumulation can be further dissected into rate and duration of net synthesis. As such, similar values for SPC can result from a variety of developmental and metabolic strategies. We have identified two such strategies to achieve high SPC within a population of F2:3 lines segregating for SPC. A subset of 'high SPC' lines maintained protein content (mg seed^{-1}) fairly constant but decreased the content of other seed components (Strategy 1). A second subset of 'high SPC' lines increased protein accumulation per se (mg seed^{-1}) (Strategy 2). These lines are being screened with SSR markers to identify genomic regions associated with these two unique strategies. We hypothesize that different suites of genes determine these alternative strategies for achieving high SPC, and that these different genes are not necessarily linked to those associated with reduced oil synthesis or seed yield. If so, identifying these genes opens the possibility to overcome the commonly observed negative correlations between SPC, oil, and grain yield.

19. Microarray analysis of nitrogen use efficiency in transgenic *Brassica napus* plants

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Overuse of nitrogen fertilizer to increase agricultural yields is becoming cost prohibitive and has deleterious environmental consequences. Increasing a plant's nitrogen utilization efficiency (NUE) would lower the farming costs and limit negative environmental impacts. We have demonstrated increased NUE in transgenic canola (*Brassica napus*) plants expressing alanine aminotransferase (AlaAT), a downstream enzyme in nitrogen assimilation. Transgenic plants have been tested in excess of seven field trials and consistently show yields equivalent to controls with as much as 50% less applied nitrogen fertilizer. We have established a greenhouse test system for measuring biomass accumulation differences in canola and are using this system to screen new events and analyze NUE phenotypes. During early development NUE plants accumulate as much as 15% more biomass. To gain insight into the molecular mechanisms behind this phenotype we performed microarray analyses of wild type and NUE Brassica roots and shoots from plants at a developmental stage just prior to when biomass accumulation differences manifest. Results of these analyses suggest that key molecular pathways are altered in transgenic plants.

20. Relationship between proline hyperaccumulation and metal tolerance in plants

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Heavy metal contamination is increasing at an alarming rate and thereby causing severe impact on plant metabolism and productivity. To counterbalance the adverse impact of metals, some tolerant plants equipped with some versatile defense system are needed to be developed. Since proline accumulation is an important metabolic modification during metal stress, therefore the present study designed to explore the possible relationship between metal tolerance and proline accumulation. Responses of 25 crops and 10 metals were evaluated to test the relationship between metal tolerance and proline accumulation. Results of the present study suggested a positive relationship between proline and metal tolerance measured in terms of level of oxidative stress, tolerance index and root growth inhibition. Artificial induction of proline in wheat seedlings by non-toxic concentrations of NaCl significantly improved the metal tolerance. On the basis of present findings, proline can be recommended as an excellent molecule for the metabolic engineering of plants for the development of metal tolerance.

21. Characterization of the structure-function relationship of AvrE, an effector of the plant pathogen *Pseudomonas syringae*

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AvrE and HopM1 are two virulence effector proteins secreted into the plant cell by *Pseudomonas syringae*. Deletion of genes encoding these two effectors in the the ΔCEL mutant results in significant loss of bacterial virulence. Each of these effectors can independently restore the virulence of the ΔCEL mutant and promote disease symptoms, suggesting functional redundancy of HopM1 and AvrE. Although progress has been made on understanding the molecular action of HopM1, the function of AvrE remains largely unknown.
Recently in animal pathogens, a WxxxE motif has been described in effector proteins that seems to mimic activated G-proteins to promote disease. Analysis of AvrE and its orthologues in plant pathogens brought to light two WxxxE motifs in these proteins. AvrE and orthologues also contain a C-terminal LKKxG motif that resembles an endoplasmic reticulum retrieval signal. We performed site-directed mutagenesis to determine the importance of these motifs for AvrE function in *P. syringae* virulence.
As a complementary approach, several N-terminal and C-terminal deletion derivatives of AvrE have been made. These derivatives were expressed transiently in *Nicotiana tabacum* and stably in *Arabidopsis thaliana* to determine which portions of the AvrE protein are relevant to the virulence function and whether some of them could act in a dominant negative fashion on the virulence activity of the full-length AvrE.

22. Cold-stress early in life constrains morphogenetic events later

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Plant morphogenesis is a combined developmental decision between environmental signals and genetic-programme. However, the quality of environment is an intimate cue designing developmental pathway for a phenotype. Environmental stress early in life can strongly affect later growth and development. We tested this hypothesis imposing exogenous cold-stress (CS, 4^0C) during seedling emergence of five *Triticum aestivum* and grown under optimal glasshouse conditions. CS plants flowered significantly earlier and diverged in allocational responses. Individual trait biomass varied significantly between CS and non-CS (NCS) plants as they grew and developed. At flowering, CS plants were constrained in specific leaf area (SLA) but benefited from higher leaf mass ratio (LMR). Moreover, their lower photosynthetic efficiency and increased dark respiration rates led to a reduction in net carbon assimilation and reserve carbon storage. In most cases, individual trait size differences were more pronounced at flowering compared to pre-flowering stages, indicating that CS-induced phenotypic plasticity appeared later in the growth and development. These results suggest that cold-stress early in life triggered different selective pressures on endogenous developmental pathways. Collectively, these results lay a foundation for the comprehensive understanding of how potentially complex environmental stress system(s) during early in life are connected to diversified developmental events later, including plant reproduction.

23. Quantitative imaging of leaf and root growth at the Jülich Plant Phenomics Center

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The mission of the Jülich Plant Phenomics Center is to allow rapid selection of optimal germplasm for crop and bioenergy production under specific environmental conditions and to serve as a research platform for fundamental questions of plant development by analyzing plant physiology via non-invasive techniques. The physiology of crops is to a large part determined by photosynthesis, nutrient and water uptake. Yet, a direct analysis and improved understanding of the physiology of growth provides an immediate handle to assess plant performance. Within the last years, methods have become available that allow non-invasive monitoring of plant growth, carbon allocation, photosynthesis as well as water and nutrient uptake or translocation. These non-invasive phenotyping technologies will improve selection of plant lines resistant to drought, heat and other abiotic and biotic stress factors. The large gap between physiological investigations of model plants at the lab scale and crop breeding will be closed only, if more realistic environmental conditions are simulated in lab experiments and if more light is shed on the interaction between shoot and root growth: Roots and leaves of monocot and dicot plants are connected in a different way to the fluctuations and heterogeneities of environmental parameters to which plants are exposed above- and belowground.

24. Molecular controls on crown architecture in the model perennial hybrid poplar

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Plants possess sophisticated sensory mechanisms to monitor their surroundings. Light, in particular, is a powerful environmental cue, and a sophisticated set of photoreceptors enable plants to adapt their growth in response to shaded environments. These photoreceptors – the *phytochromes* – provide plants an elegant system for detecting their position relative to others plants. A disadvantage of this sensory mechanism, however, is that when subjected to a competitive light environment, production of biomass often suffers as plants invest resources in height growth as opposed to stem increment. Our research addresses the molecular control of crown architecture and the so-called shade-avoidance syndrome in hopes of understanding how internode elongation responds to changing light environments, how genes regulate the control of this response, and how plant-plant competition for light potentially impacts crown architecture. Hybrid poplar is seen as a readily-available source of biomass for an emerging ethanol industry in this country. However, achieving high rates of biomass production is a concern given complex interactions that arise between height growth and changing light quality when plants are grown in dense plantations. Finding answers to these questions will be critical as we seek to optimize biomass production in stands established at higher and higher densities.

25. Sorghum Tilling population

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Sorghum (*Sorghum bicolor* L. Moench) is ranked as the fifth most important grain crop and serves as a major food staple and fodder resource for much of the world, especially in arid and semi-arid regions. The recent surge in sorghum research is driven by its tolerance to drought / heat stresses and its strong potential as a bioenergy feedstock. Completion of the sorghum genome sequence has opened new avenues for sorghum functional genomics. However, the availability of genetic resources, specifically mutant lines, is limited. Chemical mutagenesis of sorghum germplasm, followed by screening for mutants altered in important agronomic traits, represents a rapid and effective means of addressing this limitation. A sorghum mutant population consisting of 1,600 lines was generated from the inbred line BTx623 by treatment with the chemical agent ethyl methanesulfonate (EMS). Numerous phenotypes with altered morphological and agronomic traits were observed from M_2 and M_3 lines in the field. A subset of 768 mutant lines was analyzed by TILLING using four target genes. A total of five mutations were identified resulting in a calculated mutation density of 1/526 kb. Two of the mutations identified by TILLING were verified by sequencing in the gene encoding caffeic acid O-methyltransferase (*COMT*) in two independent mutant lines. The two mutant *COMT* lines segregate for the expected brown midrib (*bmr)* phenotype, a trait associated with reduced lignin content and increased digestibility. The diversity of the mutant phenotypes observed in the field, and the density of induced mutations calculated from TILLING, indicate that this mutant population represents a useful resource for sorghum genomic studies.

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