P-1
Remembering Winter: Vernalization as an Environmentally Induced Epigenetic Switch. RICHARD AMASINO, Department of Biochemistry, University of Wisconsin, Madison, WI 53706. Email: amasino@ biochem.wisc.edu

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 is bud dormancy; in many species that grow in temperate climates, bud dormancy is not broken until a 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 an epigenetic switch of these MADS box genes to an unexpressed state, thus rendering the shoot apical meristem competent to flower. This epigenetic switch is caused by covalent modifications to histones of the chromatin of the flowering repressors.

P-2
Role of miRNAs and siRNAs in Abiotic Stress Responses. JIAN-KANG ZHU. Institute for Integrative Genome Biology and Department of Botany and Plant Sciences, 2150 Batchelor Hall, University of California, Riverside, CA 92521. Email: jian-kang.zhu@ucr.edu

Small non-coding RNAs ranging in size between 20 and 24 nucleotides are important regulators of mRNA degradation, translational repression, and chromatin modification. These small RNAs can be broadly classified as miRNAs (microRNAs) and siRNAs (short interfering RNAs) based on their biogenesis. We found that the expression of some miRNAs and siRNAs in Arabidopsis plants are regulated by abiotic stresses such as drought, soil salinity and cold temperatures. Data on the functional analysis of several miRNAs and siRNAs using mutants and transgenic plants will be presented to support the regulatory roles of small RNAs in plant adaptation to abiotic stresses.

P-3
Diverse Small RNA-directed Pathways in Plants. ZHIXIN XIE. Dept. of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131. Email: zhixin.xie@ttu.edu

Most eukaryotic organisms possess highly conserved RNA silencing machinery that is associated with the formation of 21-~24-nucleotide small RNAs from precursor RNA molecules containing double stranded structures. These endogenous small RNAs, which include microRNAs (miRNAs) and small interfering RNAs (siRNAs) play important roles in regulation of gene expression, maintenance of genome integrity, control of heterochromatin formation, and antiviral defense. Formation or activity of small RNAs requires factors belonging to gene families that encode DICER [or DICER-LIKE (DCL)], ARGONAUTE proteins and, in the case of some siRNAs, RNA-DEPENDENT RNA POLYMERASE (RDR) proteins. Interestingly, unlike many animals, plants encode multiple DCL and RDR proteins. Recent genetic studies in Arabidopsis revealed that plants have evolved multiple functionally specialized small RNA pathways that require distinct DCL and RDR factors. Therefore, plants provide a unique system to study the evolution, diversification, and functional adaptation of small RNA pathways. Unique functions associated with distinct DCL and RDR factors in diverse small RNA-directed processes will be presented.

P-4
High Throughput Gene Assembly and Expression Using Viral RNA Replicons Delivered by Agrobacterium. Y. GLEBA, S. Marillonnet, and V. Klimyuk. Icon Genetics GmbH, Halle/Saale, D-06120, GERMANY. Email: gleba@icongenetics.de

Plant biotechnology relies on two processes for delivery and expression of heterologous genes in plants, stable genetic transformation and transient expression with viral vectors or with Agrobacterium, but only the transient routes provide a speed and a throughput necessary for fast research and development studies. We developed an efficient, versatile and high-throughput vector engineering and expression system based on in planta assembly of functional viral vectors from separate pro-vector modules. With this system, we use agrobacteria to deliver various DNA modules that are assembled inside the plant cell with the help of a site-specific recombinase, integrase. The resulting DNA is transcribed, and undesired elements such as recombination sites are spliced out, generating fully functional viral RNA replicons. The proposed protocol allows, by simply treating a plant with a mixture of two or more agrobacteria carrying specific prefabricated modules, to rapidly and inexpensively assemble and test multiple vector/gene combinations, without the need to perform various engineering steps normally required with alternative methods. The process described is very fast (3–6 days); it provides very high protein yield (up to 5 mg per g fresh leaf biomass); and it is based on over 250 prefabricated genetic modules that allow to express a specific protein by adding specific promoters, signal/transit peptides, purification tags, protein fusions, etc., in multiparallel studies.
P-5
Signaling Networks Controlling Disease Resistance Responses in Arabidopsis. J. GLAZEBROOK, Raka Mitra, and Lin Wang. Department of Plant Biology, University of Minnesota, Saint Paul, MN 55108. Email: jglazebro@umn.edu

Plants respond to pathogen attack by activation of a battery of defense responses. Activation is controlled by a complex regulatory network. Subnetworks of this network are commonly defined based on the identities of needed small molecule signals, salicylic acid (SA), jasmonic acid and related compounds (JA), and ethylene (ET). Genetic dissection of this signaling network in Arabidopsis has identified major regulatory genes involved in these three sectors. A complete understanding of defense network topology and function requires genome-scale analysis. To this end, we have studied genetic perturbations of the network by transcriptional profiling using whole-genome microarrays. Similarity relationships among the transcriptional profiles obtained from key mutants were used to create a basic model of network topology. It is obvious that effective defense requires many regulatory and effector genes which have not yet been identified. Many of these genes are expected to show increased expression in response to pathogen attack. In an effort to identify them, plants with mutations in pathogen-induced genes have been screened for enhanced disease susceptibility phenotypes. Several important genes have been identified, including a cytochrome P450 monoxygenase required for synthesis of the antimicrobial compound camalexin.

P-6
Profiles in Scourge: Gene Expression Analysis of a Crop Killer. H. CORBY KISTLER and Kye-Yong Seong. USDA-ARS, Cereal Disease Laboratory, University of Minnesota, St. Paul, MN 55108. Email: hcckist@umn.edu

Filamentous fungi are the most common and devastating causal agents of plant disease. Among the diseases of crop plants most important worldwide is Fusarium blight of wheat and barley caused by the fungus Fusarium graminearum. With the availability of genome sequences for several filamentous fungi including F. graminearum, large-scale functional genomics programs and genome-wide expression analysis is now possible. From the Fusarium sequence we have developed the first Affymetrix GeneChip microarray based on the entire genome of a filamentous fungus. To understand the early infection cycle of the pathogen, we monitored the RNA expression profiles in newly formed spores, in maturing spores and during the early stages of spore germination. We also examined fungal gene expression during infection time-courses in wheat and barley. The ability to detect fungal genes in planta is surprisingly sensitive even without efforts to enrich for fungal transcripts. These studies will help to accelerate understanding of pathogen-host interactions by elucidating expression of pathogenicity determinants in the fungus and disease response pathways in the plant.

P-7
In Planta Transcriptional and Functional Patterns of an Agriculturally Relevant R Gene. JAMES M. BRADEEN, B. P. Millett, and D. S. Mollov. Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108. Email: jbradeen@umn.edu

Potato late blight disease, caused by Phytophthora infestans, is among the most costly crop diseases worldwide. The disease affects both foliage and tubers. Breeding efforts to improve potato late blight resistance have led to the hypothesis that foliar and tuber blight resistance is conditioned by different resistance (R) genes. Previous research also documents changes in foliar blight resistance throughout plant development, suggesting R gene regulation or function is dependent on physiological stage. The cloning of the foliar blight gene RB provides tools to test and explore these phenomena. We have developed a highly sensitive RT-PCR assay to examine transgene RB expression. RB, like many R genes, is one of a cluster of sequence similar but functionally disparate gene copies. However, our assay differentiates not only between RB paralogs and RB alleles, but even between the RB transgene and the allele from which it was cloned. We have also optimized whole plant and whole tuber assays to functionally test for late blight resistance. The potato-P. infestans pathosystem is an ideal system in which to test tissue-specific transcriptional and functional regulation of R genes, since P. infestans is a natural pathogen of two distinct plant tissues, leaves and tubers. We compared transgene RB transcription in foliage and tubers with results of our functional assays. Although the RB transgene is expressed in all plant tissues, leaves of transgenic plants are late blight resistant while tubers of transgenic plants are late blight susceptible. In related studies, we are exploring transcriptional and functional regulation of transgene RB throughout plant development. We have compared pre-flowering, flowering, and post-flowering transgenics using our RT-PCR and foliar blight resistance assays. Our research provides insights into strategies for the integration of transgene RB into potato disease management schemes. Future experiments include exploration of RB protein levels and comparison the disease response transcriptomes in various plant tissues and throughout plant development.

P-8
Use of a High-performance, Custom Microarray for Elucidation of Signaling Networks Controlling Plant Defense Responses. M. Sato, R. Mitra, R. van Poecke, J. Glazebrook, and F. KATAGIRI. Department of Plant Biology, University of Minnesota, Microbial and Plant Genomics Institute, St. Paul, MN 55108. Email: katagiri@umn.edu

In systems analysis of a biological system, it is crucial to quantitatively and economically obtain a broad spectrum of information that characterizes the state of the system in detail. mRNA expression profiling could yield data with high information content for the cost. We developed a highly accurate, small-scale, oligonucleotide-spotted microarray (‘mini’-array) for the purpose of analyzing Arabidopsis responses to pathogen infection. For a broad spectrum, we selected 464 genes that represent diverse expression patterns captured through many Arabidopsis GeneChip experiments related to biotic interactions. We employed 107 normalization genes with a wide range of expression levels for accurate array-to-array normalization. A hybridization signal from each spot was calibrated by a hybridization signal from the calibration oligonucleotide that was included in each probe solution when the array was spotted. Each probe was spotted by four pens out of total 16 pens. The patterns of four pens for different probes were made overlapping in a symmetric manner so that the pen effect was removed from the signals by fitting to a linear model. We obtained the correlation between technical duplicates better than 0.98. We conclude that we can omit technical replicates for a miniarray measurement. When the expression ratios calculated from the miniarray data were compared with those calculated from the Affymetrix ATH1 GeneChip data, the correlation was 0.88. This high quantitativity was confirmed by qRT-PCR. We are currently using the miniarray for the purpose of sensitive screening and detailed characterization of reverse genetic Arabidopsis lines and detection, classification, and mapping of naturally occurring alleles in loci controlling defense responses.
**P-9**

Testing Methods for DNA and Proteins in Transgenic Crops. RAYMOND D. SHILLITO. Bayer CropScience, Research Triangle Park, NC 27709. Email: ray.shillito@bayercrops.com

The Agricultural, Biotechnology, Grain and Food and Feed Industries test for the presence of transgenic material in plants, seeds, grain and food. Testing starts with confirmation of the transgenic nature of calli, and regenerates the plants, and follows the plant through the breeding process until it is commercial. In addition, tests are needed for ensuring purity of commercial seed, and once the crop leaves the farm gate it may be segregated for use in certain markets. Testing is performed in order to satisfy the needs of those involved in trade of grain and foodstuffs to comply with regulatory and labeling requirements that are in force in many countries. Agricultural Biotechnology companies develop methods as part of the product development cycle. These are improved as the product comes to market. Methods are also developed by commercial and government testing laboratories. This presentation will briefly describe some of the tests available for detecting and quantifying DNA and proteins in transgenic plants, seeds, grain and food. Different test methods have different costs and are used to obtain different results. Spraying plants (if they are herbicide resistant) shows if they are sensitive to herbicides. Protein-based methods such as LFS and ELISA can be used in several ways including to measure the expression of the trait (such as the PAT protein). PCR and other DNA-based methods can be used for screening or identify the actual event concerned, and RT-QPCR can estimate the amount present. It is also important to consider the resources required when deciding on the test method to use, as well as whether it needs to be done in the laboratory or the field, and whether the necessary technical skills are available.

**P-10**

Applications of Testing Methods in the Grain Industry. R. W. GIROUX. Cargill Incorporated, Wayzata, MN, 55391. Email: randal.giroux@cargill.com

Several countries have adopted or are in the process of developing legislation related to the approval of genetically modified grain and grain products and/or the mandatory labeling of foods containing these products. Most countries that have adopted an approval process or mandatory labeling schemes have set tolerances for events or thresholds for the adventitious presence of transgenic material in grain products or the final foods based on a %GM content. Once such regulations are enacted, industry and government require analytical methods to monitor supply chains, certify product compliance, and enable enforcement. To test for the presence of transgenic events or to measure the %GM requires validated methods that are fit for the purpose and are suited to the testing environment. To meet this need, the food and feed supply chain are adopting different strategies to make %GM determinations, including protein and DNA-based testing (PCR). During this presentation, several of the approaches that have been implemented will be discussed. In addition, discussion of emerging information on the impacts of genetics, processing, and method performance on these measurements will be discussed.

**P-11**

A Global Perspective on the Economic Impact of Transgenic Crop Varieties. GREG TRAXLER. Department of Agricultural Economics, Auburn University, AL 36849. Email: traxl@auburn.edu

Commercial transgenic crop varieties have been available in the US and other countries since 1996. Twenty-one countries grew genetically modified (GM) crops in 2005, but the countries of North and South America accounted for 94%, of world GM crop area. Diffusion has been concentrated among crops and traits as well; four crops (soybean, maize, cotton and canola) and two traits (herbicide tolerance and insect resistance) account for 99% of GM crop area. This presentation reviews the adoption of GM varieties, and surveys studies that have measured the level and distribution of economic benefits from GM crops. The economic benefits of the diffusion of GM crop varieties have been widely shared among farmers, industry, and consumers even though delivery has been through the private sector. GM crops have had a favourable environmental impact by facilitating reduced pesticide use and adoption of conservation tillage. Key institutional factors influencing GM diffusion, and the potential for developing countries to benefit from GM technology are discussed.
P-13

Status of Commercial Tropical Foliage Plant Micropropagation. GARY HENNEN, Oglesby Plants International, 2664 SR 71N, Altha, FL 32421-2848. Email: garyh@oglesbytc.com

As the world’s appetite for tropical ornamental plants continue to grow, regional production systems are becoming increasingly larger, sophisticated and complex. Micropropagation has been an integral part of tropical foliage production for decades and as the tropical ornamental industry grows so does the requirements for larger volumes of high quality plants. At the same time, large consumers of tropical ornamentals are constantly demanding lower price points, pushing commercial propagators to look for alternative production methods and technologies requiring significant capital investments. This presentation will look at the great success story the micropropagation industry has enjoyed working with tropical ornamentals and some of the challenges facing the industry in the near future.

P-14

Temporary Immersion Bioreactor: An Efficient Technology For Scaling-up Plant Production. M. ESCALONA1, J. González-Olmeado1, I. Cejas1, C. Aragón1, I. Capote1, R. Rodríguez2, M. J. Cañal1, J. Sandoval1, S. Roels2, P. Debergh2. 1Laboratory for Plant Cell and Tissue Culture, Bioplant Centre, University of Ciego de Avila, CUBA; 2Dept. B.O.S, University of Oviedo, SPAIN; and 1CORBANA, COSTA RICA; and 2Dept. of Plant Production University Gent, BELGIUM.

Temporary immersion has been shown to reduce problems usually encountered in liquid culture. Based on this concept, a collective of researcher belong to BioplantCenter adapted a semi-automated system for large-scale propagation of plants. This bioreactor has been named as Twin Flasks system (BIT®) and grouped into the systems with complete immersion by pneumatic driven transfer of liquid medium without medium replenishment. BIT® is relatively simple and easy to use. They enable contact between all parts of the explants and the liquid medium. The culture environment is renewal by forced ventilation during each immersion period. For special type of plants, a forced aeration in the culture recipient can be used. The injection of CO₂ permits to improve the photomixotrophic culture. BIT® has been used for in vitro commercial propagation of a wide range crops: Ananas, Saccharum sp, Musa sp, Colocasia sp, Arecaceae, Eucaliptys sp, Rosaceae, Bromelias, Paeony. In order to establish a micropropagation procedure and increase the efficacy of BIT®-technology, different parameters should be optimized. Among them, the immersion time, immersion frequency, the volume of nutrient medium, the volume of culture container, the duration of proliferation phase, the use of plant growth retardant, the number of cycle in BIT®. Plants regenerated by BIT® have not showed somaclonal variation detected by molecular probes and evaluations in the field. The simplicity and low cost of BIT® is compatible with large-scale propagation. It permits important lower labor, better biological yield and consecutively reduces production cost.

P-15

Manufactured Seed—An Efficient Method for Delivery of Somatic Embryos to Nurseries. W. C. CARLSON, Weyerhaeuser Technology Center, WTC1B10, PO Box 9777, Federal Way, WA 98063-9777. Email: bill.carlson@weyerhaeuser.com

Manufactured seed can provide a delivery mechanism for cost effective implementation of clonal forestry. Manufactured Seed technology is designed to sow somatic embryos into bare root and container nurseries, enabling seedling growing in conventional facilities. Seed design, automation and seedling cost near that of current orchard seed are all critical to implementing the technology widely in forestry. Weyerhaeuser is in late stages of development for low-cost, automated manufactured seed production processes for its seed design. The seed design incorporates many analogs of natural seed, including biodegradable materials. Manufacturing processes involve automating all stages of the process, from somatic embryogenesis through sowing in the nursery. Demonstration machinery for manufacturing and advanced automation for many of the steps in seed manufacturing are completed and operating.

P-16

Therapeutic Protein Expression in the Plant-based LEX System. VINCENT P. WINGATE, Biolex, 158 Credle Street, Pittsboro, NC 27312. Email: vwingate@biolex.com

Lemna, or duckweed, is a small aquatic plant that can be quickly transformed to produce recombinant proteins in a contained and controlled bioprocessing environment. The benefits of the LEX System™ include a high transformation efficiency, rapid clonal growth in a contained and controlled environment with high expression using a simple and inexpensive production format. To date, over 25 different human therapeutic proteins have been produced by the LEX System™, including several hard-to-make proteins and 10 different monoclonal antibodies.
P-17
Production of Biodefense-Related Proteins in Tobacco. K. WYCOFF
Planet Biotechnology. Email: kwycoff@planetbiotechnology.com

Botulism and anthrax are lethal bacterial diseases whose symptoms are caused mainly by biological toxins. Botulinum neurotoxin and anthrax spores are considered potential bioweapons, and current defenses and treatments are inadequate to protect a large target population that is likely to include civilians. Both therapeutic and prophylactic treatment with monoclonal antibodies have been shown to be more effective and safer than alternatives, but stockpiling sufficient doses is likely to be expensive. Plants as a production system offer the promise of lower costs. We have demonstrated that plant-made monoclonal antibodies to botulinum neurotoxin A are effective and safe in a mouse model. We have also produced in plants a fusion protein based on a human anthrax toxin receptor and shown it to be an effective decoy, blocking the toxic effect of anthrax lethal toxin in vitro.

P-18
Transgenic Expression and Recovery of Biologically Active Recombinant Human Insulin from Arabidopsis thaliana Oilseeds. ELIZABETH W. MURRAY, Cory L. Nykiforuk, Joseph G. Boothe, Richard G. Keon, H. Joseph Goren, Nancy A. Markley, Maurice M. Moloney. SemBioSys Genetics Inc, 110, 2985-23 Ave NE, Calgary, Alberta. T1Y 7L3 CANADA and 1Department of Biochemistry and Molecular Biology, University of Calgary, Faculty of Medicine, Calgary, Alberta T2N 4N1 CANADA. Email: murraye@sembiosys.com

With the emergence of new delivery technologies and the rise in incidence of diabetes, the demand for affordable insulin will soon exceed the current manufacturing technologies. To address this potential production shortfall, we have developed a novel expression and purification technology to produce human insulin in oilseed plants. Recombinant human precursor insulin was expressed in Arabidopsis oilseeds and was found to accumulate at a level of 0.13% of the total seed protein. The precursor protein was digested with trypsin, in vitro, to produce mature insulin with a mass identical to that of the predicted DesB26-insulin product. In addition, we confirmed the biological activity of this plant produced insulin using cell based assays of receptor phosphorylation and insulin tolerance tests in mice.

P-19
An Overview of the Orchid-fungal Symbiosis in Nature, and its Application In Vitro to Promote Conservation. L. W. ZETTLER. Orchid Recovery Program, Department of Biology, Illinois College, Jacksonville, IL 62650. Email: lwzettle@ic.edu

In nature, the orchid life cycle is initiated and closely tied to the availability of fungi in various substrates (e.g., decaying wood, Sphagnum moss). For reasons not yet understood, fungi infect orchid seeds forming coils of hyphae (pelotons) within the embryo, protocorm, seedling, and mature plant. Once lysed, these structures serve as a critical energy (carbohydrate) source until photosynthesis is initiated, and give these plants an alternative nutritive strategy (=mycotrophy) into adulthood. In terrestrial orchids, mycotrophy is believed to supplement photosynthesis, but serves as the primary source of carbon for achorophyllous species. Epiphytic orchids, which may rely on mycotrophy to a lesser extent, utilize fungi as a source of free water to resist desiccation on arboreal substrates. Given the importance of fungi in situ, the preservation of orchid seeds alone has raised conservation concerns, and has prompted interest in using fungi in vitro for propagation (=symbiotic seed germination). This presentation will discuss ongoing global efforts to cultivate orchids threatened with extinction using fungi (e.g., Platanthera holochila, endemic to Hawaii), and will discuss the potential ecological consequences of this practice.

P-20
Symbiotic and Asymbiotic Orchid Seed Germination as Tools in Conservation. S. L. STEWART. Environmental Horticulture Department, University of Florida, Gainesville, FL 32611. Email: stewart@ifas.ufl.edu

The loss of orchid-rich habitat worldwide, as well as the restoration of many historical orchid habitats has demonstrated the need for researchers to develop appropriate propagation methods for the Orchidaceae. Traditionally, seed germination has been seen as the most efficient and effective method of plant production for orchids-asymbiotic seed germination historically being the most commonly used method for both conservation or commercial ends. While asymbiotic germination does represent a simple method for the mass production of orchids, it does not take into account the physiological need for fungal mycorrhizae during seed germination and, possibly, during later life stages. Only symbiotic orchid seed germination accounts for this fungal partner in both germination and subsequent development of the orchid plant. Both the symbiotic and asymbiotic methods represent possible avenues in producing orchid seedlings for use in conservation and restoration efforts. The benefits and shortcomings of both methods will be discussed.
P-21
In Vitro Strategies for Conservation of Madagascar’s Endemic Orchids. MARGARET MARY FROM. Omaha’s Henry Doorly Zoo, Center for Conservation and Research, 3701 S 10th Street, Omaha, NE, 68107. Email: psm@omahazoo.com

Madagascar’s orchid species represent some of the most critically endangered members of the family on earth today. The high degree of endemicism; estimated at more than 80%, the richness of diversity among Madagascar’s orchids, and the continuing threats to the country’s remaining natural areas, make the country’s conservation action plan imperative if these species are to survive for the future. Ex situ conservation measures present an opportunity to help preserve the island’s more than one thousand orchid species. Few orchids are being propagated in Madagascar, and in vitro germination protocols were not previously worked out for the majority of the native orchid species. Reintroduction of orchids to the country’s remaining natural areas was virtually non-existent prior to this collaborative effort. Orchid populations have been rapidly declining in many parts of the country. A partnership between Omaha’s Henry Doorly Zoo in the USA; which provided all in vitro biotechnology training, and the University of Antananarivo, Madagascar, successfully propagated many endemic Malagasy orchids at a great distance from the seed source. A strategy of in vitro culture that allows the orchids to be returned to Madagascar while still in sterile cultures, minimizes the risk of introducing pathogens to the natural environment. The micropropagated orchids are treated to lower nutrient and sucrose levels in vitro prior to their return to protected areas, and are acclimatized to ex vitro conditions when they arrive in Madagascar. Timing the reintroductions to coincide with the wet season helps assure a higher survival rate. Additionally, cryopreservation protocols were developed at Omaha’s Henry Doorly Zoo laboratory, which are preserving orchid seed germplasm for long-term conservation purposes. Species representing 14 separate orchid genera have been successfully micropropagated with more than 600 individual orchids already returned to Madagascar from the zoo, where they have been acclimatized and reintroduced directly into Ranomafana National Park through cooperation with the government and local residents in Madagascar. In some cases no natural seedling recruitment has occurred near the mother plants first observed in 2000, and the seedlings successfully reintroduced in 2004 and 2005 represent the only juvenile specimens for some of the populations being studied.

P-22
Expanding the Utility of Alfalfa. R. A. DIXON, F. Chen, Y. Pang, and G. Peel. Plant Biology Division, Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, Oklahoma, 73401. Email: radixon@noble.org

Alfalfa (Medicago sativa) is perhaps the world’s major forage legume. Forage quality itself is an important target for biotechnological improvement. Many years of research have identified lignin as an impediment to forage digestibility, and lack of condensed tannins as promoting pasture bloat and limiting nitrogen nutrition. Recent progress on understanding and manipulating the pathways leading to lignin and condensed tannins will facilitate engineering of alfalfa and other forage legumes for reduced bloating potential and improved digestibility and palatability. The above traits involve natural products, which are made by plants for their own health care (ie to ward off pests and pathogens). However, the impact of plant natural products on human health is increasingly recognized. With the advent of genetic and genomic approaches, the synthesis of many plant natural products is now understood at a level to permit their engineering in crop plants. Alfalfa has been engineered as a delivery vehicle for isoflavone phytoestrogens and the antioxidant epicatechin. Many other compounds of nutraceutical or pharmaceutical value could be profitably made in alfalfa. Forage crops with genetically improved quality (output) traits will benefit both the health of the animals that consume them and the environment through reductions in waste excretion and greenhouse gas emission. Furthermore, the same modifications to lignocellulose that improve digestibility may also improve the processing ability of forage crops for biofuel production.

P-23
From Crops to Biorefineries. O. V. SELIFONOVA. Biotechnology Development Center (BioTDC), Cargill Incorporated, Minneapolis, MN 55440. Email: olga.selifonova@cargill.com

Alternative or genetically modified crops have become an integral part of modern biotechnology. Current methodology for introduction of foreign genes into economically important plant species can be used not only for crop improvement and production of novel products in plants, but also for alteration of biomass composition that is tailored for needs of industrial bioprocessing. Bio-based industrial chemicals derived from biomass, vegetable oils and carbohydrates have all prerequisites to compete with the scale, flexibility and efficiency of the petrochemical industry. 3-Hydroxypropionic acid (3-HP) is one of the examples of biobased industrial chemicals that can be produced from renewable sugars. The chemical is not commercially available in large quantities, but has the potential to be a new industrial platform chemical. 3-HP has two functional groups and can be readily converted to a range of important chemicals, such as acrylic acid and 1,3-propanediol. Currently, no known organism makes 3-HP as a metabolic end-product. Using various sources of genetic information we designed and constructed several different metabolic routes to 3-HP. We then selected one route for further development based on a series of design criteria, including high theoretical yield from sugars. This route also required significant use of directed evolution to modify and improve enzyme activities. This work provides an example of the use of modern biotechnology to develop sustainable routes to industrial chemicals from crop-based renewable resources.

P-24
Development of Roundup Ready® Alfalfa Varieties. MARK MCCASLIN, Peter Reisen, Holly Deery, Sharie Fitzpatrick, and Stephen Temple. Forage Genetics International, P.O. Box 339, Nampa, ID 83653-0339. Email: mccaslin@forgegenetics.com

Roundup Ready® Alfalfa (RRA) was jointly developed by Forage Genetics International (FGI) and Monsanto Company. In 1998 FGI produced ~150 transgenic alfalfa plants expressing the CP4 EPSPS gene, driven by an enhanced version of the Figwort mosaic virus promoter. Alfalfa is a cross pollinated autotetraploid plant. To achieve high trait purity (i.e. >90% Roundup tolerant plants in a RRA variety) a two transgenic event, dihomogenic breeding strategy was adopted. From the initial 150 transgenic plants two commercial transgenic events were selected based on their agronomic performance, agronomic performance, molecular characterization and reproductive stability of the transgene insert. Event-specific PCR markers were developed to genotype Roundup Ready® plants carrying one or both transgenic events. Both Roundup Ready® Alfalfa (RRA) events were introgressed into elite FGI breeding populations using a modified backcrossing breeding strategy (MBCs). Four to six cycles of modified backcrossing were used to develop FD3 to FD9 type breeding populations. Advanced MBCs lines containing the two commercial events (RRA event B and RRA event D) were kept separate in the field and in the greenhouse. Elite parents were selected from breeding nurseries established with seedlings from these advanced MBCs lines. Crossovers between elite parents containing event B were crossed to elite parents containing event D. The 1:1 dihomogenic progeny, containing a single copy of each event, were identified using event specific markers. Based on pedigree and agronomic characteristics sets of these dihomogenic plants became Syn0 parents for RRA experimental varieties. In 2003 Syn1 seed of several RRA experimental varieties (FD3-FD8) was produced in isolation near Nampa, ID. Trait purity of these populations ranged from 92.5 to 94.8%, with a mean of 93.8%—very near the theoretical, expected value of 93.7%. In August and September 2003 multiple location variety trials were established to evaluate agronomic performance and crop safety of RRA experimental varieties. Forage yield and quality data collected in 2004 and 2005 showed performance of the RRA experimental varieties, under conventional herbicide treatment, equal to or better than the commercial check varieties. Crop safety of the RRA experimental varieties sprayed with maximum labeled rate of Roundup herbicide was excellent. Following US. deregulation of the two transgenic events in June 2005, fifteen RRA varieties were commercially released in August, 2005. *Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.
P-26

Development and Characterization of Alfalfa Populations Tolerant to Glycophosphate. GLEN R. ROGAN, Shane Fitzpatrick, Todd Pester, Daniel Kendrick, Michael Horak, Melinda McCann, Karu Kaminanandu, Stephen Temple, and Mark McCaslin. Monsanto Company, 800 N Lindbergh Blvd, St. Louis MO 63167. Email: glennon.j.rogan@monsanto.com

Monsanto Company and Forage Genetics International have developed varieties of Roundup Ready® alfalfa that are tolerant to glyphosate, the active ingredient in Roundup® agricultural herbicides. Roundup Ready alfalfa was developed using Agrobacterium-mediated plant transformation to stably incorporate into the alfalfa genome a coding sequence that encodes for a glyphosate-tolerant form of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). Roundup Ready alfalfa varieties enable growers to apply Roundup agricultural herbicides from planting through five days before cutting, providing an additional tool for improved weed control, excellent crop safety and preservation of yield potential and forage quality. Characterization of Roundup Ready alfalfa plants was performed as part of the food, feed and environmental safety assessment performed prior to regulatory submissions. Key questions addressed were whether the introduced trait or the transformation and regeneration process impacted the phenotype of alfalfa or composition of forage and whether there were any significant environmental impacts associated with the introduction of Roundup Ready alfalfa. Information and data on the introduced trait indicate that the CP4 EPSPS protein is safe for consumption. Other than the introduction of tolerance to glyphosate, there were no biologically meaningful phenotypic differences between Roundup Ready alfalfa populations and the alfalfa control or conventional reference varieties. The levels of key nutrients and components in Roundup ready alfalfa forage were comparable to the control and within the population of commercially available alfalfa varieties. Collectively, these results establish that Roundup Ready alfalfa is safe for use as feed or food and for release into the environment. **Roundup and Roundup Ready are registered trademarks of Monsanto Technology LLC.

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Impact of Yieldguard Rootworm on Corn Rootworm Control, S. C. JOHNSON, G. R. Heck, and T. T. Vaughn. Monsanto Company, Chesterfield, MO 63017. Email: ty.t.vaughn@monsanto.com

In 2003, the Monsanto Company commercialized YieldGuard® Rootworm (YGRW) protected corn hybrids. These hybrids use a variant Bt Cry3Bb1 insecticidal protein which is known to be biologically active against several species within the Coleoptera family Chrysomelidae, including western corn rootworm, Diabrotica virgifera virgifera LeConte, northern corn rootworm, D. barberi Smith and Lawrence, and Mexican corn rootworm, D. v. zeae Krysan and Smith. YGRW expresses the Cry3Bb1 protein in the root system where larvae feed and inflict damage. Thus, the level of expression is critical to the control of the insects that come into contact with the root system. Through a series of experiments, it was shown that while the level of Cry3Bb1 protein may vary between hybrids and over time, the level of protection is not affected by such variation; that is, the expression level in commercial hybrids is sufficient to provide consistent protection for CRW larval feeding. There are many benefits for growers, consumers and the environment associated with the commercialization of YGRW. YGRW hybrids are more efficacious than soil and seed-applied insecticides in protecting roots from larval feeding damage. The Cry3Bb1 protein produced in the root does not require activation (as many conventional insecticides do), and its performance is unlikely to be impacted by severe environmental conditions. Varieties containing the Cry3Bb1 protein are also combined through conventional breeding with other genetically enhanced maize varieties. Combinations with herbicide tolerance and lepidopteran insect protection provide hybrids to growers that offer more complete crop protection from the most economically damaging insect and weed pests. This performance and grower satisfaction has been demonstrated over the past 2 years of commercial experience. YGRW technology was initially launched in 2003 on approximately 400,000 acres. The number of acres has steadily increased over the past 2 years to approximately 4.5 to 5 million acres in 2005. Growers that have adopted YGRW technology have been extremely pleased with the performance and yield protection generated from planting these hybrids. In 2005 for example, most growers realized a significant yield advantage over other control options. This advantage was likely augmented due to the severe drought in 2005 across much of the corn belt. The YGRW technology protected the roots from larval feeding allowing the roots to access water from a deeper zone within the soil profile.

P-28

Wide-cross Whole-genome Radiation Hybrid Mapping in Cotton. DAVID M. STELLY. Dept. of Soil and Crop Sciences, Texas A&M University, College Station, TX USA 7843-2474. stelly@tamu.edu

The development of animal genomics was catalyzed strongly by the advent of whole-genome in vitro radiation hybrid (RH) mapping. In lieu of a comparable in vitro plant system, we opted to develop an in vivo system, based on interspecific hybridization between Gossypium hirsutum L. and G. barbadense L. using irradiated pollen. The underlying rationale was that [1] an egg cell nucleus could be used to “rescue” the irradiated sperm nucleus, [2] the interspecific nature of hybridization could provide allelic diversity, [3] the gamma irradiation could segment the inherited paternal genome, and [4] the unique origin of each radiation hybrid would greatly reduce and potentially eliminate chimerism within individual panel members. Initial experiments established a workable irradiation treatment and culminated in the establishment of a 5-Krad wide-cross whole-genome radiation hybrid (WWRH) panel of G. hirsutum. RH maps were derived by analysis of SSR data using RHMAP software, and were compared to locally available linkage maps and hypoaneploid cytogenetic stocks. While the 5-Krad (50-Gy) RH panel was capable of detecting synteny among unlinked primitive linkage maps, the map resolution seemed to be too low in some areas. We therefore re-investigated higher dosages, and constructed and characterized a second WWRH panel after 8-Krad segmentation of the G. barbadense L. genome. Limited numbers of strategically chosen markers were used to compare WWRH mapping results to the 5-Krad WWRH maps and to linkage maps. The results indicate WWRH mapping can contribute significantly to cotton genomics.

P-29

Evolution of Chromatin Structure and Function. S. M. Kaeppler. Department of Agronomy, University of Wisconsin, Madison, WI 53706. Email: smkaepl@wisc.edu

DNA packaging into chromatin is necessary for stable chromosomal segregation, and also affects transcription. DNA is packaged around octamers of histones, and histone and DNA modifications determine local chromatin states. Transitions among chromatin states occur via chromatin remodeling proteins. Protein motifs involved in chromatin modification and remodeling are conserved across plants and animals. However, important differences are observed when comparing plant and animal proteins, and monocots versus dicots proteins. In this presentation, I will discuss how our consortium has discovered putative chromatin proteins in plants using queries from diverse species. DNA methyltransferases, SET-domain proteins, and methyl-binding domain proteins will be used to exemplify evolution of chromatin proteins. Functional consequences will be discussed, and patterns of divergence will be highlighted.
P-30

Allium Genomics: Exploiting Model Plants for Analyses of Enormous Nuclear Genomes. M. J. HAVEY. USDA-ARS and University of Wisconsin, Madison, WI 53706. Email: mjhavey@wisc.edu

Enormous genomic resources have been developed for the grasses, culminating with the complete genomic sequence of rice and reduced-representation sequencing of maize. These extensive resources may be applicable to other major groups of monocots outside of the grasses. The order Asparagales (carries the Alliums and asparagus) and the commelinids (carries the grasses) are sister monophyletic groups within the monocots. The Alliaceae (onion, garlic, leek, chive, bunching onion, among others) is the second most economically important family in the monocots, following only the Poaceae. The huge nuclear genomes of the Alliaceae are major constraints to the development of genomic resources. We sequenced asparagus and onion BACs and revealed high densities of retroelements and transposons with few open-reading frames. We also observed little synteny on the recombinational and sequence levels among asparagus, onion, and rice, as might be expected given that the Asparagales and commelinids split at least 130 million years ago. Nevertheless, genomic resources developed for the grasses are useful for translational genomics of the Alliums. Occasionally physically linked sequences in rice show genetic linkage in onion and this microsynteny across shorter genomic regions aids in the identification and mapping of candidate genes. Single-copy expressed regions in the rice genome show significant similarities and share most introns with coding regions in onion, allowing the development of PCR-based markers carrying indels or single nucleotide polymorphisms to evaluate for associations between candidate genes and economically important traits.

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High Efficiency and High Throughput Transformation of Cereals Mediated by Agrobacterium for Functional Genomics. T. KOMARI. Plant Innovation Center, Japan Tobacco Inc., 700 Higashibara, Iwata, Shizuoka 438-0802, JAPAN. Email: toshihiko.komari@ims.jti.co.jp

Major cereals joined the list of plants that can be transformed by A. tumefaciens a decade ago. Then protocols for rice and maize have been tremendously improved. Overall efficiency has been increased at least 10 times, range of transformable genotypes widened, time for tissue culture shortened, workload for tissue culture lessened, and related techniques developed like elimination of selectable markers from transformants and reduction of vector backbone transfer. The progress in transformation technology, which plays indispensable roles in applications including construction of T-DNA tagged lines, map-based cloning, characterization of cloned genes and large-scale screening for gene effects, is a key factor in current and future advances in cereal functional genomics. Throughput of transformation in functional genomics must be very high. Rice is ideal in this context because of remarkable efficiency, short tissue culture periods, and small workloads for tissue culture. Maize is also high in efficiency of transformation but falls short of rice. Thus a possible option is to screen gene effects initially in rice and to characterize selected genes when appropriate induction reagents are applied. The use of mesoporous silica nanoparticles (MSNs) was shown to deliver marker genes into animal cells (Radu et al., 2004). The distinct feature of this nanoparticle is that it can both deliver DNA as well as chemicals encapsulated in the particles. Controlled release of the filling substance is also possible using this material (Gruenhagen et al., 2005). Here we show that this material can be used for transforming tobacco mesophyll protoplasts and immature maize embryos. Transgene expression was observed both transiently and stably. In addition, chemicals encapsulated in the MSN can be controlled-released in planta when appropriate induction reagents are applied. The use of mesoporous silica nanoparticles to deliver transgenes and various substances simultaneously into plant cell opens a wide range of applications for future plant genomic study.

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Periodic arrays of vertically aligned carbon nanofibers (VACNFs) have been demonstrated as effective vectors for delivery of large molecules, including DNA, into a variety of cell types. Using an approach that combines the massive parallelism of whisker-mediated delivery and the precision of microinjection, arrays of VACNF needles may be surface modified with material and simultaneously pressed into the intracellular domains of large numbers of mammalian and plant cells. While a variety of micromachined materials have been studied as gene delivery arrays for both plant and animal cells, these materials often have been found to have inadequate aspect ratio or lack mechanical strength to effectively penetrate the plant cell wall. In contrast to these micromachined materials, VACNFs feature a covalent bonding structure that provides strong, but flexible, vertical elements well suited to the rigors of cellular interfacing. They also have extremely high aspect ratio, with tip diameters of typically less than 100 nm and lengths up to many tens of microns. Mechanical strength and high aspect ratio provides for effective penetration into cells, including those protected by rugged cell walls such as yeast and pollen. The surface of nanofibers may be modified with adsorbed or covalently attached biomolecules and interfaced into cellular targets, including direct penetration into the nuclear domain. In this overview, we will describe the fabrication and functionalization of VACNFs and the application of these modified VACNFs as massively parallel delivery vectors for both plant and animal cell transformation.

P-33

DNA-coated Nanoparticles Mediated Transgene Expression in Plant Cells. François Torney, Brian Trewny, Supratim Giri, Victor Lin and Kan Wang, Center for Plant Transformation, Iowa State University, Department of Agronomy Ames, IA 50011 and Department of Chemistry, Iowa State University, Ames, IA 50011. Email: ftorney@fastate.edu

Plant genetic engineering relies mostly on biolistic and Agrobacterium-mediated transformation technologies. Both techniques allow DNA delivery into plant cells and subsequent integration into the genome. Recently, the development of nanomaterials such as mesoporous silicate nanoparticles (MSN) was shown to deliver marker genes into animal cells (Radu et al., 2004). The distinct feature of this nanoparticle is that it can both deliver DNA as well as chemicals encapsulated in the particles. Controlled release of the filling substance is also possible using this material (Gruenhagen et al., 2005). Here we show that this material can be used for transforming tobacco mesophyll protoplasts and immature maize embryos. Transgene expression was observed both transiently and stably. In addition, chemicals encapsulated in the MSN can be controlled-released in planta when appropriate induction reagents are applied. The use of mesoporous silica nanoparticles to deliver transgenes and various substances simultaneously into plant cell opens a wide range of applications for future plant genomic study.


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Transfection: A Reliable and Efficient Method for Maize Transformation.
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Maize has historically been amongst the most difficult of plant species to genetically engineer. Currently, particle bombardment (biolistics) and Agrobacterium tumefaciens are used to insert foreign genes into maize in a stable manner. Both of these methods are covered by patents making them expensive to practice for commercial purposes. We have developed a third method, one that is free of patent encumbrance, is reasonably efficient, and produces regenerated transformed plants in a reasonable mount of time. Calloused immature zygotic embryos are incubated with naked DNA in a sterile cuvette for a given length of time and then electroporated at a specified voltage, capacitance, and pulse length and wave shape. A decay wave was successful whereas a square wave was not. The protocol was optimized for transient expression of the GUS gene and those parameters were used to produce stable transformants at a frequency of ~0.5%. The use of tobacco Rb7 matrix attachment regions (MARs) gave a frequency of nearly 4% using the optimum transient parameters. Further optimization of the electroporation parameters for stable transformation resulted in a transformation frequency of nearly 4% even without MARs. Further optimization could raise the transformation frequencies even higher. Without MARs, single copy insertion events composed about 50% of the total. With MARs, the proportion of single copy insertion events was 90% of the total. This data implies that Transfection is a viable transformation system for maize.

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A Novel Plant Transformation Technology—Lipoic Acid. YINGHUI DAN*, Monsanto Company, 700 Chesterfield Parkway, St. Louis, MO 63017 and *Current address: Institute for Advanced Learning and Research, and Departments of Horticulture and Forestry, Virginia Polytechnic Institute and State University, 150 Slayton Avenue, Danville, VA 24540. Email: ydan@vt.edu

For the first time a breakthrough in plant transformation technology has been discovered in the antioxidant, lipoic acid (LA), found in most living organisms. Utilizing LA in Agrobacterium-mediated transformation processes across five different plant species has significantly improved the transformation methods, even for previously recalcitrant genotypes. Frequencies of soybean independent plant transgenic events were increased from 0.6 to 3.6%, potato from 3 to 19%, tomato from 28 to 94%, and wheat from 2.9 to 5.4%, and putative transgenic embryo frequency of cotton from 41 to 61%; frequency of escapes was reduced in soybean from 92 to 72%, potato from 50 to 16% and tomato from 91 to 53% under the optimal conditions. This study also demonstrated that the increase of the transformation frequency and reduction of escapes in tomato were accompanied by 2-fold reduction in severity of browning/necrosis of Agrobacterium-infected cotyledonary tissues, 2-fold increase in the survivability of the transformed cotyledonary tissues, 4-fold increase in the percentage of transgenic shoots and 3-fold reduction of the percentage of non-transgenic shoots when using LA under optimal conditions. LA application in plant transformation has dramatically resolved the three common problems in plant transformation: recalcitrance, tissue browning/ necrosis of the transformed cells/tissues, and escapes, which severely limit the number of transgenic plants that can be regenerated.

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Evaluation of an Automated Image Analysis System for Factors which Stabilize Gene Expression. J. M. CHIERA and J. J. Finer. Department of Horticulture and Crop Science, OARDC/The OhioState University, Wooster, OH 44691. Email: chierea.4@osu.edu

A commonly observed phenomenon after transformation of plant tissues, regardless of delivery method, is variable gene expression. This phenomenon requires the time consuming task of creating a large number of clones in order to increase the probability of obtaining a successful transformation event. The reasons for this variability in gene expression are unknown, but it is likely that protein or message instability, or transgene silencing is involved. With the advent of green fluorescent protein (GFP) and other fluorescent proteins, it is now possible to track transgene expression from the time of DNA introduction to plant recovery. The use of fluorescent proteins in combination with automated image collection and analysis software allows for the continuous monitoring and quantification of in vivo gene expression in multiple tissue samples over time. With these tools, we can begin to dissect factors that positively or negatively affect transgene expression in transiently and stably transformed tissues. We have evaluated sequences that appear to stabilize GFP expression in a cotyledon transient expression system. In control bombardments, without the additional sequences, GFP expression peaks 24 hours post bombardment and declines to minimal levels within 72 hours. Use of GFP fused to some sequences led to extended GFP expression through 168 hours. Co-introduction of these same sequences on different vectors extended expression slightly but not to the same extent as the GFP-fusions. These factors may influence gene expression by stabilizing protein, mRNA, or by suppression of host silencing.

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Agrobacterium Mediated Gene Transfer in Plants. MARC VAN MONTAGU. Institute Plant Biotechnology for Developing Countries (IPBO), Universiteit Gent, BELGIUM. Email: mamon@psb.Ugent.be; Website: www.psb.Ugent.be

The discovery that some Rhizobiaceae had evolved into pathogens capable to genetically engineer a plant cell became an interesting chapter in the study of plant microbe interactions. The exploitation of this capacity, now a good twenty years ago, to engineer novel traits into crop plants, was a major breakthrough in fundamental and applied plant sciences. The possibility to add and alter, thanks to the iRNA-technology, to silence at will a set of genes in most plant species, got molecular plant sciences off the ground. It also allowed the engineering of new traits in some of the major crops. Now, global agriculture requires the extension of this technology to the improvement of barely domesticated crops. Unraveling the molecular base of plant growth and development, of stress response and of biomass production is now becoming possible. Industry already plans the production of new compounds and new materials in plants. To do the research and these applications successfully, we need a substantial improvement of the efficiency of our universal gene vector Agrobacterium. We can ineed not rely on engineering of some model plants. We should be able to engineer the highest yielding cultivars and make that they can be grown in a sustainable way, this means with less water and nutrients than in today’s agriculture. It is urgent that specialist in “Plant Cell and Tissue Culture” take up this challenge.