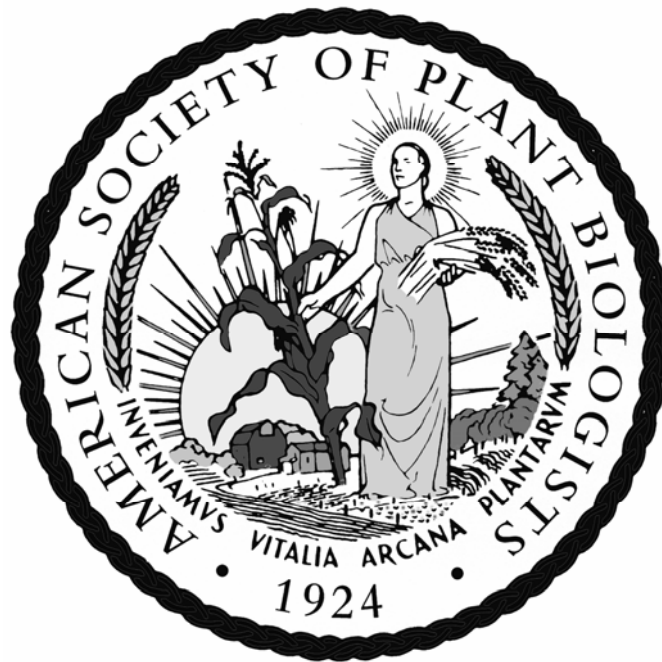


71st Annual Meeting of the
Northeast Section of the
American Society of Plant Biologists



"Fueling the Future through Plant Biology"
SUNY College of Environmental Science and Forestry
Syracuse, NY
June 1-2, 2007



**71st Annual Meeting of the
Northeast Section
American Society of Plant Biologists**

**State University of New York
College of Environmental Science & Forestry
Syracuse, New York
June 1 – 2, 2007**

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Schedule of Events
NEASPB Meeting, June 1-2, 2007

Friday, June 1, 2007

Registration: **Baker Foyer** (Poster setup in 319 Baker Hall)

11:30 am - 1:00 pm

Symposium: **140 Baker Hall**

1:00 – 1:15 pm Welcome – Provost Bruce Bongarten, SUNY-ESF

1:15 – 2:00 pm **Dr. Sharon Regan**, Queen’s University
Activation Tagging in Poplar: Gene Discovery for Improved Biomass

2:00 – 2:45 pm **Dr. Yi Li**, University of Connecticut
Genetic Improvement of Biomass Production and Development of a GM-Gene-Deletor Technology for Energy Crops

Break: **Baker Foyer**

2:45 – 3:15 pm

Symposium: **140 Baker Hall**

3:15 – 4:00 pm **Dr. Wilfred Vermerris**, University of Florida
Towards Sustainable Energy Production: Designing Maize and Sorghum for the Ground Up and Down

Reception: **319 Baker Hall and 3rd Floor Lounge**

4:00 – 6:30 pm Wine-cheese reception and poster session
(odd numbers 4:00-5:00 pm; even numbers 5:00-6:00 pm)

**Executive
Committee
Meeting:**

310 Baker Hall

5:45 – 6:30 pm

Banquet: **Alumni Lounge, Marshall Hall**

6:30 – 8:00 pm Buffet style banquet
Live Music: **Lost Time**

Saturday, June 2, 2007

Breakfast: Baker Foyer

8:00 – 9:00 am Continental Breakfast

Platform

Session I: 140 Baker Hall

9:00 am **IMPACT OF MYCORRHIZA AND GROWTH RATE ON VASCULAR MATURITY IN ROOTS**

Jeff H. Taylor, Slippery Rock University

9:15 am **THE EVOLUTION OF PHOTOSYNTHETIC GENES IN RESPONSE TO POLYPLOIDY**

J. Coate, D. Ilut, J. Doyle, Cornell University

9:30 am **BREEDING AND COMMERCIALIZATION OF NEW, HIGH-YIELDING VARIETIES OF SHRUB WILLOW AS A FEEDSTOCK FOR BIOENERGY, BIOFUELS AND BIOPRODUCTS**

Kimberly Cameron, Timothy Volk, Lawrence Abrahamson, Lawrence Smart, SUNY-ESF

9:45 am **CELL WALL BIOSYNTHESIS IN SHRUB WILLOW: ANALYSIS OF WOOD COMPOSITION AND GENE EXPRESSION PATTERNS**

Michelle Serapiglia, Kimberly Cameron, Arthur Stipanovic, Lawrence Smart, SUNY-ESF

10:00 am **PUTRESCINE OVERPRODUCTION CHANGES THE OXIDATIVE STATE OF POPLAR CELLS IN CULTURE AND AIDS IN ALUMINUM TOLERANCE**

S. Mohapatra, R. Minocha, S.C. Minocha, University of New Hampshire

Break: Baker Foyer

10:15 - 10:45 am

**NEASPB
Business
Meeting:**

140 Baker Hall

10:45 - 11:15 am

**Platform
Session II:**

140 Baker Hall

11:15 am

**ISOLATION AND CHARACTERIZATION OF ARSENIC
INDUCED GENES FROM *CRAMBE ABYSSINICA***

Bibin Paulose, Azma Zulfiqar, Om Parkash (Dhankar),
University of Massachusetts

11:30 am

**RESPONSE OF FOLIAR PHYSIOLOGY OF TREES TO
CALCIUM ADDITION AT HUBBARD BROOK
EXPERIMENTAL FOREST, N.H., USA**

Rakesh Minocha, Stephanie Long, P. Thangavel, Subhash C.
Minocha, Christopher Eagar, Charles T. Driscoll, U.S. Forest
Service and University of New Hampshire

11:45 am

**A BIOTECHNOLOGICAL APPROACH TOWARDS
RESTORATION OF THE AMERICAN ELM**

Nicholas Kaczmar, Charles Maynard, William Powell, SUNY-
ESF

12:00 pm

**CHARACTERIZATION OF *SHL6* (SUPPRESSOR OF *hrl1*
PHENOTYPE) GENE OF *ARABIDOPSIS THALIANA*
INVOLVED IN REGULATION OF CELL DEATH AND
DEFENSE AGAINST PATHOGENS**

Aditya Dutta and Ramesh Raina, Syracuse University

12:15 pm

Drawing for book give-away

ABSTRACTS – SYMPOSIUM “Fueling the Future through Plant Biology”

S1 - ACTIVATION TAGGING IN POPLAR: GENE DISCOVERY FOR IMPROVED BIOMASS

Dr. Sharon Regan, Department of Biology, Queen’s University, Kingston, Ontario

Activation tagging is an insertional mutagenesis technique which results in the dominant upregulation of an endogenous gene. A large-scale *Agrobacterium*-mediated transformation protocol was used to transform the pSKI074 activation-tagging vector into *Populus tremula* x *P. alba* hybrid poplar. As a result of generous funding by Genome Canada, we have produced the largest population of activation-tagged poplar trees in the world, approximately 1800 independent lines. In the first 1000 lines we have screened the trees for developmental abnormalities and have a visible mutant frequency of 2.4%, with alterations in leaf and stem structure as well as overall stature. Most of the phenotypes represent new phenotypes that have not previously been identified in poplar and in some cases not in any other plant as well. Molecular analysis of the T-DNA inserts of a subpopulation of mutant lines reveal both single and double T-DNA inserts with double inserts more common in lines with visible phenotypes. The broad range of developmental mutants identified in this pilot screen of the population reveals that it will be a valuable resource for gene discovery in poplar. The full value of this population will only be realized as we screen these lines for a wide range of phenotypes.

Dr. Sharon Regan is a Canada Research Chair in Fundamental Plant Biology and Associate Professor at Queen's University. She has been an investigator on two large-scale genomics projects, one on potato (www.cpgp.ca) and the second on trees (www.arborea.ulaval.ca). Her research is focused on understanding the molecular mechanisms controlling plant development with a specific focus on wood formation in trees and disease resistance in potato.

S2 - GENETIC IMPROVEMENT OF BIOMASS PRODUCTION AND DEVELOPMENT OF A GENE-DELETOR TECHNOLOGY FOR ENERGY CROPS

Dr. Yi Li, Department of Plant Science, University of Connecticut, Storrs, CT

Gibberellins (GAs) and brassinosteroids (BRs) play important roles in many growth and developmental processes in higher plants. We have recently produced transgenic poplar plants that overexpress a BR biosynthetic gene and a GA biosynthetic gene. The poplar plants overexpressing the BR gene or both the BR and GA genes exhibit many desirable phenotypes such as increased biomass production, improved root systems and higher survival rates. On the other hand, overexpression of the GA gene alone results in poor root systems and low survival rates under both greenhouse and field conditions. Our data suggest that manipulation of BR or both BR and GA contents in poplar may lead to improvement in biomass production of poplar.

Transgene flow is a serious concern in plant biotechnology, particular for transgenic bioenergy crops such as poplar and willow plants. To address the gene flow and food safety problems of transgenic plants, we have recently developed a highly efficient 'gene-deletor' system to remove all functional transgenes from pollen, seeds or both, leading to production of non-transgenic pollen and seeds. The gene-deletor system may provide a bioconfinement tool for transgenic crops, with special applicability towards bioenergy crops like poplar and willow trees.

Dr. Yi Li is a professor in the Department of Plant Science, University of Connecticut. He currently serves as the director and principal investigator for the New England Center for Invasive Plants

Yi Li obtained his B.S. degree from Beijing Forestry University, China, in 1982 and his Ph.D. from SUNY College of Environmental Science and Forestry, Syracuse, NY in 1989. He completed his postdoctoral training at University of Missouri, Columbia, MO from 1990-1993.

Yi Li was an Assistant Professor at Kansas State University, Manhattan, KS from 1994 to 1998, and then moved to the University of Connecticut in 1998 as an Assistant Professor, an Associate Professor and is now a Professor.

S3 – TOWARDS SUSTAINABLE ENERGY PRODUCTION: DESIGNING MAIZE AND SORGHUM FROM THE GROUND UPAND DOWN

Dr. Wilfred Vermerris, Associate Professor of Agronomy, University of Florida, Gainesville, FL

Political and environmental concerns have stimulated the interest in the production of transportation fuels from renewable resources. In the US the majority of fuel ethanol is currently produced from corn starch, but this will not be sustainable in the future. Other crops that can be used for ethanol production include species that produce sugar (e.g. sugar cane) or large amounts of vegetative biomass. Vegetative biomass, such as stover, straw or wood chips, consists largely of plant cell walls. The plant cell wall is a complex matrix that consists of cellulose and hemicellulose, lignin, pectin, hydroxycinnamic acids, and proteins. Enzymatic saccharification of lignocellulosic biomass results in the formation of hexose and pentose sugars that can be converted to ethanol via microbial fermentation. While production of cellulosic ethanol is feasible from an energy-balance perspective, it is currently not economically competitive. Along with improvements in bioprocessing and fermentation, enhancing the yield and composition of the biomass itself offers a tremendous potential to make ethanol production more cost-effective.

The genetic control of cell wall biosynthesis is, however, poorly understood. As part of an NSF-funded plant genome project, novel cell wall mutants of maize have been generated through both forward and reverse genetics approaches. A high-throughput spectroscopic screen aimed at the identification of changes in chemical composition was developed to screen the Uniform*Mu* population. This population was created by introgressing the *Mutator* transposable element system into an inbred line, thus ensuring a high forward mutation rate *and* genetic uniformity. A set of 39 mutants with so-called spectrotypes but otherwise no distinct visual phenotype were identified. Some of these mutants look promising with respect to cellulosic ethanol production. See <http://cellwall.genomics.purdue.edu> for further details.

Sorghum has a number of characteristics that make it ideally suitable as a dedicated bioenergy crop. We are developing *brown midrib* sweet sorghum as a dual-source feedstock for ethanol production: sugar-rich juice from the stalk will be directly processed into ethanol through microbial fermentation, while the sorghum bagasse is processed for the production of cellulosic ethanol. The *brown midrib* trait has been shown to enhance the yield of fermentable sugars. We are using comparative genomics approaches and the recently released sorghum genome sequence to identify and isolate the genes underlying these and other useful traits. This includes genes controlling root size and architecture. Roots are often overlooked, but can make a significant contribution to soil organic matter content as above-ground plant parts are being harvested for bioprocessing. Compared to current practice, development of dedicated bioenergy crops will require selection for entirely different traits, offering many opportunities and challenges.

Dr. Wilfred Vermerris is Associate Professor of Agronomy at the University of Florida Genetics Institute and Adjunct Associate Professor of Agricultural & Biological Engineering at Purdue University. He has an M.S. degree in biomolecular engineering from Wageningen Agricultural University and a Ph.D. in genetics from North Carolina State University. He co-authored the book *Phenolic Compound Biochemistry* (Springer '06).

ABSTRACTS – PLATFORM SESSIONS

O1 IMPACT OF MYCORRHIZA AND GROWTH RATE ON VASCULAR MATURITY IN ROOTS

Jeff H. Taylor. Department of Biology, Slippery Rock University, Slippery Rock, PA 16057

It has long been accepted that the distance behind the tip at which the tracheary elements become mature is positively correlated with the rate of root growth. However, this finding is based on a few seminal studies. Likewise, how the presence of a mycorrhizal association impacts this has received little consideration. As mycorrhiza tend to produce smaller, and therefore potentially slower-growing root systems, our hypothesis was that mycorrhizal root systems would induce tracheary elements to mature closer to the tip. In the current experiment, both ectomycorrhizal roots (*Pinus banksiana*) and roots associated with arbuscular mycorrhizal fungus (AMF); (*Zea mays*, *Lycopersicon esculentum* and *Fragaria virginiana*) were investigated. In each system, anatomical maturity was determined via chlorazol black E staining to reveal lignification, and functional maturity was assessed by cellufluor conductivity. In the case of the ectomycorrhizal roots, growth of mantle-bearing roots was absent, and the tracheary elements matured much closer to these tip than in their non-mycorrhizal counterparts. Conversely, AMF status had no impact on the distance behind the root tip at which the tracheary elements matured. In considering the rate of root growth alone, there was generally no correlation within a particular species between root growth and xylem maturity. However, when multiple species were analyzed together, a significant positive correlation between root growth rate and a greater distance between the tip and the mature tracheary elements was observed. Impacts of these findings on water and nutrient acquisition will be discussed.

O2 THE EVOLUTION OF PHOTOSYNTHETIC GENES IN RESPONSE TO POLYPLOIDY.

J. Coate, D. Ilut, and J. Doyle. Dept. of Plant Biology, Cornell University, Ithaca, NY 14853.

The effect of polyploidy varies among classes of genes. We are using a comparative bioinformatics approach to investigate patterns underlying the evolution of photosynthetic genes in response to polyploidy in *Arabidopsis* and soybean (*Glycine max*). In both species, photosystem I has retained fewer duplicates from polyploidy than photosystem II or the Calvin cycle, suggesting that photosystem I genes are “duplication resistant.” In *Arabidopsis*, the Calvin cycle has the highest fraction of gene families retaining duplicates from polyploidy. In soybean, photosystem II exhibits the greatest retention rates. In both species, polyploid-derived duplicates of photosystem genes maintain highly correlated expression profiles, providing no evidence for sub- or neofunctionalization, and perhaps supporting the “gene balance” hypothesis. In contrast, Calvin cycle genes duplicated by polyploidy in *Arabidopsis* seem to follow one of two extreme fates – maintaining tight co-regulation or diverging dramatically. Two gene families have retained duplicates from polyploidy in which one gene product is chloroplast localized and the other is cytosolic, providing strong evidence for neofunctionalization. These gene pairs exhibit pronounced expression divergence. Gene pairs for which both copies encode cytosolic isoforms also exhibit greater expression divergence than their chloroplast counterparts. In conjunction with the persistent coexpression of duplicated photosystem genes, this suggests that the expression of photosynthetic genes is highly constrained, making sub- or neofunctionalization unlikely.

03 BREEDING AND COMMERCIALIZATION OF NEW, HIGH-YIELDING VARIETIES OF SHRUB WILLOW AS A FEEDSTOCK FOR BIOENERGY, BIOFUELS AND BIOPRODUCTS

Kimberly D. Cameron¹, Timothy A. Volk², Lawrence P. Abrahamson^{1,2}, and Lawrence B. Smart¹. Departments of ¹Environmental and Forest Biology and ²Forest and Natural Resources Management, SUNY College of Environmental Science and Forestry, 1 Forestry Drive, Syracuse, NY 13210.

The development of perennial energy crops as feedstocks for biorefineries and bioenergy will combat global warming and contribute to petroleum independence, while stimulating rural economic development. Dedicated energy crops, such as fast-growing shrub willow (*Salix* spp.), are ideally suited for growth in the Midwest and Northeastern U.S. SUNY-ESF has established and maintains the largest willow breeding program in North America with a genetically diverse collection of >700 varieties. Many of these individuals have been used as parents in our breeding program and, through controlled crosses, have produced in excess of 4000 new varieties. Breeding compatibility was species- and gender-dependent with intra-specific crosses generally successful more often than inter-specific crosses. From among >2000 individuals produced in 1999, 82 were chosen for further study based on stem height and total stem area after two growing seasons. In 2002, selected individuals were planted in a replicated, randomized selection trial with four current production varieties. After the second two-year rotation, 24 varieties had yields greater than the current production variety, 'SV1'. Individuals displaying exceptional growth were scaled-up and planted in two regional yield trials in 2005 using the double row production-style spacing. Concurrently, SUNY-ESF has worked closely with Double A Willow, a commercial nursery, to promote deployment of shrub willow crops. Presently, over 100,000 plants representing 16 elite SUNY-ESF varieties have been established at Double A Willow to produce planting stock needed to meet the projected scale-up demand in the next few years.

04 CELL WALL BIOSYNTHESIS IN SHRUB WILLOW: ANALYSIS OF WOOD COMPOSITION AND GENE EXPRESSION PATTERNS

Michelle J. Serapiglia¹, Kimberly D. Cameron¹, Arthur J. Stipanovic², and Lawrence B. Smart¹. (1) Department of Environmental and Forest Biology, (2) Department of Chemistry, State University of New York – College of Environmental Science and Forestry, 1 Forestry Dr. Syracuse, NY 13210.

The cultivation of shrub willow (*Salix* spp.) in North America is being scaled-up commercially as a dedicated energy crop. Due to its high genetic diversity, shrub willow has great potential for genetic improvement through traditional breeding. The SUNY – College of Environmental Science and Forestry (SUNY-ESF) has an extensive breeding program for the genetic improvement of shrub willow for biomass production and since 1998 breeding efforts have produced over 200 families resulting in over 5,000 progeny. Several progeny have reached a 40% increase in harvestable biomass over check varieties. With the success of the breeding program thus far, the selection of shrub willow for optimum processing in a biorefinery is promising. The separation of the wood components, cellulose, hemicellulose, and lignin, involves costly and time-consuming pretreatment methods that can be minimized based on the ratio of these three components. The goal for this project was to utilize a high-throughput method for the compositional analysis of willow wood to aid in the rapid screening of willow clones developed in the breeding program. A select group of willow clones was analyzed using high resolution thermogravimetric analysis (HR-TGA) and nuclear magnetic resonance (NMR). Significant differences in wood composition were observed among the willow clones. In addition, expression patterns of genes involved in primary and secondary cell wall biosynthesis will be correlated with wood composition among selected parent and progeny genotypes. Gene expression analysis of lignin biosynthetic genes and selected carbohydrate active enzymes (CAZs) is currently in progress.

O5 PUTRESCINE OVERPRODUCTION CHANGES THE OXIDATIVE STATE OF POPLAR CELLS IN CULTURE AND AIDS IN ALUMINUM TOLERANCE

S. Mohapatra¹, R. Minocha², S.C. Minocha¹

¹. Department of Plant biology, University of New Hampshire, Durham, NH 03824

². USDA Forest Service, NERS, Durham, NH 03824

The effect of enhanced putrescine metabolism on the oxidative state of poplar (*Populus nigra x maximowiczii*) cells with enhanced putrescine metabolism was studied. The cell lines used in this study were either transformed with a *GUS* gene (control) or with a mouse *ornithine decarboxylase* (*mODC*) gene; the latter resulted in over-production of putrescine and a concomitant enhancement in its catabolism, and is called HP (high putrescine) line. Activities of some of the important enzymes of the reactive oxygen species (ROS) scavenging machinery were measured over a seven day culture period in the two cell lines. The cellular contents of a common reductant glutathione and amino acids proline and glutamate were also analyzed. Glutathione reductase and monodehydroascorbate reductase activities increased in response to enhanced putrescine metabolism, and the activity of ascorbate reductase remained mostly unchanged. Contents of reduced glutathione were lower in the HP cells. Proline content increased on some days, and glutamate content was lower on all seven days in the HP as compared to the control cells. While no significant difference in the mitochondrial activity was detected, enhanced putrescine metabolism resulted in increased membrane damage in the cells. Treatment with aluminum (a potential inducer of mitochondrial oxidative damage) was better tolerated by the HP cells as compared to controls. We conclude that, while enhanced putrescine metabolism in itself makes poplar cells vulnerable towards increased oxidative damage, high putrescine has a protective role against aluminum toxicity.

O6 ISOLATION AND CHARACTERIZATION OF ARSENIC INDUCED GENES FROM CRAMBE ABYSSINICA

Bibin Paulose, Azma Zulfiqar and Om Parkash (Dhankar). Dept. Of Plant, Soil and Insect Sciences, University of Massachusetts Amherst, MA 01003.

Arsenic(As) has been known as a carcinogen as well as an acute poison for centuries. Well-known high-As groundwater areas have been found all over the world. Phytoremediation renders an eco-friendly and sustainable method to remediate the As polluted sites. Crambe (*Crambe abyssinica*) is reported to be able to tolerate and accumulate unusually high amount of As. Understanding the molecular mechanism of high As tolerance is essential to improve the efficiency of uptake thereby exploiting the plant for commercial phytoremediation. PCR select subtraction hybridization was employed to isolate arsenate induced genes from Crambe seedlings. After differential screening, 105 positive clones from the subtracted library were sequenced. The sequences were categorized based on their similarity with reported sequences in the databases. Heat shock proteins dominated the subtracted library. Many novel sequences were present in the library, which were hitherto uncharacterized or not reported. A variety of reductases *viz.* peptide methionine sulfoxide reductase, oxophytodienoate reductase, dioxygenases, aldo-keto reductase and sulfite reductase, were found to be differentially expressed implying that the As altered the redox potential at cellular level. Glutathione transferases including the Tau and Phi subfamily that are involved in cellular detoxification were also represented in the library. Other important sequences were ATPases, drug transporter/antiporter, phosphosulfate kinases, adenylyl transferase, methionine synthetase, proteins involved in ubiquitin proteolytic pathway along with transcription factors and RNA binding proteins. Reverse subtracted colonies were also sequenced and found that genes involved in photosynthesis are the most down regulated in arsenate stress. Expression analysis and functional characterization are being carried out to elucidate the mechanisms of arsenic tolerance in plants.

07 RESPONSE OF FOLIAR PHYSIOLOGY OF TREES TO CALCIUM ADDITION AT HUBBARD BROOK EXPERIMENTAL FOREST, N.H., USA

Rakesh Minocha, Stephanie Long, P. Thangavel, Subhash C. Minocha, Christopher Eagar and Charles T. Driscoll. US Forest Service and University of New Hampshire, Durham, NH

Acidic deposition has depleted calcium (Ca) from soils in northeastern forests. Calcium silicate (wollastonite) was added to watershed 1 (WS1) of the Hubbard Brook Experimental Forest in October 1999 to study its effects on various aspects of ecosystem function. In the present study, changes in foliar physiology of red spruce (at high elevation only) and hardwoods (sugar maple, yellow birch, and American beech at three different elevations) were studied in order to evaluate the effects of Ca addition on stress physiology. The effects of Ca addition were species-specific and varied with elevation. In general, the physiology at high elevation was significantly different from low elevation. At mid to high elevations, an increase in Ca lowered free putrescine in the foliage of sugar maple indicating remediation from stress caused by Ca deficiency. A lack of inverse relationship between putrescine and Ca at low elevation suggests the absence of Ca deficiency at this elevation. However, the increase observed in total chlorophyll and glycine in sugar maple at low and mid elevations was caused by positive indirect effects of Ca addition on soil base cation status and mycorrhizal associations as reported previously. Except for a significant decline in soluble Mg and Mn, red spruce physiology was not affected by Ca addition. Interannual variations in physiology, possibly caused by changes in climatic and edaphic factors, were observed for several metabolites at both the reference (east of watershed 3) and Ca-treated (WS1) sites during 2004 and 2005.

08 A BIOTECHNOLOGICAL APPROACH TOWARDS RESTORATION OF THE AMERICAN ELM

Nicholas Kaczmar, Charles Maynard, and William Powell. SUNY-ESF, Syracuse, NY

Dutch Elm Disease (DED), caused by the fungal pathogen *Ophiostoma novo-ulmi*, has pushed the American elm to the brink of extinction. Efforts to combat DED, such as the use of fungicides and vector control, have provided limited results. Efforts to combat DED have recently focused on the development of disease-tolerant trees. Genetic transformation offers an attractive alternative to breeding because it offers the potential to transfer specific traits into selected genotypes without affecting their desirable genetic background. A synthetic antimicrobial peptide, ESF39a, has been developed by our lab and successfully expressed within the vascular tissue of American elm via *Agrobacterium* mediated transformation. Preliminary results suggest that expression of the antimicrobial peptide reduces symptoms of DED in transgenic plants compared to in the wild type. Four confirmed transgenic events are currently being grown under field conditions. We are currently comparing these trees with wild type trees for resistance levels as well as phenotypic differences such as relative growth rate and xylem vessel diameter.

O9 CHARACTERIZATION OF *SHL6* (SUPPRESSOR OF *hrl1* PHENOTYPE) GENE OF *ARABIDOPSIS THALIANA* INVOLVED IN REGULATION OF CELL DEATH AND DEFENSE AGAINST PATHOGENS

Aditya Dutta and Ramesh Raina

Department of Biology, Syracuse University, Syracuse, NY 13244

Lesion mimic mutants spontaneously develop lesions mimicking pathogen-associated cell death in plants. These mutants are powerful tools to decipher programmed cell death pathways. The *hrl1* (*Hypersensitive Response-like Lesions 1*) mutant of *Arabidopsis* is characterized by spontaneous lesion formation, constitutive expression of defense genes, and enhanced resistance to virulent bacteria and oomycete pathogens. These results suggest that HRL1 is a negative regulator of cell death and pathogen defense in *Arabidopsis*. To identify additional components of HRL1-mediated defense pathway(s), T-DNA insertional knockout mutants were created in the *hrl1* background to identify the mutants that would suppress the *hrl1* phenotypes. One such mutant, *shl6 hrl1*, had greatly suppressed *hrl1*-associated spontaneous lesions and defense-associated phenotypes. The *SHL6* gene was cloned and found to code for a transcription factor. The *shl6* mutation segregates in a non-mendelian fashion. We were unable to identify the homozygous null alleles of *shl6*, suggesting that null mutants of *shl6* are lethal. Constitutive overexpression of *SHL6* was also found to be lethal. Together these results suggest that plants must maintain a precise amount of SHL6 protein for their survival. Therefore, to better understand the role of SHL6, transgenic plants over- and under-expressing *SHL6* gene from a Dex-inducible system are being constructed. This should allow us to fine-tune the level of expression of *SHL6* in plants and should help us in determining its role in cell death and defense activation.

ABSTRACTS – POSTER SESSION

P01 ANALYSIS OF CANDIDATE VTC3 GENES IDENTIFIED VIA A GENETIC APPROACH

C. Euler and P.L. Conklin. Dept. of Biological Sciences. SUNY Cortland, Cortland, NY 13045.

Ascorbic acid biosynthesis in plants proceeds via the intermediates D-mannose and L-galactose. Although alternative pathways clearly exist, several lines of evidence suggest that this D-mannose/L-galactose – based pathway is crucial. Three ascorbic acid – deficient *Arabidopsis* mutants (*vtc1*, *vtc2*, and *vtc4*) led to the discovery of three of the ascorbic acid biosynthetic genes in this pathway, therefore genetically validating the pathway. The other proposed biosynthetic genes in this pathway have also been functionally identified. Remaining to be identified is the VTC3 gene. The *vtc3-1* and *vtc3-2* EMS-generated mutants have a significant ascorbate deficiency and appear to be unaffected in ascorbate recycling. Map-based cloning with over 3,400 individual F2 mapping lines has narrowed the VTC3 locus to a ~147 kb region bounded by a centromere distal Cereon In/Del and a centromere proximal SSLP near the bottom of chromosome II. Forty candidate VTC3 genes lie within this region. None of the candidate genes appear to be related to known ascorbic acid biosynthetic genes. It is possible that VTC3 encodes a polypeptide involved in the regulation of ascorbate biosynthesis. Segregating populations of insertion mutant lines representing many of the candidate genes have been tested for ascorbate deficiency with the use of a simple qualitative nitroblue tetrazolium - based assay. The results of this analysis will be presented and discussed.

P02 ROLES OF THE ARABIDOPSIS YSL (YELLOW STRIPE-LIKE) FAMILY OF METAL TRANSPORTERS

Heng-Hsuan Chu, Brian M. Waters, and Elsbeth L. Walker, Biology Department, University of Massachusetts, Amherst

Here, we describe two members of the *Arabidopsis* (*Arabidopsis thaliana*) Yellow Stripe-Like (YSL) family, *AtYSL1* and *AtYSL3*. The YSL1 and YSL3 proteins are members of the oligopeptide transporter family and are predicted to be integral membrane proteins. YSL1 and YSL3 are similar to the maize (*Zea mays*) YS1 phytosiderophore transporter (ZmYS1) and the *AtYSL2* iron (Fe)-nicotianamine transporter, and are predicted to transport metal-nicotianamine complexes into cells. *YSL1* and *YSL3* mRNAs are expressed in both root and shoot tissues, and both are regulated in response to the Fe status of the plant. β -Glucuronidase reporter expression, driven by *YSL1* and *YSL3* promoters, reveals expression patterns of the genes in roots, leaves, and flowers. Expression was highest in senescing rosette leaves and cauline leaves. Whereas the single mutants *ysl1* and *ysl3* had no visible phenotypes, the *ysl1ysl3* double mutant exhibited Fe deficiency symptoms, such as interveinal chlorosis. Leaf Fe concentrations are decreased in the double mutant, whereas manganese, zinc, and especially copper concentrations are elevated. In seeds of double-mutant plants, the concentrations of Fe, zinc, and copper are low. Mobilization of metals from leaves during senescence is impaired in the double mutant. In addition, the double mutant has reduced fertility due to defective anther and embryo development. The proposed physiological roles for YSL1 and YSL3 are in delivery of metal micronutrients to and from vascular tissues.

P03 CHLOROPLAST PROTEASE SPPA1 IS NON-ESSENTIAL FOR NORMAL PLANT GROWTH AND DEVELOPMENT.

Carolyn Wetzel, Laura Harmacek, Lee Yuan, Judith Wopereis, Anna Naito, Rhiannon Chubb, Paula Turini. Biology Dept. Smith College, Northampton, MA 01063.

Chloroplasts have a variety of proteases required for protein processing and turnover under non-stress and stress conditions. Mutation of some of the proteases results in dramatic changes in phenotype demonstrating that they play an essential function in the plastid. It has been proposed that chloroplast SppA1, associated with stromal lamellae of the thylakoid, is involved with turnover of proteins during acclimation to high light conditions. We identified two *Arabidopsis thaliana* T-DNA insertional lines with insertions in the single copy *SPPA1* gene. Both lines lack detectable *SPPA1* mRNA and protein. Insertional mutants were compared to wild type plants regarding their general life cycle, morphology, chloroplast morphology, pigmentation, quantum efficiency, NPQ, and acclimation to high light. In some cases, slight differences were detected between the genotypes but in general disruption of the *SPPA1* gene had little effect compared to wild type plants. The substrate for SppA1 remains enigmatic. Continued analyses of the plants under different stress conditions and stages of development are ongoing.

P04 ENGINEERED RICE PLANTS FOR ENHANCED RESISTANCE AND DECREASED UPTAKE OF ARSENIC

Indrajit Dutta, Denise Debrito, and Om Parkash Dhankher, Department of Plant, Soil, and Insect Sciences, University of Massachusetts-Amherst, MA 01003

The specific aim of this project is to engineer rice plants for enhanced resistance to and decreased uptake of arsenic in seed grains and rice straw. Arsenic is an extremely toxic carcinogenic metalloid pollutant that adversely affects the health of more than 450 million people worldwide. Recently, several studies have shown that rice grown on arsenic contaminated soils accumulates high levels of arsenic in seed grains and straw. Thus arsenic uptake by rice plants plays an important role in the transfer of this toxic element into the food chain. The basis of this proposed research stems out from my current research on phytoremediation of arsenic in model plant system *Arabidopsis*. Previously, we examined the *Arabidopsis* endogenous arsenate reductase, AtACR2, activity that affects the electrochemical state and binding of arsenic in roots to further understand arsenic movement in plant tissues (Dhankher et al., 2006, PNAS, 103(14): 5413-18). We have a proof-of-concept in *Arabidopsis* that transgenic plants overexpressing an arsenate reductase, AtACR2, were highly resistant to arsenate (6-7 fold more biomass) and accumulated 3-fold less arsenic in aboveground tissues as compared to wild type control plants, when grown on media containing very high levels of arsenic. Now we are transferring this knowledge from this successful work in model plant *Arabidopsis* to rice plants for increased resistance to and reduced uptake of toxic elements. This project will lead to the development of strategies for blocking the entry of toxic arsenic and other toxic metals into rice plant roots and to further prevent the translocation of arsenic from roots to shoot tissues and seeds. Therefore, the engineered rice will have less arsenic in seed grains, which will be safer food for human consumption, and rice straw will be safer feed for livestock.

P05 ENDOGENOUS LEVELS OF ABA AND PLANT PHOSPHORUS CONTENT IN DROUGHT STRESSED SOYBEANS (GLYCINE MAX) INOCULATED WITH *GLOMUS FASCICULATUM*

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The present study compared the growth and endogenous level of the abscisic acid in VAM and non VAM soybean plants subjected to drought. The results showed a significant negative correlation between ABA and shoot dry weight by VAM as well as by drought (F-test: $P < 0.001$). VAM plants had much lower root/shoot dry weight ratio as compared to non VAM plants. The presence of VAM caused a variation of 58.3 % in the seed dry weight of soybean plants (F-test: $P < 0.001$). There was a negative correlation between the seed dry weight (gms)/plant and the ABA content (F-test: $P < 0.001$). A positive linear correlation between the shoot dry weight and the phosphorus content was observed ($P < 0.001$). The ABA level and phosphorus content (mg)/gm of the plants were negatively correlated by VAM as well as by drought. The ABA levels in plant shoots across all the treatment combinations were found to be positively correlated to arbuscular + vesicular colonization of roots ($P < 0.001$). ABA and phosphorus content had a significantly negative linear correlation (F-test: $P < 0.001$). The ABA and total seed weight/plant by VAM had a significantly negative correlation (F-test: $P < 0.001$). Test show a significantly negative correlation between the endogenous levels of ABA and the shoot dry weight/plant (F-test: $P < 0.001$). The VAM plants subjected to drought showed lower levels of ABA as compared to droughted non-VAM plants.

P06 IDENTIFICATION OF SUBTILASES IN ARABIDOPSIS THALIANA SEEDLINGS

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The *Arabidopsis thaliana* genome has 56 genes coding for proteolytic enzymes belonging to the subtilisin gene family. Although mRNA level gene expression can be determined by techniques such as DNA microarray and RT-PCR, there is no easy way to study protein-level expression of these genes. The approach taken here was to use antibody made to synthetic peptides, the sequences of which were derived from conserved regions of the *Arabidopsis thaliana* subtilases. These antibodies were used to monitor prefractionation of the proteins extracted from plant tissues by ion exchange spin column chromatography followed by SDS-PAGE and Western blot analysis. The pool of proteins that were detected by the antibodies was analyzed by LC-MS-MS using a Michrom C18 AQ column in-line to the Applied Biosystems Q-Star XL mass spectrometer operated in Information Dependent Acquisition mode to obtain identification of peptide ions by database matching to the fragment ion spectral data. Ten peptides of the subtilase, At5g67360, also known as ARA12, were identified. To find subtilases of much lower abundance, the pool of proteins was also concentrated and further separated by SDS-PAGE stained with Coomassie Blue. Bands and spaces between the bands were excised. Proteins were subjected to in-gel disulfide reduction, alkylation and trypsin digestion, and then analyzed by LC-MS-MS and protein identification using a combination of several programs such as ProID, Protein Prospector, and Mascot. Supported by NSF DBI-0445825

P07 ASSESSMENT OF SOIL MICROBIAL DIVERSITY AT HUBBARD BROOK EXPERIMENTAL FOREST USING 16S rRNA APPROACH

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Acidic deposition has depleted calcium (Ca) from soils in northeastern forests. Calcium was applied in the form of wollastonite (calcium silicate, CaSiO₃) at watershed 1 (WS1) of Hubbard Brook Experimental Forest (HBEF) in 1999 to address the question of improving forest growth by restoration of Ca pools. Recent studies showed that Ca treatment caused a decline in microbial biomass N, potential net N mineralization and soil inorganic N levels. However, no detailed information is available on the effects of Ca-treatment on changes in the microbial community profiles (abundance and composition) or in specific microbial function/role(s) in these soils. In this study, we provide the first detailed characterization of bacterial diversity in HBEF through analysis of small-subunit rRNA gene (16S rRNA). DNA was isolated from soil samples collected from reference and Ca-treated sites in HBEF and amplified using universal 16S rRNA primers. The amplicons were cloned and nearly 400 clones from two sites were partially sequenced. Preliminary results showed that *Acidobacterium* phylotype was the most abundant and diverse group among the analyzed clones. Other phylotypes including *Proteobacteria*, *Planctomycetes*, *Bacteroidetes* and unclassified bacteria also represent the library, while divisions such as *Verrucomicrobia*, *Genera_incertae_sedis_OP10* represented only 1% or even less in the library of clones analyzed. *Nitrospira* was found only at reference site, while *Firmicutes*, *Actinobacteria* and *Genera_incertae_sedis_TM7* were seen in Ca-treated site. Community profiling via DGGE analyses revealed that there were qualitative differences between the two sites. The excision of representative DGGE bands and their sequencing is in progress.

P08 SOLATING CHROMIUM-INDUCED GENES FROM *CRAMBE ABYSSINICA* FOR PHYTOREMEDIATION OF CHROMIUM CONTAMINATION

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Chromium (Cr) is a serious environmental pollutant due to its widespread use in industries such as tanning, corrosion, plating, pigments manufacturing and nuclear weapons production. Cr (VI) is generally considered to pose the greatest human health risk because of it being toxic, mutagenic and carcinogenic. There is no cost-effective environmental-friendly Cr remediation strategy available so far. Plants can be used to cleanup the Cr pollution by accumulating, stabilizing or transforming into less toxic form Cr(III). Previously, we have analyzed the uptake of Cr and other toxic metals in *Crambe abyssinica* and other Brassica species. Crambe accumulated high levels of Cr and As in the shoot tissues and thus has potential to be utilized as an ideal non-food crop for phytoremediation of heavy metals and metalloids. The present study was undertaken with an aim to isolate and characterize the genes induced in response to Cr stress in Crambe using a PCR-Select Subtractive cDNA Hybridization approach. After subtraction and differential screening, 69 positive cDNA clones from the subtracted library were sequenced. The sequences were categorized based on their similarity with reported sequences in the databases. Forty-five different types of genes were found to respond to Cr stress. Among these were Transcriptional factors, Chitinases, Thi-J like protein, Peroxidases, Glutathionases-S-Transferases, Aquaporins, oxidoreductases, harpins, zinc and iron binding proteins and many novel sequences with unknown functions. Currently, we are analyzing these genes for expression analysis and functional characterization using both forward and reverse genetic approaches. The candidate genes will be used to engineer non-food high biomass *C. abyssinica* plants for phytoremediation of Cr contaminated soil and sediments.

P09 ANALYSIS OF THE EXPRESSION OF KEY POLYAMINE BIOSYNTHETIC GENES IN *ARABIDOPSIS*

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Using promoter::GUS fusion approach, we studied the spatial and temporal expression of two paralogues each of arginine decarboxylase (*ADC*), S-adenosylmethionine decarboxylase (*SAMDC*) and glutamate decarboxylase (*GAD*) genes in all tissues and organs during the entire life of *Arabidopsis thaliana*. The results show that *ADC1* was expressed moderately in cotyledonary veins and lamina, hypocotyl and petiole vascular tissues, apical meristems with low expression in root tips during the first 15 days post germination (DPG). Moderate expression of *ADC2* was noticed in cotyledonary veins, hypocotyl veins and root vascular tissues (except root tips) following germination, that gradually declined by 15 DPG. In mature plants *ADC2* expression was high in stem, leaf trichomes and moderate in rachis, flowers and siliques while *ADC1* expression was more in flowers, siliques and leaves. Uniform expression of *SAMDC1* in all organs was observed at early stages, but its expression was localized in only a few tissues in mature plants, while *SAMDC2* expressed highly in rachis, rosette leaves, flowers and siliques. *GAD1* showed high expression in all parts of the plant during first 15 DPG while the expression of *GAD2* was limited to cotyledon and hypocotyl veins. Bioinformatics study of promoter regions of the six genes revealed presence of several common motifs; e.g. MYB1AT (dehydration response), GAREAT (GA response) and ABRE (ABA response). Detailed analysis of the expression of each gene in response to salinity and osmotic stress was also analyzed. This is the most comprehensive analysis of the expression of the polyamine metabolic pathway in any plant.

P10 ENHANCED POLYAMINE METABOLISM AND SULFUR AMINO ACIDS IN POPLAR CELLS

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Enhanced polyamine metabolism (via over-expression of a mouse ornithine decarboxylase - mODC gene) was seen to cause a large reduction in the cellular content of two sulfur-containing amino acids methionine (Met) and cysteine + cystine in cell suspension cultures of poplar (*Populus nigra x maximowiczii*). This was accompanied by a sharp increase in protein accumulation and slowing of growth of these cultures. We investigated the effect of exogenous cysteine (Cys) and Met on the mitochondrial activity and cell growth as well as polyamine (putrescine, spermidine and spermine) contents in the suspension cultures of control (*GUS*-transgenic) and HP (high putrescine or mODC-transgenic) cells. Addition of either Cys or Met did not have a significant effect on polyamine content of the cells, nor did it affect the mitochondrial activity and the growth of the two cell lines. To check whether inorganic sulfur was being a limiting factor responsible for decrease in sulfur amino acids and the soluble proteins, potassium sulfate was added to the growth medium, and again polyamines and total proteins were analyzed. No significant change in the polyamine content was seen with the addition of inorganic sulfur. The profile of changes in soluble protein content of the cells during the 7 d culture period was also not altered in either cell line by the addition of sulfur. We conclude that enhanced protein synthesis in the HP cells is probably responsible for the decrease in sulfur amino acids, which may then cause a rapid protein breakdown.

P11 APX1 EXPRESSION PATTERNS IN SOYBEANS GROWN UNDER ELEVATED OZONE AND CO₂ CONDITIONS

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Carbon dioxide levels in the troposphere are expected to steadily increase ca. 50% by the year 2050 from present levels. The amount of ozone is also expected to increase over this time. Most agricultural regions in the United States are exposed to elevated levels of O₃ sometime during the growing season. Soybeans (*Glycine max*) are especially sensitive to elevated levels of O₃ and show significant yield loss when continuously exposed to elevated levels of O₃. Unlike CO₂, tropospheric levels of O₃ fluctuate daily so that soybeans are exposed to varying levels of this ROS throughout the growing season. We are particularly interested in understanding how increasing levels of tropospheric O₃ effect soybeans. To this end, we measured the gene expression levels of Ascorbate Peroxidase (APX1), an enzyme involved in the antioxidant defense system, in soybean plants exposed to elevated O₃ (20% above ambient), elevated CO₂ (550 ppm), both elevated O₃ and CO₂, or ambient conditions. Soybeans were grown under field conditions at the SoyFACE field site in Champaign, Illinois. Real-time PCR analysis showed that ozone only-treated plants exhibited a slight decrease in APX1 expression after flowering. Those plants exposed to both elevated CO₂ and elevated levels of O₃ showed negligible changes in APX1 expression compared to the housekeeping gene ubiquitin. This work was supported by an HHMI summer undergraduate research award to APVL (#52005130).

P12 GENOME EVOLUTION IN HOMOELOGOUS REGIONS OF *GLYCINE*

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<http://www.bio.indiana.edu/~nsflegume/index.php>

Soybean, *Glycine max*, is a paleopolyploid, as are other crops such as maize and cotton. The current $2n = 40$ diploid genome is the result of two rounds of polyploidization estimated to have occurred 50 mya and 10-15 mya. In an effort to understand the impact of the 10-15 mya duplication event on a region of the *Glycine* genome rich in disease resistance genes (R-genes), we are studying variation in a ca. 1 Mb region and its homoeologue in soybean, and orthologous regions in a wild perennial soybean relative, *Glycine tomentella*. Previous studies of soybean indicate that homoeologous regions from the 10-15 mya duplication event remain relatively unchanged. In contrast, we find that one homoeologue is gene rich and relatively compact while the other homoeologue has experienced gene loss and transposable element expansion. These findings are similar in the two *Glycine* species, and are reminiscent of the paleopolyploid genome of *Arabidopsis thaliana*, where one homoeologue appears to have remained gene rich while the other has undergone significant change, including gene loss. One hypothesis to explain the difference between regions of the soybean genome may be the relatively quick birth and death cycles of R-genes and how genome evolution proceeds in regions flanking R-genes.

P13 EXPRESSION PROFILING OF SPERMIDINE SYNTHASE3 AND SPERMINE SYNTHASE IN ARABIDOPSIS

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Very little is known about the regulation of the expression of polyamine biosynthetic genes, an aspect that can elucidate the functions of these ubiquitous compounds. Using promoter::GUS fusion technique, we studied the expression profiles of two key polyamine biosynthetic genes, *spermidine synthase3 (SPDS3)* and *spermine synthase (SPMS)* in various tissues and organs in *Arabidopsis thaliana* during its entire life cycle. The expression of *SPDS3* was high in young developing tissues with continued, but weaker, expression in the vascular tissue of mature plants. This is consistent with the role of spermidine as a source of H₂O₂ for cell wall lignification. A similar expression profile was observed for *SPMS*; expression was high in meristematic and elongating regions of the plant, and also in the hydathodes. This corresponds with the proposed role for spermine in cell elongation and expansion. In addition, changes in expression were studied during various abiotic stress conditions, including salt, drought, and chilling, as well as mechanical injury. Overall, both *SPDS3* and *SPMS* were induced in response to drought and 100 mM NaCl. There was also a transient increase in expression in response to chilling, but expression decreased again by 24 h. *SPDS3* was also induced during wounding. A bioinformatics study of the regulatory motifs within the promoter region identified several transcription factor binding sites in the promoter sequences of the two genes that are consistent with their expression pattern during development and in response to stress.

P14 EFFECTS OF ALUMINUM AND/OR CADMIUM ON CELLULAR METABOLITES IN RED SPRUCE (*Picea rubens* Sarg.) CELL SUSPENSION CULTURES

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Aluminum (Al) and cadmium (Cd) are natural components of the biosphere that can become toxic when they exceed a critical threshold. Plants respond to metal toxicity by triggering various cellular detoxification mechanisms, including the synthesis of amines and phytochelatins (PCs). Although evidence suggests that high levels of Al or Cd individually can affect various cellular metabolites in plants, few studies for their combined toxicity are reported. Their coexistence on acid soil is very common. The present study examined the effects of Al and Cd separately and in combinations on red spruce (*Picea rubens* Sarg.) cell suspension cultures. Samples were collected after 24 h and analyzed for changes in fresh cell mass, polyamines, phytochelatins, amino acids, and soluble inorganic ions. The induction of PCs (nearly 4 fold) was observed only in Cd-treated cells. However, glutathione (GSH), a precursor of PCs, showed a declining trend with increasing concentrations of both Al and Cd. Although an increase in putrescine was observed in both the treatments, the magnitude of increase was higher only in Al-treated cells. Among the soluble ions analyzed, Al induced a significant decrease in K and increase in P on red spruce cells. Some amino acids (glutamic acid, glycine, threonine, and phenylalanine) showed an inverse trend between Al and Cd treatments. We observed that binary metal combinations of Al and Cd produced synergistic (GSH) and antagonistic (γ -glutamylcysteine and PC₂) responses in red spruce cells.

P15 CHARACTERIZATION OF *ARABIDOPSIS* GENES INVOLVED IN REGULATING DEFENSE AGAINST PATHOGENS

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Defense against pathogens in plants involves coordinated activity of several defense genes. Here we describe preliminary characterization of two *Arabidopsis* defense-related genes. The first gene, *DEFENSE-RELATED ASPARTIC PROTEINASE 1 (DAP1)*, is predicted to encode for an aspartic proteinase. Transgenic plants overexpression this gene are more susceptibility to virulent bacterial pathogen *Pseudomonas syringae* suggesting that DAP1 is a negative regulator of defense and is likely to be a target of pathogen virulence factors. Detailed characterization, including analysis of knockout mutants of *DAP1* and identification of substrates of DAP1 is in progress. The second gene, *ARABIDOPSIS NEMATODE RESISTANCE1 (ANR1)* gene has no known function but it shows homology to a nematode-resistance gene. This gene is rapidly and strongly induced in response to pathogens and oxidative stress inducing chemicals. Knockout mutants of *ANR1* are sensitive to oxidative stress-inducing chemicals. These results suggest that ANR1 plays a critical role in regulating pathogen defense and oxidative stress in plants. Detailed characterization of *ANR1* gene to determine the response to transgenic plants overexpressing *ANR1* to pathogens and oxidative stress-inducing chemicals, subcellular localization of ANR1 protein, and promoter analysis etc. is in progress. These studies should help us understand the role of ANR1 in regulating pathogen and oxidative stress in *Arabidopsis*.

P16 DEVELOPING MARKERS FOR ASSOCIATION MAPPING IN BIOFUEL GRASSES

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We are initiating association mapping studies of potential biofuel grasses, including switchgrass (*Panicum virgatum* L.), reed canarygrass (*Phalaris arundinacea* L.) and gamagrass (*Tripsacum dactyloides* L.). A critical first step in this research is the identification of molecular markers that are tightly linked to biomass-related trait loci, thus enabling marker assisted selection and greatly accelerating the breeding programs for enhanced biomass production. In perennial polyploid species such as these, effective marker assisted selection will be key to rapid selection for improved varieties and adaptation to environments across the US. Here we present the first set of results from our marker development study, in which we used complete digestion by *HpaII*, an enzyme that preferentially cuts unmethylated DNA within low-copy gene regions, followed by DNA fragment size selection (100-600bp) to eliminate the large fragments containing the hypermethylated retrotransposons, as the first steps to enrich for the genic fraction. These fragments were ligated together and non-selectively amplified using a GenomiPhi™ kit. Four sequence libraries (switchgrass, reed canarygrass, and gammagrass (diploid and tetraploid)) were generated. Cloned DNA sequences were electronically digested at CCGG sites and systematically screened against several databases to assess levels of potential contaminants (vector sequences and bacteria genomes), organellar DNA, repetitive sequence (TIGR Gramineae repeats), and sequence homologous to annotated genomic sequence of related organisms (maize and rice). The percentage of known grass repetitive DNA sequence was very low in all four libraries, ranging from 0.3-2.6% of sequences analyzed. These preliminary data show that this *HpaII* library preparation approach works for polyploid grasses. Using next generation sequencing technologies and methylation-filtration of the genomes, we plan to identify thousands of SNPs for these polyploid grasses, for which there are currently limited genetic resources.

P17 IDENTIFICATION OF QUANTITATIVE TRAIT LOCI FOR STEM SUGAR, GRAIN YIELD, AND BIOMASS TO IMPROVE SORGHUM AS A BIOFUEL FEEDSTOCK

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Little is known about the genetics which control carbohydrate partitioning or the specific tradeoffs involved between the yield of stem sugar, grain, biomass, and the composition of these products. Recent demand for biofuel feedstocks has increased the desirability of sorghum grain, and increased interest and technology for digesting stem sugar and plant biomass as feedstocks for the future. We characterize quantitative inheritance of the leaf, stem and grain carbohydrate profile using a cross between BTx623 (low biomass grain sorghum) and Rio (a high biomass sweet stem sorghum) over three environments. A framework genetic map has been developed to identify quantitative trait loci (QTL) for these traits. Results suggest that desirable stem sugar, grain yield and biomass may be simultaneously increased. Thus, there appears to be great potential for improvement of sorghum crop residue for grain crops or as a dedicated feedstock crop.

P18 BIOENGINEERED MAIZE PRODUCING QUORUM SENSING SIGNALS TO DISRUPT BIOFILM FORMATION IN *PANTOEA STEWARTII* SUBSP. *STEWARTII*.

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The overall goal of this project is to evaluate the effectiveness of bioengineering maize to be resistance to the bacterial pathogen, *Pantoea stewartii* subsp. *stewartii* (*Pnss*). *Pnss* causes Stewart's wilt disease on sweet corn and leaf blight disease on maize by producing a biofilm in the xylem vessels. *Pnss* utilizes the bacterial cell-to-cell communication signal *N*-(3-oxohexanoyl)-L-homoserine lactone (3-oxo-C6-HSL) to regulate the production of the Stewartan capsular polysaccharide (CPS) virulence factor and *Pnss* biofilm development. Hi II maize was bioengineered to produce the 3-oxo-C6-HSL signal by expression of YenI. YenI, from *Yersinia enterocolitica*, has been shown to direct the production of 3-oxo-C6-HSL in tobacco and here we show 3-oxo-C6-HSL production by YenI in bioengineered maize. Signal separation and detection assays characterized accumulation of 3-oxo-C6-HSL in 115 RO bioengineered plants. We are currently crossing signal-producing bioengineered Hi II with pollen from untransformed inbred B73. R1 plants will be analyzed for transgene insertion and number. Most importantly, *Pnss* will be used to infect 3-oxo-C6-HSL producing maize and controls not producing signal. *Pnss* biofilm development will be visualized by confocal laser scanning microscopy in the xylem vessels to determine if premature expression of CPS biosynthesis will alter biofilm development. Results will determine if expression in maize of the cognate quorum-sensing signal to *Pnss* will disrupt xylem colonization and thus reduce the level of disease.

P19 CONSTRUCTION OF A BIOLUMINESCENT PLASMID REPORTER BASED ON *PANTOEA STEWARTII* QUORUM SENSING RECEPTOR PROTEIN, *ESAR*.

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Pantoea stewartii subsp. *stewartii* (*Pnss*) is a Gram negative bacterial pathogen that causes Stewart's wilt and leaf blight diseases on sweet corn and maize, respectively. *Pnss* grows in the plant xylem and produces an exopolysaccharide (EPS) slime layer called Stewartan that interferes with vascular transport. Production of Stewartan is cell density dependent and governed by the *EsaI/EsaR* quorum sensing (QS) system. Previous work has shown that the *EsaR* directly interacts with promoter regions upstream of the *rcsA* gene to promote Stewartan synthesis. Presented here is our strategy presented to construct an *EsaR*-dependent bacterial biosensor that will detect the *EsaR*-cognate QS signal, 3-oxo-C6-HSL. Progress to date includes PCR amplification and cloning of the *esaR lux* box sequence from promoter of *rcsA* into *luxCDABE* cassette plasmid, pBS377, resulting in pBS377-*rcsA*. The newly constructed bioluminescent plasmid consists of the inducible *EsaR* binding promoter region and reporter operon, *luxCDABE*, that is predicted to produce light in the presence certain QS signals. This reporter plasmid will be transformed into *esaI*, *esaR*⁺ *Pnss* strain to test the activity and functionality of the biosensor in the presence of various QS signals including *EsaR* cognate signal, 3-oxo-C6-HSL. This biosensor will be used in various detection assays with bioengineered corn and to characterize QS signal mimics produced by genotypes of maize that differentially respond to *Pnss* infections.

P20 MODULUS OF ELASTICITY OF SPLIT-THICKNESS CELL WALL LAYERS IN THE ALGA *CHARA CORALLINA*

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Plant cell morphogenesis is largely determined by the nature of the cell wall and the biophysics of how it stretches. In rapidly growing plant cells turgor pressure places the cell wall under high tensile stress, which leads to the question: Does the newly formed inner wall layer in growing cells really support most of the stress? Looking for an answer to this question, I carefully peeled apart the inner and outer layers of *Chara corallina* cell walls excised from growing cells and mounted them on a tensile tester. By measuring the thickness, width, and length of the excised cell wall layers (inner, outer, and full-thickness layers), it was possible to determine the modulus of elasticity of the different wall layers and determine whether the inner layer actually does support most of the stress in growing cells. Through this experiment, I hope to aid in the understanding of the viscoelastic properties of the different cell wall layers in this model organism. Understanding the physical properties of these cell wall layers will also help in the comprehension of the cell wall's structure and how it relates to the cell wall's role in growth and development.

P21 IDENTIFICATION AND CHARACTERIZATION OF TWO GENES INVOLVED IN DEFENSE SIGNALING IN *ARABIDOPSIS*

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We describe preliminary characterization of two *Arabidopsis* genes, *SR11* and *SR14*. These genes are likely to be involved in regulating defense against pathogens. *SR11* is strongly induced and *SR14* is strongly suppressed in response to pathogen infections. To determine the function of these genes in regulating defense against pathogens, we identified T-DNA knockout lines of these genes and tested the growth of virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) in these plants. We found that *sr11* knockout line is ~15-fold more resistant and *sr14* knockout line is ~10-fold more susceptible to the *Pst* DC3000. These results suggest that *SR11* functions as a negative regulator of pathogen defense, while *SR14* is a positive regulator of pathogen defense. To further analyze the role of these genes in pathogen defense we are constructing transgenic plants that express these genes constitutively at high levels. Based on the results of knockout mutants, we predict that the plants overexpressing *SR11* would be more susceptible and plants overexpressing *SR14* would be more resistant to *Pst* DC3000. In addition, experiments to characterize the promoter of these genes and other components of *SR11*- and *SR14*-mediated defense signaling are also underway.

P22 GRAVITY EFFECTS ON GROWTH FORM OF *BRASSICA RAPA* L. AND *ARABIDOPSIS THALIANA* (L.) HEYHN.

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To investigate effects of hypergravity on plant growth and development, we conducted a series of experiments on large-diameter centrifuges at NASA Ames Research Center in Moffett Field, CA, during summer and fall 2006. *Brassica rapa* L. cv. Astroplants and *Arabidopsis thaliana* (L.) Heyhn. var. 'Columbia' were grown for 16 or 11 days, respectively, inside small growth chambers mounted in powered swinging buckets or in stationary units. Responses of the plants grown in the hypergravity treatments (2-g and 4-g) were compared with the 1-g controls. For purposes of growth form analysis, *Brassica* plants were photographed daily, during short-duration rotor stops, while *Arabidopsis* plants were photographed only at the beginning and the end of the 11-d continuous runs. Stem angles were measured using Image-J software. At both 2-g and 4-g, *Brassica* plants assumed a more horizontal growth form as their exposure to hypergravity progressed, with a ~ 45° plant angle after 16 days at 4-g. A significant but less dramatic effect of g-force on growth form was seen in *Arabidopsis* (~25° from vertical at 4-g). Growth form changed less at 2-g than at 4-g. In *Brassica*, stem diameter increased in both hypergravity treatments. The results will be discussed in the context of gravitropic set point angle and how g-force, across its continuum, affects plant growth form. Supported by NASA grant NAG10-329.

P23 MDP1, A SMALL PLASMA MEMBRANE-ASSOCIATED PROTEIN OF ARABIDOPSIS IS A CRITICAL REGULATORY COMPONENT OF DEFENSE AGAINST PATHOGENS.

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In order to understand the complex defense regulatory mechanisms of plants, it is necessary to identify and characterize genes whose products are involved in the initiation and regulation of the defense response. Here we describe identification and characterization of *SMALL PLASMA MEMBRANE-ASSOCIATED DEFENSE PROTEIN 1* (*MDP1*), a novel *Arabidopsis* gene which is predicted to encode a small plasma membrane-associated protein of unknown function. Expression of this gene is strongly induced in response to pathogens, the defense hormone salicylic acid, and oxidative stress inducing chemicals. Preliminary results from RNAi knockdown plants suggest that expression of *MDP1* is required for pathogen-inducible expression of *PR-1* and possibly other defense genes. Together these results suggest that *MDP1* is a critical regulatory component of defense against pathogens in *Arabidopsis*. Response of the RNAi knockdown plants to virulent pathogens is being studied. In addition, experiments to determine the sub-cellular localization of *MDP1* protein, temporal and spatial expression pattern of the *MDP1* gene, and identification of other components of *MDP1*-mediated defense signaling are underway. These studies should help us better understand the mechanism of *MDP1*-mediated defense signaling in *Arabidopsis*.

P24 GENETIC COMPARISON OF CASTANEA MOLLISSIMA CANKER TISSUE AND CASTANEA DENTATA CANKER TISSUE BY SUBTRACTIVE HYBRIDIZATION

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To better understand the genetic differences between *Cryphonectria parasitica* resistant Chinese chestnut (*Castanea mollissima*) and the susceptible American chestnut trees (*C. dentata*), two subtracted cDNA libraries were constructed. One cDNA library was enriched for genes that are upregulated in Chinese chestnut canker tissue. The other was enriched for American chestnut canker tissue genes, some of which might be related to the increased fungal susceptibility seen in this species. DNA sequencing was done on 288 randomly selected clones from the Chinese chestnut enriched library. Initial sequence analysis has revealed that many of these genes are involved in plant pathogenesis resistance. Several genes will be further investigated by Northern blot to determine whether there is differential gene expression between the two different chestnut species. In addition it may be possible to use these sequences to determine if polymorphisms exist between homologous genes and how these differences affect response to *C. parasitica* infection. Identified polymorphisms will also be used to help map the chestnut genome as part of the *Fagaceae* genome project.

P25 COMMON SEQUENCES AFFECT RNA EDITING OF TWO MAIZE CHLOROPLAST TRANSCRIPTS

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RNA editing is a biological process that alters the sequence of transcripts from diverse organisms. Cytidine to uridine editing of chloroplast and mitochondrial transcripts of land plants involves both the RNA sequence element (the *cis*-element) that identifies an editing site, and the *trans*-acting factor(s) that recognize the editing site and perform the editing reaction. There is no consensus sequence near all Cs that are converted to Us in chloroplasts. However, the ~30 chloroplast editing sites in a given species can be divided into small groups, called clusters, which share short regions of sequence identity 5'- of the editing site, when gaps are introduced in the sequences. The relevance of two of these clusters have been shown *in vivo*, in that overexpression of sequence from an editing site cluster member causes reduction in editing of the endogenous transcript containing the site being overexpressed, as well as at the other sites in the cluster. We set out to uncover the nature of the competition phenomena using mutant RNA editing site substrates for editing reactions *in vitro*. We have determined the location of the *cis*-element region responsible for competition in one of these clusters, containing the ZMrpob C467 and ZMrps14 C80 sites, by comparative competition. The -20 to -16nt region, relative to the edited C, is critical for competition in both sites, and mutating this region ablates competition. We propose this region is likely a binding site for a shared *trans*-factor, necessary for editing of both sites.

P26 FACTORS AFFECTING STORAGE VACUOLE ACIDIFICATION DURING SOYBEAN GERMINATION.

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The initial mobilization of the α' and α subunits of the β -conglycinin storage protein of soybean is due to a serine protease with optimum pH of 4. Protease C1 in mature form was localized in the protein storage vacuoles (PSV) even during seed fill. Methylamine sensitive acridine orange accumulation in the cotyledon PSVs only upon germination suggests that protease C1 acts only when PSVs are acidic. In this study, seeds were split such that the embryonic axis was attached to only one cotyledon. The early steps of reserve protein mobilization as described above were observed by SDS-PAGE to occur only in the cotyledons with attached axis. Correspondingly, acridine orange accumulation was observed only in the PSVs of the cotyledons with attached axis. To examine the possible role of pyrophosphate-dependent proton pumps in PSV acidification, surface-sterilized seeds were imbibed in water containing 50 μ M sodium imidodiphosphate, an inhibitor specific for H(+)-PPases and examined by SDS-PAGE 3 days later. Proteolysis of the α' and α subunits of β -conglycinin were 56% and 68% inhibited compared to water controls. When treated with 100 μ M imidodiphosphate, the percentage inhibition increased to 78% and 100% for the α' and α subunits, respectively.

P27 IN VITRO SEED MATURATION IN BRASSICA RAPA L.: RELATIONSHIP OF SILIQUE ATMOSPHERE TO STORAGE RESERVE DEPOSITION

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The *Brassica rapa* L. silique is a self-contained environment that maintains hypoxia around developing seeds, and in which carbon dioxide accumulates to very high concentrations (>30,000 ppm). How the silique microenvironment modulates storage reserves in the seeds is of interest because of the important role played by canola as an oilseed. To study this, we have developed a silique culture system that permits maturation of seed *in vitro*. Siliques excised from plants just 11 days after pollination ripen their seeds after 20 days in light (200 $\mu\text{mol}/\text{m}^2/\text{sec}$) on MS medium containing 30 g/l sucrose, 0.25 mg/l BAP, and 0.025 mg/l NAA. Cytochemical localization and biochemical analyses revealed that storage reserves were affected by the *in vitro* maturation system. Although following a comparable ripening timeline to that occurring on the plant, and producing fully germinable seeds, *in vitro* maturation re-sulted in 40% lower seed weight, and the seeds contained less lipid, but more protein, starch and soluble carbohydrates. Analysis of the internal silique atmosphere showed that *in vitro* siliques gave seeds a less oxygenated environment than they experience attached to the plant. Carbon dioxide concentrations remained high later into the maturation sequence *in vitro* than on the plant. Our *in vitro* maturation system is useful since both the gaseous and hormonal environments can be readily manipulated. Supported by NASA grant NAG-10-329.

P28 POSTTRANSCRIPTIONAL CONTROL OF CHLOROPLAST GENE XPRESSION: 5' UNTRANSLATED REGION CIS AND TRANS-ACTING FACTORS

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Chloroplast gene expression is strongly influenced by posttranscriptional mechanisms, many involving protein-RNA complexes. The focus of our research is the identification and study of RNA-protein complexes that form in the 5' untranslated regions (UTRs) of chloroplast-encoded mRNAs. Here we present experimental results that examine the effects of *cis*-acting elements in a 5' UTR on reporter gene function *in vivo*. A series of plasmids containing a reporter gene (*uidA*) under control of a single chloroplast 5' UTR were constructed and transformed in tobacco. We analyzed RNA abundance using RNase protection analysis and protein expression using β -glucuronidase assays. Replacement of a region containing the 5'-most of the two conserved elements in the 5' UTR with a random sequence did not significantly change the amount of protein as compared to that expressed under control of the wild type 5' UTR. In contrast, replacement of the 3'-most of the two conserved regions decreased protein expression to 28% of wild type levels. We are currently focusing on an 11-nt sequence within the 3' most conserved sequence that is 100% conserved among the 25 sequenced angiosperm genomes as a potential target for our binding factors.

P29 ANALYSIS OF SIGNIFICANT POLYMORPHISMS WITHIN CANDIDATE ALUMINUM TOLERANCE GENES

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Aluminum (Al) toxicity is a major constraint to maize productivity on acidic soils throughout the world. Phototoxic Al becomes soluble at pH < 5.5, inhibiting root growth and function, thus severely reducing yields. Al exclusion and intracellular tolerance are important mechanisms involved in achieving Al tolerance in maize; however, little is known about the underlying genetics. Diverse maize inbred lines, with varying tolerance to Al, provide a useful resource for studying the genetics behind this complex trait. An association approach was used to evaluate candidate genes involved in Al tolerance, which were selected using comparative and physiological genomics-based approaches. We scored polymorphisms from 22 candidate genes across ~300 diverse inbred lines. Six of these candidate genes produced significant associations with net seminal root growth under toxic Al stress conditions: malic enzyme (ME), isocitrate lyase (ICL), S-Adenosylhomocysteinase hydrolase (SAHH), aluminum activated malate transporter (ZmALMT), multidrug and toxin exclusion transporter (ZmALS), and pectin methylesterase (PME). We will discuss the significant polymorphisms identified and the haplotype diversity contributing to Al tolerance in maize

P30 HIGH DENSITY SNP MARKERS FOR COMPLEX TRAIT DISSECTION IN MAIZE

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In our ongoing efforts to bridge the gap between plant genome studies and crop breeding strategies, association approaches offer the most direct means of identifying genes and alleles of agronomic importance. With high levels of naturally occurring genetic diversity and low levels of linkage disequilibrium (LD), diverse maize inbred lines are tremendous resources for association mapping of quantitative traits down to the gene level. An estimated 1 million SNP markers, however, are required to capture most of the LD structure in 27 maize inbred lines. These 27 inbred lines encompass 80% of the common SNP variation in maize and are parents of a 5,000 recombinant inbred line nested-association mapping (NAM) population- an assemblage of 26 populations in the process of being phenotyped for more than 35 traits at 6 field locations. In NAM, only the founder lines need to be genotyped at high resolution; then lower resolution markers track the chromosomal segments in their offspring. To accomplish these goals, we developed a library preparation method built on the differential cytosine methylation pattern of genes and retrotransposons, which are discriminated by the methylation-sensitive restriction enzyme *HpaII*. This gene-biased *HpaII* library construction approach, in conjunction with 454 sequencing, is being used for high-throughput SNP discovery via library oversampling. Almost 1 billion bases of sequence have been collected by 454 GS-FLX sequencing of *HpaII* libraries prepared from maize inbred lines, B73 and Mo17. Preliminary results from our bioinformatics analyses are presented.

P31 XLYEM VESSEL DIAMETER IN TRANSGENIC *ULMUS AMERICANA* EXPRESSING SYNTHETIC ANTIMICROBIAL PEPTIDES WITHIN VASCULAR TISSUES

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Dutch Elm Disease (DED), caused by the fungal pathogen *Ophiostoma novo-ulmi*, has killed most of the mature American elms (*Ulmus Americana*) within the United States. Natural resistance in *Ulmus americana* to *Ophiostoma novo-ulmi* depends on several factors. Previous research has shown a correlation between disease susceptibility and increasing xylem vessel size in several *Ulmus* species. It is believed that elms with smaller xylem vessels can quickly compartmentalize infected vascular tissue in order to minimize further spread of the disease. We have produced transgenic American elms expressing a synthetic antimicrobial peptide gene, ESF39, within the vascular tissue. Preliminary research has shown that these trees exhibit higher resistance to *Ophiostoma novo-ulmi* compared to wild type trees. We are currently comparing xylem vessel diameter in the branches of two year old field grown transgenic and wild type American elms. This research has potential to provide us with insights on how expression of the antimicrobial peptides in the vascular tissues is potentially affecting tree anatomy and disease resistance.

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