PROGRAM AND ABSTRACTS

Western
Sectional Society

AMERICAN SOCIETY OF PLANT BIOLOGISTS
WESTERN SECTION ANNUAL MEETING

APRIL 25 – 26, 2009

FOUR POINTS SHERATON

TUCSON, ARIZONA, USA

MEETING ORGANISER:

Dr. Ravi Palanivelu, University of Arizona, Tucson, USA

MEETING OVERVIEW

Saturday, April 25, 2009

7:30 AM – 9:00 AM	Registration
7:30 AM – 9:00 AM	Breakfast
9 AM – 9:15 AM	Opening Remarks
	Dr. Ken Feldmann, Head, Department of Plant Sciences, University of Arizona
9:15 AM-10:30 AM	**Session I: Plant Development**
10:30 AM-11:00 AM	Coffee break
11:00 AM-12 NOON	**Session II: Physiological processes**
12 NOON-2 PM	Lunch break
2 PM-2:45 PM	**Session III: Towards Genetic Improvement of Plants**
2:45 PM-3:30 PM	**Session IV: Genomics**
3:30 PM-4:00 PM	Coffee break
4:00 PM-6:00 PM	Poster session
6:00 PM-7:00 PM	Dinner break
7:00 PM-8:00 PM	**Key note Address**
	"The iPlant Collaborative: Cyberinfrastructure and Collabortive Tools to Address Grand Challenges in Plant Science"
	Dr. Stephen Goff **Project Director, iPlant Collaborative,** **University of Arizona**

Sunday, April 26, 2009

7:30 AM – 9 AM	Breakfast
9 AM – 10:30 AM	**Session V: Plant Environment Interactions**
10:30 AM	Prize winner announcement & award
10:45 AM	**END OF MEETING**

ORAL PRESENTATIONS OVERVIEW

Saturday, April 25, 2009, 9 AM-10:15 AM

Plant Development session (Chair: Dr. Hanjo Hellmann, Washington State University)

9:15 AM Dongfang Wang, U of Arizona

PARTIAL-LOSS-OF FUNCTION PHENOTYPE FOR A POLYCOMB-GROUP GENE GENERATED THROUGH AN ARTIFICIAL MICRORNA STRATEGY

9:30 AM Jasmine King, U of California, San Diego

DEVELOPMENT OF A TRANSIENT ASSAY TO MONITOR CIRCADIAN RHYTHMS IN PLANTS

9:45 AM Amanda Durbak, U of Arizona

THE RECEPTOR KINASE CLV1 REGULATES FRUIT ORGAN NUMBER VIA STEM CELL REPRESSION IN ARABIDOPSIS

10:00 AM Tatsuya Tsukamoto, U of Arizona

REF1/LORELEI GENE FUNCTIONS IN THE FEMALE GAMETOPHYTE AND REGULATES POLLEN TUBE RECEPTION AND REPULSION

10:15 AM Tie Liu, Stanford University

ORTHOLOGUES OF ARABIDOPSIS THALIANA STOMATAL BHLH GENES AND REGULATION OF STOMATAL DEVELOPMENT IN GRASSES

10:30 AM - 11:00AM COFFEE BREAK

Saturday, April 25, 2009, 10:45 AM-11:30 AM

Physiological processes session (Chair: Dr. Judy Brusslan, California State University)

11:00 AM Hanjo Hellmann, Washington State University

THE DDB1A INTERACTING PROTEINS CSA AND DDB2 ARE CRITICAL FACTORS FOR UV-B TOLERANCE IN ARABIDOPSIS THALIANA

11:15 AM Lisa Patrick, U of Arizona

EMPIRICAL EVALUATION OF EXTERNAL AND INTERNAL BRANCHING ARCHITECTURE PATTERNS IN PINUS EDULIS

11:30 AM John Belletto, California State University

IDENTIFICATION OF PEPTIDE SUBSTRATES FOR ACYL-AMINO-ACYL PEPTIDASES IN ARABIDOPSIS.

12 NOON - 2:00 PM LUNCH BREAK

Saturday, 25 April 2009, 2:00 PM-3:30 PM

Towards Genetic Improvement of plants Session (Chair: Dr. Zhongguo Xiong, U of Arizona)

2:00 PM Tamara Miller, U of California, Berkeley

IMPROVING AGROBACTERIUM TUMEFACIENS-MEDIATED GENE DELIVERY IN SORGHUM BICOLOR

2:15 PM Jun Won Kang, University of Washington

GENETIC ANALYSIS OF TRICHLOROETHYLENE (TCE) METABOLISM IN HYBRID POPLAR AND TRANSGENIC POPLAR

2:30 PM Hyun Jo Koo, U of Arizona

EVOLUTION AND BIOSYNTHESIS OF MEDICINALLY IMPORTANT TERPENOIDS SUCH AS CURLONE AND THE TURMERONES IN TURMERIC AND GINGER

Genomics session (Chair: Dr. Ramin Yadegari, U of Arizona)

2:45 PM Elizabeth Waters, San Diego State University

COMPARATIVE GENOMIC ANALYSIS OF CLP/HSP100 PROTEINS

3:00 PM Andrea Zuccolo, U of Arizona

ASSESSMENT OF THE EXTENT OF SUBSTITUTION RATE VARIATION OF LONG TERMINAL REPEAT SEQUENCES IN ORYZA SATIVA AND ORYZA GLABERRIMA

3:30 PM – 4:00 PM **COFFEE BREAK**

4:00 PM – 6:00 PM **POSTER SESSION**

6:00 PM – 7:00 PM **DINNER**

7:00 PM – 8:00 PM **KEYNOTE ADDRESS**
DR. STEPHEN GOFF
Project Director, iPlant Collaborative,
University of Arizona

<u>Sunday, 26 April 2009, 9 AM-10:30 AM</u>

Plant Environment Interactions session (Chair: Dr. Elizabeth Waters, San Diego State University)

9:00 AM Salme Timmusk, Uppsala Biocenter, Sweden

DETECTION AND QUANTIFICATION OF PLANT DROUGHT TOLERANCE ENHANCING BACTERIUM PAENIBACILLUS

POLYMYXA IN THE RHIZOSPHERE OF WILD BARLEY (HORDEUM SPONTANEUM) WITH REAL-TIME PCR

9:15 AM Helton Camara Sao Paulo University, Brazil

SPODOPTERA FRUGIPERDA HERBIVORY AND MECHANICAL INJURY ON CEREAL GRASSES A. VOLATILE ORGANIC COMPOUND INDUCTION IS UNCOUPLED WITH INJURED LEAF PHOTOSYNTHETIC RESPONSES- DAY 1

9:30 AM Juliana Medeiros, University of New Mexico

DROUGHT INCREASES FREEZING TOLERANCE OF LEAVES AND XYLEM IN L. TRIDENTATE

9:45 AM Johnny Maruthavanan, New Mexico State University

PHYSIOLOGICAL AND YIELD RESPONSES OF FIELD-GROWN ALFALFA (MEDICAGO SATIVA L.) TO WATER DEFICIT

10:00 AM Fushi Wen, U of Arizona

FUNCTIONS OF EXTRACELLULAR PROTEINS AND EXDNA IN ROOT TIP RESISTANCE TO FUNGAL INFECTION

10:15 AM Minsoo Kim, U of Arizona

A DOMINANT HSP101 MUTATION IS SUPPRESSED BY MUTATIONS IN AN MTERF-RELATED PROTEIN

CONCLUSION OF MEETING

Oral Presentations - Session I

Plant Development

Saturday, April 25, 2009, 9:15 am – 10:30 am

Chair: Dr. Hanjo Hellmann

Washington State University

PARTIAL-LOSS-OF FUNCTION PHENOTYPE FOR A POLYCOMB-GROUP GENE GENERATED THROUGH AN ARTIFICIAL MICRORNA STRATEGY

Mayuko Ikehara, Dongfang Wang, Changqing Zhang, Kirsten N. Wallace, Karen S. Schumaker, and Ramin Yadegari
Department of Plant Sciences, University of Arizona, Tucson, Arizona 85721-0036

Study of gene function is often hindered by a lack of mutants. Although mutant resources have been developed for the model organism *Arabidopsis thaliana*, suitable mutants are still lacking for many genes. To study the function of genes expressed in the female gametophyte, we adapted an artificial microRNA (amiRNA) strategy to generate loss-of-function mutants in Arabidopsis. As a proof of concept, we tested this method on *FIS2*, a gene encoding a zinc-finger Polycomb-group protein required for proper seed development. *FIS2* is first expressed in the central cell within the female gametophyte before fertilization and in early endosperm after fertilization. A *fis2* null mutant allele that was isolated previously produces seeds that abort early in development with embryos arrested at the heart stage. We generated transgenic plants harboring a *FIS2*-promoter-driven amiRNA against the coding region of *FIS2*. T1-generation plants displayed a wide range of phenotypes from those with no discernable defect in seed development to those that exhibited seed abortion levels that were comparable to the reference null mutant. We also obtained amiRNA lines with intermediate phenotypes suggesting identification of partial-loss-of-function mutants. These plants showed embryos that were aborted at torpedo or early bent-cotyledon stage. The amiRNA transgenes led to seed abortion only when they were inherited through the maternal parent. The mutant phenotypes were stable in T2 generations. Therefore, amiRNA-generated mutants have similar genetic behaviors as the *fis2* null mutant despite different degrees of penetrance. Our results indicate that amiRNA can be used to generate complete- and partial-loss-of-function mutants for genes expressed in the female gametophyte.

DEVELOPMENT OF A TRANSIENT ASSAY TO MONITOR CIRCADIAN RHYTHMS IN PLANTS

Jasmine King, Steve Kay
Division of Biological Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, USA

The circadian clock plays a critical role in regulating essential aspects of growth, metabolism, and behavior in many species. The goal of this project is to develop a method to rapidly screen transcription factors for effects on the circadian clock in plants. Through the use of agrobacterium-mediated infiltration, we were able to transiently transfect Arabidopsis core-clock promoter driven luciferase reporters into tobacco leaves. These bioluminescent reporters were shown to display robust, ~24 hour oscillations consistent with previous data from stable Arabidopsis transgenic lines. The oscillatory behavior of the bioluminescent reporter was observed for several days after the leaves were excised and imaged using an automated CCD-camera system. By co-injecting known effectors of the clock and the reporters, we were able to observe changes for both period and phase consistent with prior knowledge from stably transfected Arabidopsis lines. This method is currently being used to test both novel promoters and effectors of the clock, leading to a significant increase in the rate at which new clock components can be identified and studied.

THE RECEPTOR KINASE CLV1 REGULATES FRUIT ORGAN NUMBER VIA STEM CELL REPRESSION IN ARABIDOPSIS

Amanda Durbak,[1] *Frans E. Tax*[2]
[1]*Dept. of Plant Sciences, University of Arizona, Tucson, USA*
[2]*Dept. of Molecular and Cellular Biology and Dept. of Plant Sciences, University of Arizona, Tucson, USA*

In crops such as tomatoes and peppers, the largest fruit also have the greatest number of fruit organs. Studies in tomato show that increases in both cell division and organ number are responsible for larger fruit, but the molecular mechanisms through which these components integrate and contribute to overall patterning and size is still not clear. Fruit arise from floral meristems (FM) derived from inflorescence meristems (IM), stem cell-containing structures, and studies in Arabidopsis indicate that when extra cells are present in FM, extra fruit organs are produced. Mutation of individual components of the stem cell-repressing CLAVATA signaling pathway results in an increase of cell number in both IM and FM. Through the characterization of new alleles in the receptor-kinase *CLAVATA1* (*CLV1*), we identified a second role for CLV1 in the regulation of fruit organ number via the repression of stem cell proliferation during later stages of fruit development. This later role is temporally distinct from the previously known role of CLV1 in IM and FM, and our analysis of *CLV1* expression indicates that CLV1 is not limited to the meristem. We show that loss of clv1 activity in developing fruit leads to an accumulation of stem cells as shown by the ectopic activation of the stem cell-promoting factor *SHOOTMERISTEMLESS* in developing fruit, and that extra organs are produced from these stem cell containing regions. Our results indicate that in addition to its role in the FM, CLV1 is also acting to repress stem cell proliferation in developing fruit.

REF1/LORELEI GENE FUNCTIONS IN THE FEMALE GAMETOPHYTE AND REGULATES POLLEN TUBE RECEPTION AND REPULSION

Tatsuya Tsukamoto, Yuan Qin, Yiding Huang and Ravi Palanivelu.
Department of Plant Sciences, University of Arizona, Tucson, AZ 85721.

Mutations affecting gamete function exhibit non-mendelian inheritance pattern. In flowering plants, distorted segregation mutant screens have identified mutations that cause either male or female gametophytic defects. However, since the gametophytic defect is completely penetrant in many of these mutants, homozygous plants could not be recovered. Lack of homozygous mutants with gametophytic defects prohibits facile functional analyses. To identify mutations that do not completely abolish gametophyte functions, we undertook a forward genetics screen to isolate semi-sterile mutants that segregated homozygotes. From this screen, we identified a female gametophyte-specific, reduced fertility defect 1 (*ref1*) mutant. Characterization of the defects using a variety of assays revealed that in the *ref1* female gametophyte, pollen tube fails to undergo growth arrest (pollen tube reception) and is not induced to lyse and release the two sperm cells. In addition, the mutant ovules are defective in pollen tube repulsion and consequently mutant ovules attracted multiple pollen tubes. Using map-based cloning and TAIL-PCR procedures, we identified the gene (LORELEI, AT4g26466) that was disrupted in *ref1* mutant. *ref1* defects were phenocopied by three newly characterized mutant alleles (*ref1-2*, *ref1-3* and *ref1-4*) confirming that disruptions in *REF1/LORELEI* led to the pollen tube reception and repulsion defects. *REF1/LORELEI* encodes a putative GPI-anchor containing protein of unknown function. Our current efforts are directed at deciphering the role of *REF/LORELEI* in pollen tube reception and repulsion.

ORTHOLOGUES OF ARABIDOPSIS THALIANA STOMATAL BHLH GENES AND REGULATION OF STOMATAL DEVELOPMENT IN GRASSES

Tie Liu, Dominique Bergmann
Stanford University

Stomata are adjustable pores in the plant epidermis that regulate gas exchange between the plant and the external environment. Stomata are present on most aerial parts of higher plants, but display significant diversity in their morphology and distribution patterns. Genetic pathways controlling stomatal development and distribution have been described in some detail for one dicot species, Arabidopsis, where three paralogous bHLH transcription factors, FAMA, MUTE and SPCH control discrete sequential stages in stomatal development. Orthologues of FAMA, MUTE and SPCH are present in other flowering plants; of particular interest are the homologues in the grasses because grass stomatal guard cell structure and distribution differ substantially from Arabidopsis. By examining gene expression patterns and insertional mutants in rice and by cross-species complementation studies, we find evidence that FAMA function is conserved between monocots and dicots, despite their different stomatal morphologies, whereas MUTE and SPCH roles may have diverged.

Oral Presentations- Session II

Physiological Processes

Saturday, April 25, 2009, 11:00 am – 12 NOON

Chair: Dr. Judy Brusslan

California State University, Long Beach

THE DDB1A INTERACTING PROTEINS CSA AND DDB2 ARE CRITICAL FACTORS FOR UV-B TOLERANCE IN ARABIDOPSIS THALIANA

Sascha Biedermann, Anne Bernhardt, Sutton Mooney, Hanjo Hellmann
Washington State University, Pullman

Genotoxic stress imposed by UV-irradiation or chemical treatment is a permanent threat potentially affecting the genomic integrity of all live forms, but especially that of sessile organisms like plants. Consequently, several DNA repair mechanisms have evolved, one of which is the nucleotide excision repair (NER). NER comprises two separate pathways, the global genomic repair (GGR) and the pathway of transcription-coupled repair (TCR). Critical for recognition of UV-induced DNA damages to initiate GGR and TCR dependent repair processes are the proteins DDB2 (Damaged DNA Binding 2), and Cockayne Syndrome A (CSA), respectively. Both DDB2 and CSA assemble with an ubiquitin E3 ligase that contains the cullin CUL4 and the substrate adaptor DDB1. This assembly leads to the ubiquitination and subsequent degradation of DDB2, CSA and additional proteins via the 26S proteasome. Although GGR and TCR have been intensively described in mammalian cells, only poor knowledge is present for plants. Here, we report that loss of the CSA and DDB2 orthologs from Arabidopsis thaliana leads to an increased sensitivity of affected plants to UV stress. We provide evidence that these plants exhibit an impaired NER-dependent repair of UV induced thymidine dimers. Additionally we demonstrate that CSA assembles into a CUL4-DDB1a complex and that it is degraded by the 26S proteasome in a CUL4 and UV-B dependent manner. Furthermore we describe tissue specific expression patterns of Arabidopsis CSA and DDB2 using promoter:GUS constructs and RT-PCR analysis, and investigate subcellular localization of the two proteins. In summary, this work describes for the first time functions of Arabidopsis CSA and underscores the significance of CUL4-based E3 ligases, DDB2, and CSA for DNA repair in higher plants.

EMPIRICAL EVALUATION OF EXTERNAL AND INTERNAL BRANCHING ARCHITECTURE PATTERNS IN *PINUS EDULIS*

Philippe Gregoire[1,2], Vanessa R. Buzzard[1], and Lisa D. Patrick[1]
[1]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, USA
[2]Department of Biology, University of Sherbrooke, Quebec, Canada

Metabolic scaling models have proven useful across a broad range of species to quantitatively link form and function and explore community and ecosystem level patterns. However, there is significant uncertainty regarding model predictions of plant dynamics and behavior due to a lack of empirical data on the internal vascular design and external network architecture of diverse plants. As such, our objectives were to measure external internode length, external node diameter, branching ratio (n), needle number, needle area, internal conducting area, and internal conduit diameter throughout a *Pinus edulis* tree. The external node diameter decreased from 57.3 mm at the oldest level (k=1) to 1.2 mm at the youngest level (k=20), while external internode length was relatively constant across k (2.45 ± 0.65 cm). Across k, n was not constant. For example, in a sample of 77 nodes at k=10, the distribution of n was observed to exponentially decrease from n=1 to n=6. The external daughter/parent cross-sectional area ratios (for k=1 to k=4) ranged from 1.013-0.904, suggesting this tree is area-conserving. Throughout the tree, there was an increase in the number, weight and area of needles at each node as k increased. Further, the internal conducting area was variable at each level k. These results will be critical for fine tuning predictions of how variability in network architecture of plants influences fluxes of carbon and water.

IDENTIFICATION OF PEPTIDE SUBSTRATES FOR ACYL-AMINO-ACYL PEPTIDASES IN ARABIDOPSIS.

John V. Belletto, Julian Whitelegge, Naser Farrokhi, Judy A. Brusslan,

The Department of Biological Sciences, CSULB, Long Beach, CA 90840-3702 and The Pasarow Mass Spectrometry Laboratory, David Geffen School of Medicine, UCLA, CA 90024-1759

Acyl-Amino-acyl peptidases (AAPs) are a class of catabolic enzymes that are conserved among photosynthetic organisms from cyanobacteria and green algae to angiosperms. Their evolutionary perseverance suggests that their function has physiological significance. In Arabidopsis thaliana, the catalytic domains of two cytosolic enzymes (AAP1 & 2) and one stromal localized enzyme (cAAP) are similar to other eukaryotic peptidases that serve to cleave the N-terminus and the first amino acid of N-terminal acytelated peptides. Once deacetylated, other peptidases will complete the peptide breakdown, aiding in amino acid recycling and redistribution. Our lab has screened for homozygous T-DNA knock-out mutants for these three genes and has generated a caap mutant as well as a triple mutant aap1aap2caap. None of the mutations exhibited any phenotype under normal growth chamber conditions. We hypothesize that the loss-of-function mutants, caap and aap1aap2caap will accumulate acytelated peptides that will not be present in wild type. Chloroplasts were isolated from WT, caap and triple mutant plants, and lysed into 1% TFA to prevent artifactual proteolysis. Larger proteins, above 10 kD, were removed by a Centricon filter. Size exclusion chromatography and HPLC reverse phase chromatography were used to fraction peptide pools based upon size and hydrophobicity. Differences in peptide accumulation between the mutants and WT will be identified by tandem MS/MS. These results may identify cAAP enzyme substrates, and place cAAP's activities within specific physiological pathways.

Oral Presentations - Session III

Towards Genetic Improvement of Plants

Saturday, April 25, 2009, 2:00 pm – 3:30 pm

Chair: Dr. Zhongguo Xiong

University of Arizona

IMPROVING *AGROBACTERIUM TUMEFACIENS*-MEDIATED GENE DELIVERY IN *SORGHUM BICOLOR*

Tamara Miller, Eric Trieu, Cindy Lee, and Peggy G. Lemaux.
Department of Plant and Microbial Biology, University of California, Berkeley 94720

Grain sorghum (*Sorghum bicolor*), the fifth most important cereal worldwide, possesses multiple agronomic traits that make it a desirable future crop. These include its ability to survive abiotic stresses like drought, flood, and high temperatures while using far fewer inputs than other crops, such as maize. Not only is sorghum an important food for many in semi-arid regions, but the grain and stems, and sugars from the stalks of sweet sorghum, can be used for biofuel production. Despite these positive traits, its grain is lacking in the amino acids, lysine and tryptophan, essential elements of the human diet, and it is the least digestible of all cereals. Genetic improvement of sorghum could not only benefit millions of people in semi-arid regions of Africa and Asia, but also help meet energy demands in more developed countries. However, the recalcitrant nature of its *in vitro* culture makes sorghum difficult to engineer due to low transformation efficiencies and production of phenolics that adversely affect callus culture. Our laboratory has developed more efficient procedures to genetically transform sorghum via *Agrobacterium* infection (Gurel et al., 2009). We tested multiple treatment methods to induce a stress response prior to *Agrobacterium* inoculation, which led to higher embryo survival rates, increased callus initiation and an ~8% transformation frequency. To reduce the time to generate and characterize transgenics, most recently we used a short-season sorghum variety, N247, and achieved 85% transformation efficiency, observing GFP expression six days post-inoculation. Selection and regeneration of these tissues is currently underway.

GENETIC ANALYSIS OF TRICHLOROETHYLENE (TCE) METABOLISM IN HYBRID POPLAR AND TRANSGENIC POPLAR

Jun Won Kang[1], Sharon Doty[1], Jasmine Wilkerson[2], Dick Beyer[2], Theo Bammler[2], Fred Farin[2], & Stuart Strand[3]
[1]College of Forest Resources, University of Washington, Seattle, WA 98195;
[2]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA 98195;
[3]Department of Civil and Environmental Engineering, University of Washington, Seattle, WA 98195, USA

Trichloroethylene is one of the most commonly detected organic compounds and is suspected of causing human cancer. To remove trichloroethylene, various conventional treatments have been applied. One of the most promising methods is phytoremediation which uses plants to remove pollutants. There is a lot of interest specifically in the use of poplar trees (*Populus sp.)* for phytoremediation because poplar has a small genome, ease of vegetative propagation, and availability of genetic transformation. Poplar trees are able to take up trichloroethylene and degrade it by variety of plant enzymes such as cytochrome p450s, glutathiones, and peroxidases. Also in our previous study, we have shown that transgenic hybrid poplar plants expressing mammalian cytochrome P450 2E1 (CYP2E1) had greatly increased metabolism of TCE.

In this research, we compared the poplar genes that may be involved in TCE metabolism between wild-type hybrid poplar and transgenic poplar by microarray analysis. Through this analysis, we found many putative genes that are likely to be involved in TCE metabolism. Also, the overexpressed mammalian CYP2E1 gene triggered altered expression patterns of numerous putative detoxification genes. To determine specific CYP450s or other genes that may be involved in the degradation of TCE, we are currently identifying the function of putative genes by using a yeast system for expression of the plant genes. These putative genes may have biotechnological potential for degradation of organic pollutants.

EVOLUTION AND BIOSYNTHESIS OF MEDICINALLY IMPORTANT TERPENOIDS SUCH AS CURLONE AND THE TURMERONES IN TURMERIC AND GINGER

Hyun Jo Koo, David R. Gang
The University of Arizona, Department of Plant Sciences

Turmeric and ginger are well known for their anti-inflammatory and anti-cancer activities, which have been mostly attributed to the presence of the gingerols, curcuminoids and related diarylheptanoids. Curlone and the turmerones are sesquiterpenoids from turmeric that have been shown to possess antioxidant and antimutagenic properties. α-Zingiberene and β-sesquiphellandrene, which also possess important biological activities, have been found in both turmeric and ginger. We have investigated the biosynthesis of such compounds in these plants using a biochemical genomics-based approach. In a database of over 50,000 ESTs from these two species, we were able to identify 20 putative monoterpene synthases and 10 putative sesquiterpene synthases, as well as P450 monooxygenases that are good candidates for the oxidation step leading to curlone and the turmerones. The corresponding cDNAs were isolated from the respective species and the recombinant proteins were expressed in E. coli or yeast. In vitro enzyme assays were able to determine the function of most of these enzymes. The terpene synthases identified were: camphene synthase, α-phellandrene synthase, β-phellandrene synthase, 1,8-cineole synthase, *p*-mentha-1,4(8)-diene (terpinolene) synthase, α-zingiberene/sesquiphellandrene synthase, β-selinene synthase, β-elemene synthase, γ-amorphene synthase, and α-humulene synthase. A comparison of metabolite profiling and gene expression profiling data across tissues, plant varieties, and developmental stages suggested that the curlone/turmerone synthesis pathway is initiated by α-zingiberene/sesquiphellandrene synthase in turmeric. Evolution of terpenoid metabolism in these species will also be discussed.

Oral Presentations - Session IV

Genomics

Saturday, April 25, 2009, 2:45 pm – 3:30 pm

Chair: Dr. Ramin Yadegari

University of Arizona

COMPARATIVE GENOMIC ANALYSIS OF CLP/HSP100 PROTEINS

Elizabeth R. Waters
Dept. of Biology San Diego State University

The Clp/Hsp100 proteins are members of the superfamily of AAA+ ATPases. The Clp proteins include HSP101 in Arabidopsis and HSP104 in yeast. Both of these proteins are not required at normal conditions but are required for survival at heat shock temperatures. The Clp/Hsp100 proteins work closely with the HSP70 chaperone system. Specifically, these proteins act to disaggregate proteins so that they can be refolded. We have been examining the phylogenetic distribution, genomic organization, and sequence evolution of this large protein family. We searched a variety of databases including Genbank and databases not yet linked or deposited in Genbank. Our analysis of plant/chloroplast ClpB proteins includes homologs from all available, cyanobacterial, algal and plant genomes. Our results include an analysis of the Clp/Hsp100 proteins of the moss Physcomitrella patens and the spikemoss Selaginella moellendorffii. We will present a comparative evolutionary analysis of this large and complex family of proteins. We have examined the patterns of protein domain evolution and amino acid conservation. We will present evidence of differential types and patterns of natural selection on domains and between Clp/Hsp100 subfamilies. These patterns will be discussed in light of known expression patterns, 3-D protein structures, and current models of protein function. In order to gain a more complete understanding of the evolution of the chaperone system these evolutionary patterns will also be compared to known patterns of evolution of other chaperone protein families.

ASSESSMENT OF THE EXTENT OF SUBSTITUTION RATE VARIATION OF LONG TERMINAL REPEAT SEQUENCES IN ORYZA SATIVA AND ORYZA GLABERRIMA

Andrea Zuccolo, Aswathy Sebastian, Yeisoo Yu and Rod A. Wing
Arizona Genomics Institute, Department of Plant Sciences, BIO5 Institute, University of Arizona, Tucson, AZ 85721

Long Terminal Repeat retrotransposons (LTR-RTs) are widespread and ubiquitous in the plant kingdom where they constitute significant portions of several genomes. Useful information about the "history" of these elements in a genome is provided by the comparative study of their insertion times that can be inferred through the comparisons of the two retrotransposon LTRs, if the appropriate mutation rate is known.

Over the past several years, different mutation rates have been proposed for LTRs in crop plants. However very little is known about the extent of the mutation rate variation and the factors contributing to this variation, so the rates currently used are generally considered rough estimators of the actual rates.

To evaluate the extent of substitution rate variation in LTRs, we mined all orthologous LTRs on the chromosome 3 short arms of both Oryza sativa and Oryza glaberrima species. Seventy orthologous LTRs were isolated: since they were present in the common ancestor before the two species separated, the mutations in these regions have accumulated during the time elapsed from speciation event. This applies to all the orthologous sequences mined. This gives the opportunity to study the variation of LTR substitution rate in different elements across the short arm of the chromosome 3. For comparison purposes we investigated a similar amount of not repeat related sequences collected near the orthologous LTRs.

We demonstrated that the extent of the substitution rate variation in LTRs is greater than 5 fold, is positively correlated with GC content, and is not correlated with LTR-RT position along the chromosome. We confirmed that in the vast majority of cases, that LTRs mutate faster than their corresponding not repeat related neighboring sequences. Finally we discussed the effects of methylation on LTR mutation rate variation.

Oral Presentations - Session V

Plant Environment Interactions

Sunday, April 26, 2009, 9:00 am – 10:30 am

Chair: Dr. Elizabeth Waters

San Diego State University

DETECTION AND QUANTIFICATION OF PLANT DROUGHT TOLERANCE ENHANCING BACTERIUM PAENIBACILLUS POLYMYXA IN THE RHIZOSPHERE OF WILD BARLEY (HORDEUM SPONTANEUM) WITH REAL-TIME PCR

Salme Timmusk[1] *and Eviatar Nevo*[2]
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Paenibacillus polymyxa is a plant growth-promoting bacterium which has shown several interesting qualities. Many research groups have studied the species to exploit its useful functions in agricultural systems, specifically in plant growth promotion and biological control against plant pathogens. P. polymyxa strains produce several secondary metabolites including auxins and cytokinins and antimicrobial compounds useful for biotechnological applications (1, 3)and references therein). The bacterial qualities are rather common among plant growth promoting rhizobacteria. P. polymyxa is also known to enhance plant drought stress tolerance (4). This rhizobacterial ability to interfere with osmotic stress regulation has been rarely reported and holds huge potential for agricultural applications (2, 4).

Real-time PCR was developed to detect and quantify P. polymyxa in the rhizosphere of wild barley (H. spontaneum) at the 'Evolution Canyon' ('EC'). Primers and FAM-TAMRA probe targeting 16S rDNA gene were designed and used to detect and quantify the target. Two commercial kits Bio101 Fast Spin and Mo Bio Ultra Clean Soil DNA kits were tested for DNA isolation from the rhizosphere and surrounding soil. With the assay we were able to detect 1 pg of DNA per PCR corresponding to 100 cells per ml. Population densities of P. polymyxa were studied in the rhizosphere of wild barley and surrounding soil from the contrasting climatic slopes at the 'EC'. Significantly higher P. polymyxa number was detected the rhizosphere of arid 'African' microclimate indicating a role of adaptive co-evolution with plants.

References

1. Haggag, W., and S. Timmusk. 2007. Colonization of peanut roots by biofilm forming Paenibacillus polymyxa intiates biocontrol against crown rot disease. J Appl Microbiol 104, 961-969.
2. Timmusk, S., N. Grantcharova, and E. G. H. Wagner. 2005. Paenibacillus polymyxa invades plant roots and forms biofilms. Appl Environ Microbiol 11, 7292-7300.
3. Timmusk, S., B. Nicander, U. Granhall, and E. Tillberg. 1999. Cytokinin production by Paenibacillus polymyxa. Soil Biol Biochem 31, 1847-1852.
4. Timmusk, S., and E. G. Wagner. 1999. The plant-growth-promoting rhizobacterium Paenibacillus polymyxa induces changes in Arabidopsis thaliana gene expression: a possible connection between biotic and abiotic stress responses. Mol Plant Microbe Interact 12, 951-959.

SPODOPTERA FRUGIPERDA HERBIVORY AND MECHANICAL INJURY ON CEREAL GRASSES A. VOLATILE ORGANIC COMPOUND INDUCTION IS UNCOUPLED WITH INJURED LEAF PHOTOSYNTHETIC RESPONSES- DAY 1

Helton P. Camara[1,2], Kevin J. Delaney[2], Robert K. D. Peterson[2], David K. Weaver[2]

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Herbivory on plants has been shown to affect photosynthesis and volative organic compound (VOC) production, but both are typically not examined in the same study. Here, we measure gas exchange parameters from two main stem leaves and VOC production from the main stem at 4h and 28h. Uninjured cultivated wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) plants were compared to plants that experienced fall armyworm attack (*Spodoptera frugiperda*) or mechanical injury. Only leaf photosynthetic rate was significantly reduced by both types of injury at 4h post-injury and only for wheat, while no gas exchange parameters were affected at 28h post-injury for all three grasses. The VOC profiles in response to injury differed between these three grasses. *S. furgiperda* herbivory resulted in qualitative and quantitative differences in VOC induction compared mechanical injury, with the most dramatic differences for wheat. Unique (only for these three grasses) injury induced VOC's include indole for wheat, copaene for barley, and decanal for oats. Commonly injury induced VOC's among the three tested grasses include the sesquiterpenoid furan-3-(4,8-dimethyl-3,7-nonadienyl(E)) and β-ocimene from *S. frugiperda* herbivory. Results with day-1 shows that the compound furan-3-(4,8-dimethyl-3,7-nonadienyl(E)) and β-ocimene appeared in all three grasses in the *Spodoptera frugiperda* treatment. Our results initially suggest that plant primary physiological responses (e.g., photosynthesis) were not coupled with plant secondary physiological responses (VOC induction) with the oats and barley, as main stem VOC induction does not appear to lead to a trade-off with injured leaf photosynthetic activity. Results with day-2 to be covered in a different talk also suggest no VOC induction trade-off with injured leaf photosynthesis for wheat.

DROUGHT INCREASES FREEZING TOLERANCE OF LEAVES AND XYLEM IN *L. TRIDENTATA*

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Freezing may limit the high latitude expansion of warm desert shrub populations; however, because drought and freezing often occur together in deserts, it is important to determine their interactive effects on plant performance and survival. We made measurements of greenhouse-grown droughted and well-watered *Larrea tridentata* from Chihuahuan Desert. We measured survival, leaf loss and re-sprouting as well as plant water potential (ψ), gas exchange, electrolyte leakage, freezing point depression, leaf specific xylem hydraulic conductance (k_l) and following freeze/thaw events at -8°C, -15°C, -19°C and -24°C. For all measures, drought plants (mean ψ -3.5 MPa) showed lower performance than well-watered (mean ψ -1.8 MPa) plants before freeze/thaw events, but the reduction in performance due freezing in drought plants was lower than well-watered plants. The presence of mild drought significantly increased leaf retention following low temperatures as cold as -19°C (X^2 =0.0443). A repeated measures ANOVA revealed significant differences between drought and well-watered plants in photosynthesis ($p<0.0001$). Drought also prevented cell death in leaves and green stems following freezing to -15°C but not -19°C, and two-way ANOVA indicated a significant water by temperature interaction ($p<0.0001$). Furthermore, drought plants showed significant freezing point depression in leaves and green stems ($p = 0.0208$) and significant differences in percent loss of k_l following freezing in both green stems and wood ($p = 0.0011$). These data indicate that dry, cold winters could increase establishment of the species, while increases in the number of wetter, warmer winters could lead to reductions in recruitment, productivity and survival at high latitudes. In addition, lower k_l in the green stems of well-watered plants was associated with greater electrolyte leakage compared to plants, suggesting a possible role for living cells in the maintenance or repair of freeze-thaw xylem embolism.

PHYSIOLOGICAL AND YIELD RESPONSES OF FIELD-GROWN ALFALFA (MEDICAGO SATIVA L.) TO WATER DEFICIT

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Water deficit reduces forage yield in arid regions of the southern Great Plains and western United States. A study conducted at Leyendecker, NM (2006 and 2007) investigated the effect of water deficit on water relations (leaf water potential [Ψ^{pd}], relative water content [RWC]), gas exchange, forage yield and root biomass by withholding irrigation on six alfalfa (*Medicago sativa* L.) accessions varying in water use efficiency (WUE). Physiological measurements were taken on non-stressed plants (baseline) within 5-10 days after irrigation (DAI) and on visibly wilting plants (stress I and II) between 15-20 DAI; solar noon leaf RWC indicated that plots were water stressed. Forage yield was collected at the end of each harvest (2006-2007), while root biomass was collected at the end of 2007 season. Well-watered mean photosynthesis (P_{net}) was 13 μmol m^{-2} s^{-1} across alfalfa accessions in both years. Progressive drought reduced mean P_{net} by 55% to 6 μmol m^{-2} s^{-1};mean forage yield was reduced by 65 to 80 % from baseline to stress I and II. Alfalfa accessions expressed conserved drought response, where water-deficit induced stomatal closure and reduced transpiration corresponding to increased leaf temperature and vapor pressure deficit, causing drop in P_{net} and hence forage yield. Although forage yield in well-watered plots was similar across alfalfa accessions in both years, water-deficit plots yielded differently with Wilson (drought tolerant) and Falcata (low WUE) being the highest and lowest yielder, respectively, in both years. Wilson also had the higher root biomass relative to Falcata, suggesting that enhanced diversion of carbohydrates to root tissue enhances drought tolerance.

FUNCTIONS OF EXTRACELLULAR PROTEINS AND EXDNA IN ROOT TIP RESISTANCE TO FUNGAL INFECTION

Fushi Wen, Gerard J. White, Hans D. VanEtten, Martha C. Hawes
University of Arizona, Department of Plant Sciences Division of Plant Pathology and Microbiology, Tucson AZ 85721

Root diseases caused by soilborne plant pathogens continue to be an important source of crop loss. These diseases are of increasing concern as the use of pesticides is greatly reduced to protect the environment. One alternative means of crop protection is to exploit natural mechanisms of disease resistance. Root tips are highly resistant to infection by pathogens that readily invade other parts of the root. Although the mechanism for strong immunity at root tip to soilborne plant pathogens is not clear, the presence of detached root cap border cells surrounding the root tip and mucilaginous matrix or 'slime' encasing border cells and root tip have been shown to play a role in protecting root tip. Here we report that extracellular proteins and DNA (exDNA) are components of root cap slime, and that these extracellular macromolecules are required for root tip resistance to fungal infection. A complex of >100 extracellular proteins was confirmed, by multidimensional protein identification technology. By feeding pea root tips with 32P-dCTP and harvesting root cap slime, we demonstrated that living cells are the source of exDNA. Direct cloning and sequencing revealed that the vast majority of exDNA are structural “junk” DNA. Our results, for the first time, suggest that exDNA is a previously unrecognized component of plant defense; an observation which is in accordance with the recent discovery that an extracellular trap comprised of exDNA and extracellular proteins from white blood cells play a key role in the vertebrate immune response.

A DOMINANT HSP101 MUTATION IS SUPPRESSED BY MUTATIONS IN AN MTERF-RELATED PROTEIN

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HSP101 is a hexameric AAA+ protein in the ClpB/Hsp100 chaperone protein family and is required for acquired thermotolerance in plants and other organisms. The protein has two ATP-binding domains and exhibits ATP-dependent protein remodeling activity necessary for its stress-protective function. We identified a dominant negative allele of *Arabidopsis HSP101, hot1-4*, which is hypersensitive to heat. The *hot1-4* allele has a mutation in a coiled-coil domain, which is unique in the ClpB/Hsp100 family proteins. To search for factors that interact with HSP101, we screened for extragenic suppressors of *hot1-4* in an M2 population of EMS mutagenized *hot1-4* seeds. One suppressor, *shot1* (*SHOT: suppressor of hot1-4 1*), encodes a mitochondrial transcription termination factor-related protein (MTERF). The MTERF family includes diverse proteins in metazoans and plants (including 34 members in *Arabidopsis*), about which there is limited functional data. Both the *shot1-1* missense mutant (G105D) and the *shot1-2* knock out mutant (T-DNA insertion) have a short hypocotyl phenotype and suppress the *hot1-4* heat-hypersensitive phenotype. Mature *shot1-2* plants are smaller than WT or *shot1-1* plants. A SHOT1-GFP fusion protein localizes to both mitochondria and chloroplasts in transgenic plants, but not to the cytosol where HSP101 resides.

These data indicate that the SHOT1 protein does not interact directly with HSP101, but rather suppresses the *hot1-4* phenotype by an indirect mechanism. Further study of the function of SHOT1 and the mechanism by which it suppresses *hot1-4* heat-hypersensitivity are in progress.

Poster Presentations

Saturday, April 25, 2009, 4:00 pm – 6:00 pm

P20: **IDENTIFICATION OF TWO RECEPTOR -LIKE KINASES REQUIRED FOR PATTERNING AND SEEDLING VIABILITY IN ARABIDOPSIS THALIANA.**

Anthony C. Bryan, Adam Obaidi and Frans E. Tax
Molecular and Cellular Biology, University of Arizona Tucson, AZ

A key area of interest in developmental biology is how cells communicate to determine cell fate and how these cell fates are maintained and executed. In Arabidopsis thaliana, transmembrane Receptor-Like Kinases (RLKs) are the largest class of protein kinases consisting of at least 420 genes. RLKs play diverse roles including regulating differentiation, cell division, and response to biotic and abiotic stresses. Maintaining the identity of the epidermis, or outer cell layer, in adult plants has been shown to require signaling mediated by several RLKs including ACR4, ALE1 and ALE2. In a reverse genetic screen, I have determined that epidermal development in Arabidopsis embryos and seedlings requires two additional related RLKs. Disruption of both of these RLKs results in a growth-arrested seedling that fails to form a protective barrier on its exterior surface. Using transcriptional fusions to the Glucoronidase reporter gene, I have determined that both RLKs are expressed in seedlings, anthers, and during embryogenesis. Translational fusions and microarray analyses suggest these RLKs are highly expressed during early embryogenesis and predominantly found in the epidermis. Embryos with loss of both RLKs express markers for epidermal identity, though aberrant patterning of differentiated epidermal cells is observed. Additionally, mutant seedlings have defects in cell division including clustering of stomata. Although the epidermis is specified in our mutants, there is incorrect execution of epidermal cell fates. The identification of additional RLKs involved in epidermal development further shows the complexity of intercellular signaling required to correctly regulate cell division, and cell identity specification and execution.

P21: **IDENTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF ENZYMES INVOLVED IN (+)-PISATIN BIOSYNTHESIS: THE UNKNOWN STEPS**

Rhodesia M. Celoy and Hans D. VanEtten
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(+)-Pisatin, produced by pea (*Pisum sativum* L), is an isoflavonoid derivative belonging to the pterocarpan family. (+)-Pisatin was the first chemically identified phytoalexin and subsequent research has demonstrated that most legumes produce (-)-pterocarpans as phytoalexins. Studies on the biosynthesis of (+)-pisatin had shown that (-)- enantiomeric compounds are intermediates in the synthesis of this (+)-pterocarpan. However, the step(s) from a (-)-enantiomeric intermediate to a (+)-product is still unknown. Chemical reduction of (-)-7,2'-dihydroxy-4',5'-methylenedioxyisoflavanone [(-)-sophorol], the first optically active substrate in the pathway, produced two isomers of (-)-7,2'-dihydroxy-4',5'-methylenedioxyisoflavanol [(-)-DMDI]. NMR analysis has showed that the major product is the *cis* isomer and the minor product is the *trans* isomer. We believed that this is the branch point in the pathway for the production of (-)-maackiain or (+)-pisatin. Initial enzyme assays with proteins from elicited pea tissue, using *cis* (-)-DMDI isomer as the substrate, showed the production of an achiral isoflavene. The production of an achiral intermediate could serve as the step for the change in configuration that will ultimately produce a (+)-derivative like that of (+)-pisatin.

P22: **METABOLITES FROM THE ROOT CAP: IMPACT ON ROOT GROWTH, BORDER CELL PRODUCTION, AND POTENTIAL IMPORTANCE IN CONTROLLED ENVIRONMENT AGRICULTURE SYSTEMS.**

G. Curlango-Rivera, G. Albala, D.V. Duclos, J.J. Ebolo & M.C. Hawes Department of Plant Sciences, Division of Plant Pathology and Microbiology, University of Arizona. Tucson, AZ. USA. *Corresponding author.*

Roots release proteins, sugars, amino acids and other small metabolites into the soil environment. Root caps are an important source of such exudates, especially in emerging roots. The impact of these chemicals on growth, behavior, and gene expression of pathogens, symbionts and other soilborne microflora has been studied intensively. The functional impact of root exudates on the roots that produce them has received less attention. Here we report that even a transient exposure of *Pisum sativum* L. root tips to certain metabolites normally released from root caps into the rhizosphere can significantly influence not only root growth but also can alter the quantity and quality of exudation, independent of changes in root growth. Understanding how root-derived metabolites can impact root function may yield benefits in crop production, especially in controlled environment agriculture.

P23: **SPODOPTERA FRUGIPERDA HERBIVORY AND MECHANICAL INJURY ON CEREAL GRASSES A. VOLATILE ORGANIC COMPOUND INDUCTION IS UNCOUPLED WITH INJURED LEAF PHOTOSYNTHETIC RESPONSES- DAY 2**

Kevin J. Delaney[1], Helton P. Camara[1,2], Robert K. D. Peterson[1], David K. Weaver[1]

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Herbivory on plants has been shown to affect photosynthesis and volatile organic compound (VOC) production, but both are typically not examined in the same study. Here, we measure gas exchange parameters from two main stem leaves and VOC production from the main stem at 28h. Uninjured cultivated wheat (Triticum aestivum), barley (Hordeum vulgare), and oat (Avena sativa) plants were compared to plants that experienced fall armyworm attack (Spodoptera frugiperda) or mechanical injury. No gas exchange parameters were significantly changed at 28h post-injury for all three grasses. The VOC profiles in response to injury differed between these three grasses. S. furgiperda herbivory resulted in qualitative and quantitative differences in VOC induction compared mechanical injury, with the most dramatic differences for wheat. However, several VOC's that are induced during the first day of measurements do not have significant induction detected by the second day of measurements after injury. Linalool induction for wheat was much more dramatic in the second day after injury, but otherwise no additional VOC's were induced beyond those detected in the same day as injury (day-1). The tested grasses have an initial strong VOC response several hours after injury has occurred, but this response is reduced by the second day following injury. These day-2 results also suggest that VOC induction in not coupled with photosynthesis for wheat, suggesting that for all three tested grasses that a trade-off with VOC induction does not result in photosynthetic reduction on injured leaves from mechanical injury or from Spodoptera frugiperda herbivory.

P24*: ARABIDOPSIS* T-DNA INSERTION MUTANTS IN PUTATIVE CHLOROPLAST PROTEASES RETAIN RUBISCO LARGE SUBUNIT DURING NATURAL LEAF SENESCENCE

Maryann Kimoto, John V. Belletto, Eileen M. Belanger and Judy A. Brusslan,
California State University-Long Beach, Long Beach CA, 90840

Chloroplasts contain the majority of cellular nitrogen, which is recycled during leaf senescence. Thylakoid membranes are internally dismantled during leaf senescence yet chloroplasts remain distinct organelles. While senescence-associated vacuoles and RuBisCO-containing bodies contribute to the process, both allow for additional pathways. We hypothesize that chloroplast proteins are degraded *in situ* by chloroplast-localized proteases during leaf senescence. Using bioinformatics tools, our laboratory identified twenty *Arabidopsis thaliana* proteases which are predicted to reside in the chloroplast and to be up-regulated at the mRNA level in senescing leaves. Homozygous T-DNA insertion alleles in the genes encoding the protease of interest have been isolated and insertion sites have been sequenced. These lines are currently being evaluated for senescence phenotypes. Most of these proteases are stromal-localized, so levels of RuBisCO large subunit (LSU) are being quantified during leaf senescence to determine if protease mutants accumulate higher levels of stromal protein. Leaves of comparable senescent progression were harvested by chlorophyll content. RuBisCO LSU levels were measured by immunoblot and normalized to Lhcb1 protein levels. Currently, the *s33a*, *s41-3* and *m41a-2* mutants have consistently shown a 1.2-1.3 fold increase in Lhcb1 normalized RuBisCO LSU protein levels at the middle stages of senescence when compared to wildtype. This increase is not observed in the early stages of senescence. Other mutants (*a1ua-1*, *a1-1*, *a1-2*, *a1-3*) have shown a smaller (≤1.1 fold) increase. While additional work needs to be done, these data suggest that chloroplast-localized proteases, in particular S33a, S41 and M41a-2, likely contribute to turnover of protein in the chloroplast during natural leaf senescence.

P25: **METALLOTHIONEINE IN COPPER AND ZINC DETOXIFICATION IN PLANTS**

Kuznetsov Vl.V. Ivanova E.M. Volkov K.S. Grinin A.L. Kholodova V.P.

Metallothionein (MT) participation in Cu and Zn excess detoxification was investigated in *Mesembryanthemum crystallinum*, *Brassica napus*, *Brassica juncea* plants. Expression of genes *McMT2, McMT2a, BnMT2, BjMT2* was evaluated with RT-PCR technique using 18S as the control. The prolonged action of HM excess in the nutrient medium (7-10 days) increased MT mRNA levels compare to controls in leaves of all plant studied. Maximal values 6-fold higher then in the control - were obtained in leaves of *Mesembryanthemum crystallinum* plants under 50 uM $CuSO_4$, $ZnSO_4$ (500uM)increased *McMT2* expression not more than 3 times. Detailed dynamic of HM effect to gene activation was followed for rape plants. Significant activation of *BnMT2* was registeredin 24 hours of HM action, later the effect was enhansed and maintained to the level exceeding 2-times the control variant during 5-7 days then mRNA content dropped almost to the control level. In distinction to *BnMT2*, activation of *BjMT2* expression started later but retained longed in leaves of rape plants. It was determined that sum mRNA content of two genes investigated correlated much stronger to the rates of Cu accumulation than to its total content in leaves. It may witness that the role of MT in HM detoxification is limited to HM active centre blockage until they were in cytoplasm, in the zone of an active metabolism. After HM sequestration for prolonged storage in vacuole or apoplast, phytochelatins seems to serve as HM chelators. The conclusion was supported by increased activation by Cu and Zn of PCS, gene encoding phytochelatinsynthase during the later stage of *B. napus* adaptation to HM excess. The work was partially supported by the RFBR (project 07-04-00995; 08-04-90111-Mol_a) and by Presidium of RAS program (cell and molecular Biology).

P26: **EMBRYONIC CELLULAR SIGNALING IN ARABIDOPSIS THALIANA**

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Receptor like kinases (RLKs) are a family of structurally related proteins that consist of an extracellular domain, a transmembrane domain, and a kinase domain. The extracellular domain of RLKs is suggested to be very important in the perception of secreted signals. Two RLKs, RPK1 and TOAD2, are known to play a role during *Arabidopsis thaliana* embryogenesis, and when one or both of these receptors are knocked out, embryo lethality results. Both of these receptors have overlapping expression in the protoderm and ground tissue initials of the late globular stage of embryo development, which is when the three primary radial layers of the embryo are formed. Furthermore, embryos homozygous for *rpk1* and *toad2* mutations do not properly express markers specific for the protoderm and ground tissue at the globular stages. Putative ligands of RPK1 and TOAD2 belong to the CLE gene family. The CLE genes encode for small peptides, which have a conserved 14 amino acid sequence. However, redundancy within the CLE family has so far made it difficult to characterize the function of these ligands. Several experiments have pointed towards CLE peptides interacting with RPK1 and/or TOAD2. Normally, exogenous treatment of Arabidopsis roots with a CLE peptide inhibits root growth. However, when *toad2* mutants are treated with exogenous CLE peptide, no inhibition of root growth is observed, suggesting that the TOAD2 receptor is responsible for perception of and response to the CLE peptide. In addition, using a nuclear-localized GFP promoter fusion as well as a GUS promoter fusion, several CLE genes have been found to be expressed during the globular and late globular embryonic stages. These findings suggest that there may be some interaction taking place between the CLE peptides and RPK1/TOAD2 during radial patterning in embryogenesis.

P27: **COMPARATIVE TRICHOMICS REVEALS GENES IMPORTANT FOR PELTATE TRICHOME FUNCTION**

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Department of Plant Sciences, University of Arizona

The peltate glandular trichome is an important extra-epidermal structure broadly conserved across the angiosperms. With a wide assortment of shapes and sizes, this structure plays important roles in plant defense, pollination and seed dispersal. Many important developmental, biological and biochemical processes occurring within this evolutionarily essential specialized cell type remain largely uncharacterized. Several projects have produced collections of expressed sequence tags (ESTs) from the secretory cells of glandular trichomes, including our project that seeks to investigate metabolism in the glandular trichomes of Solanum species. By comparing the EST sequences of a specific and physically similar peltate glandular trichome type across ten species of plants (Solanum habrochaites, Solanum pimpnellifolium, Solanum lycopersicum, Solanum peruvianium, Mentha piperita, Salvia divinorum, Salvia fruticosa, Ocimum basilicum, Cannabis sativa, and Humulus lupulus), even though some of the EST collections were rather limited, we were able to identify eight genes within the reference species S. lycopersicum that are common to all of the species examined. Some of these genes are implicated in basic biochemical pathways such as one carbon metabolism, glycolysis, and nitrogen recycling. Other uncharacterized genes appear to be involved with maintenance of cellular redox potential as well as transport within and between the biosynthetically active trichome secretory cells. All together, this small collection of genes highlights the potential of the glandular trichome as a model system to study previously untestable basic biological and biochemical questions as well as the functions of novel genes.

P28: **THE IDENTIFICATION OF ENZYMES USED IN SALVIA DIVINORUM TO PRODUCE SALVINORIN A**

Angela Schlegel[1], Hyun Jo Koo[1], Eric McDowell[1], Lukasz Kutrzeba[2], Franck Dayan[2], David R. Gang[1]
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Salvia divinorum (Labiateae) is a sage found in Oaxaca, Mexico where it has been used by the Mazatec Indians to treat such ailments as headaches, rheumatism, coughs, and digestive issues. The main compound it produces is the diterpenoid salvinorin A, a potent and exclusive Kappa-opioid (KOP) receptor agonist. KOP receptor antagonists could represent treatments for a variety of diseases, including diseases where hallucinations are prominent, certain dementias with hallucinogenic aspects, and various drug addictions. Previous research indicates that salvinorin A is produced through the MEP/DOXP pathway and a biosynthetic pathway for salvinorin A and its analogs has been proposed, although most of the enzymes involved have not been fully identified and characterized. This study attempts to elucidate the remaining sequences of each enzyme and characterize those enzymes by utilizing 5' and 3' RACE and nested RACE or Genome Walking to amplify unknown portions of each gene, cloning and sequencing the resulting amplicons, constructing full-length genes from the resulting data, and amplifying, expressing, and testing the full-length enzymes for substrate specificity. RACE and nested RACE have produced products of the expected size for three genes; of those, two acetyltransferases revealed incomplete 5' ends and one dehydrogenase showed a complete 3' end.

P29: **DIFFERENTIAL SUPPRESSION OF RNAI-MEDIATED RESISTANCE TO AN RNA VIRUS BY A HETEROLOGOUS VIRUS ENCODING A STRONG RNAI-SUPPRESSOR**

Chrystine Solofoharivelo and Zhongguo Xiong
Department of Plant Sciences, Division of Plant Pathology and Microbiology, and BIO5, University of Arizona, Tucson, AZ 85721

Engineered resistance mediated by RNA interference (RNAi) has shown promise in controlling viral diseases in crops. However, the discovery of virus-encode RNAi suppressors raises the question that this resistance may not be stable in mixed viral infections. A heterologous virus encoding a strong RNAi suppressor may neutralize an RNAi-mediated resistance engineered to a specific virus. To test this hypothesis, *Nicotiana benthamiana* plants were genetically engineered to confer resistance to *Red clover necrotic mosaic virus* (RCNMV). The resistant plants were then co-inoculated with RCNMV and *Potato virus Y* (PVY), which encodes a strong RNAi-suppressor, HcPro. We demonstrated that PVY differentially affected RNAi-mediated resistances induced by different mechanisms. In dsRNA-induced RNAi-mediated resistance, PVY did not break the resistance as the transgenic lines are completely immune to RCNMV infection. However, our data showed that PVY did interfere with RNAi, resulting in an increase of the 1.2 kb transgene mRNA. Further, PVY infection increased accumulation of 21-nt si-RNA and did not alter the transcription of the transgene. In contrast, PVY infection suppressed resistance to RCNMV mediated by virus-induced RNAi. In these lines, PVY infection did not alter the accumulation of the 1.2 kb transgene mRNA nor the 21 nt siRNA corresponding to the transgene. The differential effects of PVY infection on the two types of RNAi-mediated resistances suggests that properly designed resistant plants might withstand mixed viral infections and overcome a strong virus-encoded suppressor of RNAi. In addition, these data suggests that parallel but distinct pathways exist in dsRNA-induced RNAi and virus-induced RNAi.

P30: **EFFECT OF THE ABSENCE OF S-NITROSOGLUTATHIONE REDUCTASE AND ALTERED NO METABOLISM ON STOMATAL FUNCTION**

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The enzyme S-nitrosoglutathione reductase (GSNOR) is involved in the reduction of S-nitrosoglutathione (GSNO) and impacts the level of cellular nitric oxide (NO). This enzyme is found in bacteria, yeast, and humans. We have isolated mutants of the single gene encoding GSNOR in the model plant *Arabidopsis* (*hot5* mutants). *Hot5* mutants exhibit several detrimental phenotypes. The null mutants are smaller, produce very few seeds, and have higher levels of NO. In addition, these mutants have a higher amount of nitrogen containing compounds compared to carbon containing compounds. The discrepancy in the levels of the two classes of compounds may be due to altered stomatal function, which is affected by NO. We compared the stomatal aperture of mutant plants to that of wild type plants. When exposed to light followed by dark, the stomata of mutant plants were more open than wild type plants. Future directions of this work are proposed.

P31: **DEVELOPMENTAL CHARACTERIZATION OF A FAMILY OF LRR-RLKS IN *ARABIDOPSIS THALIANA***

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The ability of cells to perceive extracellular signals is a key component in the coordination of complex biological processes. In plants, receptor-like kinases (RLKs) have been shown to be a primary means for the transduction of these signals across the plasma membrane. RLKs are the largest class of *Arabidopsis thaliana* protein kinases. This class consists of at least 610 members and thus represents about 2.5% of the protein encoding genes in *Arabidopsis thaliana*. In a reverse-genetic screen of previously uncharacterized Leucine-Rich-Repeat (LRR) - RLKs, I have found mutations in two genes, PROGERIC (PROG) and PROGERIC-LIKE1 (PGL1), produce few, if any, lateral roots individually. Double mutants display a reduction in primary root growth. Based upon kinase domain sequence homology, these genes form a family with two other LRR-RLKs, PGL2 and PGL3. Preliminary results indicate that single loss-of-function mutants in PROG and PGL3 cause severe and weak vegetative defects, respectively, reminiscent of defects in the brassinosteroid receptor, the LRR-RLK BRI1. The severe vegetative phenotype of PROG mutants is partially suppressed when combined with mutants in the BRI1 interacting LRR-RLK BAK1. Results suggest complex interaction between members of the PROG gene family and BAK1 depending on developmental context. In this study I am characterizing their morphological defects in growth and development, as well as exploring PROG family interactions with BAK1.