20th Anniversary

Mid Atlantic Plant Molecular Biology Society

National Wildlife Visitors Center
Patuxent Research Refuge

August 7 & 8th
2003

CONTENTS

INTRODUCTION

On behalf of the Organizing Committees I would like to welcome you to the twentieth meeting of the Mid-Atlantic Plant Molecular Biology Society, MAPMBS 2003. The goal of this society is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. This meeting is designed to bring some of the best scientific minds in the country to the Mid-Atlantic region to present interesting advances in plant molecular biology at a reasonable price and at an accessible location. We hope to encourage students, postdocs, and senior scientists to actively participate in presentations and discussions. In addition, the meeting is designed to promote interaction among scientists in an informal atmosphere therefore we provide on-site lunches and breaks so that each participant has the opportunity to meet invited speakers and other presenters.

As you know, the meeting encompasses a broad range of research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests. Please contact members of the organizing committees, if you have any thoughts or comments for consideration in the planning of future meetings. Also, if you are interested, you can join next years organizing team and volunteer your services to improve upon what we did this year. All are welcome at any stage of the planning or organizing process.

Many people were involved in the planning and organization of this meeting, and we give them our hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date products, and help to defray costs. Please be sure to visit our exhibitors in the multipurpose room.

Since this is the twentieth meeting of the MAPMBS we would also like to take this opportunity to give a special thanks to Dr. Benjamin Matthews and members of his laboratory for their continued commitment to this society, and to past chairs and co-chairs of the meeting, Dr. Susan Koelher, Dr. Ellen Reardon, Dr. James Saunders, Dr. Dennis Schaff, Dr. Janet Slovin, Dr. David Straney, Dr. Mark Tucker, Dr. John Watson, Dr. Madeline Wu, and Dr. Lynn Zimmerman

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society.

Frank J. Turano
Chair

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2003 MAPMBS MEETING SCHEDULE

Thursday, August 7

9:30 am Registration and Poster set-up

9:40 am Introductions

Session I – **Plant Signaling and Interactions**
Moderator: Janet Slovin

9:50 am Dave Straney, Department of Cell Biology and Molecular Genetics, University of Maryland College Park, College Park, MD
Fungal perception of host isoflavonoids in signaling pathways that control pathogenesis

10:20 am Leslie Wanner, USDA-ARS, Vegetable Lab, Beltsville, MD
Molecular and pathogenic variation in Streptomycetes causing potato or radish scab

10:40 am Break - Coffee, Exhibitors, Poster Set-up and Viewing

Session II – **Genomics, Bioinformatics, and New Technologies**
Moderator: Rana Khan

11:10 am Theresa Taylor, Arcturus, North Carolina
A novel platform for high sensitivity analysis of cell specific gene expression using microarrays

11:40 am Benjamin Matthews, USDA/ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD
Alterations of gene expression and metabolite levels in soybean roots during invasion by the soybean cyst nematode

12:00 pm Lunch - Exhibitors and Poster Viewing

Session III – **Plant Signaling and Interactions**
Moderator: Janet Slovin

1:00 pm Ramesh Raina, Biology Department, Syracuse University, Syracuse, NY
How plants sense the environment: functional genomics of receptor-like kinases in *Arabidopsis*

1:30 pm Richard Sicher, USDA-ARS, Plant Sciences Institute, Beltsville, MD
Exogenous trehalose induces carbohydrate accumulation, and stress-responsive genes and proteins in *Arabidopsis thaliana*

1:50 pm Glenda Gillaspy, Center for Biotechnology, Virginia Tech, Blacksburg, VA
Turning OFF inositol signal transduction

2:20 pm Elena del Campillo, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD
An Arabidopsis cellulase required for root hair development

2:40 pm Jiman Kang, Department of Biological Sciences, The George Washington University, Washington, DC
The role of putative glutamate receptor 1.1 (AtGLR1.1) in the regulation of abscisic acid signaling in *Arabidopsis thaliana*

3:00 pm Break - Sodas, Exhibitors and Poster Viewing

Keynote Address
Introduction: Leslie Wanner

3:30 pm Jen Sheen, Department of Genetics, Harvard Medical School, Boston, MA
Plant sugar sensing and signaling networks

4:30 pm Close of day

Friday, August 8

Session IV – **Genomics, Bioinformatics, and New Technologies**
Moderator: Rana Khan

9:30 am John Quackenbush, The Institute for Genomic Research, Rockville, MD
Whole genome expression analysis in plants: moving beyond arrays

10:00 am Tara VanToai, USDA-ARS, Soil Drainage Research Unit, Columbus, OH
Insights into flooding tolerance of *SAG12:ipt* Arabidopsis plants through transcriptome analyses

10:20 pm Anik Dhanaraj, USDA/ARS, Fruit Laboratory, Beltsville, MD
EST analysis for the study of cold hardiness in blueberry

10:40 am Break - Coffee, Exhibitors and Poster Viewing

Session V – **Plant Signaling and Interactions**
Moderator: Janet Slovin

11:10 am Terrence Delaney, The Department of Plant Biology, Cornell University, Identification in Arabidopsis of novel forms of inducible resistance against pathogens

11:40 am Malcom Livingstone, Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA
Improved resistance to *Sclerotina minor* in transgenic peanut

12:00 pm **Business Meeting**

12:10 pm **Lunch: Exhibitors and Poster Viewing**

Session IV – **RNA biology**
Moderator: Frank Turano

1:30 pm Stephen M. Mount, University of Maryland, College Park, MD
Genetic approaches to understanding pre-mRNA slice site selection in plants

2:00 pm Carole Bassette, USDA/ARS, Appalachian Fruit Research Station, Kearneysville, WV
A novel TATA box element controls expression of the peach dehydrin gene, *PPDHN2*, in ripe fruit and potentially alters the intracellular distribution of the cognate polypeptide

2:20 pm **Closing Remarks**

Poster Take-down

2003 MAPMBS Poster session

Poster Page

5 32 **Janet C. Daniel**, Matthew Seward and Douglas Wilson
Department of Biology, James Madison University, Harrisonburg, VA
Understanding the functional significances of conserved sequence motifs in the *Arabidopsis thaliana* hexose transporter, STP1

6 33 Changhe Zhou, Sunandini Sridha, Ling Zhang, Lining Tian, Daniel Brown, Brian Miki and **Keqiang Wu**
Department of Biology, West Virginia University, Morgantown, WV
Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario, Canada
Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada
Regulation of gene expression by HD2-type histone deacetylases

7 34 **Amanda R. Stiles** and Elizabeth A. Grabau
Department of Plant Pathology, Physiology and Weed Science
Virginia Polytechnic Institute and State University, Blacksburg, VA
Identification and characterization of inositol 1,3,4-trisphosphate 5/6-kinase and inositol 1,4,5-trisphosphate 3/6-kinase in *Glycine max*

8 35 **Joseph M. Chiera**, John Finer and Elizabeth A. Grabau
Department of Plant Physiology, Pathology, and Weed Science,Virginia Polytechnic Institute and State University, Blacksburg, VA
Department of Horticulture and Crop Science, OARDC/The Ohio State University, Wooster, OH
Ectopic expression of phytase in soybean

FUNGAL PERCEPTION OF HOST ISOFLAVONOIDS IN SIGNALING PATHWAYS THAT CONTROL PATHOGENESIS

David Straney, Reynold Tan, and Rana Khan
Department of Cell Biology & Molecular Genetics,University of Maryland, College Park 20708.

Soilborne microorganisms adapted to a certain plant can use the metabolites released from that plant as cues to coordinate their interaction. Although such chemical signaling has been well characterized in model bacterial pathogens and symbionts, much less is known about the basis of the phenomenon in fungal-plant interactions. We are studying one signaling pathway through which a fungal pathogen may be able to "smell" its host by recognizing its specific defense compounds. Pea (*Pisum sativum* L.), like many legumes, exudes its isoflavonoid phytoalexin from its roots under fairly normal conditions. The phytoalexin may be released in order to influence the rhizosphere community. Strains of *Fusarium solani* that are pathogenic on pea are able to perceive the host's isoflavonoid phytoalexin, pisatin, and induce several virulence traits. These traits include the germination of quiescent spores and the expression of a group of genes that constitute a pea-specific virulence gene cluster. One of these pisatin-induced genes is a cytochrome P450 that detoxifies pisatin, PDA1. The perception of the host phytoalexin appears to be used as a host-specific cue to coordinate pathogeneisis. How does the fungus perceive this compound that is encountered only near its host? The gene-specific response, studied with the cytochrome P450 gene, is mediated through a binuclear zinc (C6-Zn2) transcription factor. Studies of its binding site on the PDA1 promoter shows it to be both necessary and sufficient for pisatin-responsive transcription. This transcription factor is able to confer a pisatin response to heterologous fungal systems. The specificity indicates that it acts through perception of pisatin rather than through a stress imposed by pisatin. Thus the transcription factor may serve as a nuclear receptor, much like the different gene family of nuclear receptors that regulate detoxification-associated cytochrome P450s in metazoans. With the recent genome sequencing of plant-pathogenic fungi, it has become apparent that these fungi possess an unusually large number of genes encoding cytochrome P450s and transporters. The phytoalexin-responsive cytochrome P450 in *F. solani* may be just one case where chemical perception of the plant is tied to these chemical tolerance and virulence genes. It will be interesting to determine if other fungi have similar "noses" attuned to detect their hosts as well. If so, a plant's chemical defenses may be considered as much a liability as a protection.

MOLECULAR AND PATHOGENIC VARIATION IN STREPTOMYCETES CAUSING POTATO OR RADISH SCAB

Leslie A. Wanner, USDA-ARS, Vegetable Lab, 10300 Baltimore Ave. Beltsville, MD 20705 USA, e-mail: **wannerl@ba.ars.usda.gov**

Common scab is the fourth most important potato disease, and affects root and tuber crops world-wide. Scab is caused by streptomycetes, a diverse group of soil-inhabiting gram-positive bacteria. Most are not plant pathogens. To better understand the basis for plant pathogenesis, and the variability in disease symptoms seen in laboratory and field situations, we are investigating pathogenicity determinants and virulence factors among plant pathogenic streptomycetes. Streptomycetes that cause scab are phylogenetically diverse, but production of the toxin thaxtomin is considered a pathogenicity determinant. Pathogenicity is also associated with a proposed pathogenicity island (PAI) containing a gene for a pathogenicity factor (*nec1*) within a larger conserved region that may be horizontally transferred into distantly related streptomycetes to produce new plant pathogenic strains. We isolated streptomycetes from scabby potatoes from several regions of the USA and assessed their pathogenicity in radish and potato. Disease symptoms varied in severity and appearance. Several pathogenic isolates do not produce the pigment melanin, and a few do not contain the *nec1* gene. No isolate missing the *txtA* gene encoding thaxtomin biosynthesis was pathogenic, although some isolates not producing melanin were pathogenic. We have further characterized the putative PAI regions of the isolates using PCR, with the *nec1* gene and a second linked gene as anchor points. In contrast to our expectations for structural conservation in this region, we find large variation among plant pathogenic strains. We plan to identify additional molecular and biochemical/physiological characteristics of streptomycetes contributing to variability in scab disease symptom development.

A NOVEL PLATFORM FOR HIGH SENSITIVITY ANALYSIS OF CELL SPECIFIC GENE EXPRESSION USING MICROARRAYS

Theresa B. Taylor
Arcturus, North Carolina

It is now possible to perform genomic analysis from as few as 10-50 cells and proteomic analysis from just a few thousand cells selected and isolated from tissue sections. This exciting new platform from Arcturus combines advanced laser capture microdissection (LCM) with sophisticated methods for small sample preparation and molecular analysis.

Microgenomics makes it possible to obtain the cellular specificity and sensitivity needed to understand the mechanisms underlying complex biological processes, such as plant biology, organism development, disease pathology, and neuronal functioning. The presentation will highlight the technologies comprising Arcturus' integrated microgenomics platform from sample preparation, through cell selection, biomolecule extraction, isolation and amplification. Specific examples will demonstrate applications of microgenomics in combination with PCR, microarrays and other detection platforms in the fields of plant, cancer, neuroscience and developmental biology.

ALTERATIONS OF GENE EXPRESSION AND METABOLITE LEVELS IN SOYBEAN ROOTS DURING INVASION BY THE SOYBEAN CYST NEMATODE

Benjamin Matthews[1], Rana Khan[1], Nadim Alkharouf [1], and Lloyd W. Sumner[2]
[1]USDA, ARS, Plant Science Institute, Soybean Genomics & Improvement Laboratory, BARC-West, Beltsville, MD 20705, USA; [2]Noble Foundation, Ardmore, OK, USA.
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The soybean cyst nematode (SCN) is the most devastating pest of soybean in the US, causing an estimated one billion dollars in damage each year. The defense response of soybean to SCN is a multigenic trait and varies depending upon the genotypes of soybean and SCN. More than 6,000 cDNA inserts from several soybean cDNA libraries were printed and monitored using microarrays to identify genes involved in the response of soybean to SCN. RNA was harvested from roots of soybean cv. Peking resistant to SCN strain NL1-RHp (reflecting a race 3 phenotype) and susceptible cv. Kent, either not infected or at 0, 6, 12 hr, 1, 2, 4, 6, and 8 days after infection by SCN strain NL1-RHp. Two independent biological samples were used for microarrays and metabolic profiles. The RNA was fluorescently labeled as cDNA for hybridization to the microarrays. A number of defense-related genes were identified statistically as being altered due to SCN invasion, as were genes encoding potential regulatory factors, such as kinases and transcription factors, genes involved in sugar metabolism and cell wall formation, and a number of genes encoding proteins of unknown function. Alterations in metabolic profiles combined with gene expression analysis allowed a better interpretation of the events that occur in the root during nematode attack. These results are being used to identify pathways, genes, and metabolites important to the defense response of soybean against SCN attack. See our web site at http://bldg6.arsusda.gov/benlab/ for further information.

How Plants Sense the Environment: Functional Genomics of Receptor-like Kinases in *Arabidopsis*

Ramesh Raina
Biology Department, Syracuse University, Syracuse, NY 13244 (email: raraina@syr.edu)

Receptor-like kinases (RLKs) are involved in signal perception through the cell surface. In *Arabidopsis* RLKs constitute a large family consisting of more than 600 family members (1). However, the function of very few of these genes is known. We have initiated a project directed towards functional analysis of RLKs in *Arabidopsis*. The goal of this project is to develop *Arabidopsis* plants expressing chimeric RLKs, which induce visible responses (hypersensitive response-like necrotic lesions and express GFP reporter gene) when they sense various chemical and biological agents. The chimeric RLKs will have the extracellular domain of the target RLK fused to the kinase domain of an RLK involved in eliciting hypersensitive response and downstream defense responses. These chimeric RLKs will be introduced in the Col-0 plants expressing GFP from the promoter of the *PR-1* gene (a tightly regulated *Arabidopsis* defense gene). Several hundred RLK genes have been targeted for this project. Transgenic plants expressing these chimeric RLKs will constitute a "Chimeric Receptor Kit" and will be screened to identify the extracellular domain(s) involved in perception of many stimuli of interest. Recognition of the ligand by the extracellular domain of the chimeric RLK should activate the kinase, which in turn would induce HR and expression of GFP. If an RLK functions in a developmental process, transgenic plants would be expected to develop necrotic lesions and express GFP at that stage of development/tissue. Thus these transgenic plants should be useful to identify candidate *Arabidopsis* RLKs for a wide variety of biotic and abiotic environmental stimuli and possibly those involved in developmental processes.

Reference:

Shiu, S. H. and A. B. Bleecker (2001). Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proc Natl Acad Sci U S A*, 98: 10763-10768.

EXOGENOUS TREHALOSE INDUCES CARBOHYDRATE ACCUMULATION, AND STRESS-RESPONSIVE GENES AND PROTEINS IN *ARABIDOPSIS THALIANA*

Hanhong (Rino) Bae[1], Eliot Herman[2] and **Richard Sicher**[1]
[1]USDA-ARS, Plant Sciences Institute, Beltsville Agricultural Research Center, 10300 Baltimore Avenue, Beltsville, MD 20705-2350, USA. [2]USDA-ARS, Plant Genetics Research Unit, Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA
rbae@asrr.ars.usda.gov, EHerman@danforthcenter.org, sicherrr@ba.ars.usda.gov

Trehalose is a nonreducing disaccharide (1, 1 α-D glucopyranosyl α-D-glucopyranoside) that occurs in most living organisms, and functions as the principal storage carbohydrate and as an important in osmoprotectant in yeast and certain fungi. Trehalose is present in trace amounts in flowering plants and has been shown to be essential for embryo maturation. Exposure of higher plants to exogenous trehalose inhibits root growth and affects gene expression. In the current study 30 mM trehalose was applied exogenously to *Arabidopsis thaliana* (Columbia) grown in liquid culture for 2 weeks. Whole seedlings were analyzed for changes of metabolites, proteins and mRNA transcripts. Starch, sucrose and glutamate were increased, ATP levels were reduced and glutamine and chlorophyll were unchanged by trehalose treatment. Changes of gene expression were detected using DNA microarrays and proteomic analyses were performed using two-dimensional gel electrophoresis combined with MALDI-TOF. Novel trehalose-induced genes and proteins were found, including those encoding stress-responsive proteins, transcription factors, and cell wall modifying enzymes. Exogenous trehalose treatment repressed the genes for nitrilases (*NIT1, 2* and *4*). These enzymes catalyze the hydrolysis of indole-3-acetonitrile to indole-3-acetic acid (*NIT1* and *NIT2*) and are involved in cyanide detoxification (*NIT4*). Ultra-structural changes to cotyledons and roots were detected by transmission electron microscope (TEM) and densely stained particles were observed in the apoplast of trehalose-treated seedlings. Our results indicate that exogenous trehalose induced stress-responsive genes and proteins, and changed ultra-structure and carbohydrate allocation.

TURNING OFF INOSITOL SIGNAL TRANSDUCTION

Glenda Gillaspy
Fralin Biotechnology Center, Virginia Tech, Blacksburg, VA 24061

Inositol signal transduction is used by plants to respond to many signals including light, gravity, pathogens and the hormone abscisic acid (ABA). The classical inositol signal transduction pathway generates transient increases in second messenger I(1,4,5)P3 (IP3) in response to signals perceived at the cell surface. This signaling ultimately stimulates intracellular calcium release and downstream biological effects. We are interested in how plants control the accumulation of inositol-containing second messengers (including IP3), and whether manipulation of inositol-containing second messengers could effectively be used to alter signal transduction events in plants. To answer this question, we are investigating 15 genes from *Arabidopsis thaliana* that have the potential to encode inositol 5-phosphatases (5PTases), enzymes that catalyze the first step in inositol second messenger breakdown. We are taking a functional genomics approach to understanding how these enzymes control second messenger levels in plants. Our results indicate that 1. Different 5PTases have different second messenger substrates; 2. Plants regulate expression of 5PTases in response to signaling, effectively linking signal termination to second messenger generation;. 3. Overexpression of a 5PTase can block certain ABA-induced signal transduction events; and 4. Inositol signaling and/or metabolism impacts Vitamin C synthesis in plants.

AN ARABIDOPSIS CELLULASE REQUIRED FOR ROOT HAIR DEVELOPMENT

Elena del Campillo, Kaushal Shah, Ebrahim Paryavi and Kadima-Nzuji, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, cd84@umail.umd.edu

A large proportion of the Arabidopsis genome is dedicated to enzymes that change the cell wall. One example of cell wall modification is the initiation and growth of root hairs that are polarized outgrowths of specific root epidermal cells. Cells which overly two cortical cells become hair cells (H position) while cells that contact only one cortical cell develop into non hair cells (N position). Using
a promoter-reporter construct, an endo-β-1,4-glucanase (cellulase) that is specifically expressed in H root hair cells has been identified (Cel6). In addition, some transformants also showed Cel6 expression in the vascular tissue of the cotyledons and young leaves. Cel6 is a member of the glycosyl hydrolase family 9 and has a unique 100 amino acid extension at the C-terminal that resembles a cellulose binding domain, CBD II. The expression of this gene is strongly increased by the presence of ACC, and inhibited by AVG. These results suggest that Cel6 is regulated by ethylene Other abiotic factors are being tested to determine their effects on the expression of this gene. A homozygote T-DNA mutant for Cel6 was identified in the SALK collection. The mutant seedlings show normal root hairs at the junction of the root with the stem however they do not develop new root hairs in the main root as it elongates and grows. A global search of the NASCArrays database of Affymetrix microarrays confirmed the high expression of Cel6 in most experiments that included primary root tissue and, more importantly, a low expression of Cel6 in the root hair defective mutant, rh2. The function of this cellulase may be related to the modification of cellulose microfibrils, which is necessary to allow lateral growth of cells into projecting hairs.

THE ROLE OF PUTATIVE GLUTAMATE RECEPTOR 1.1 (ATGLR1.1) IN THE REGULATION OF ABSCISIC ACID SIGNALING IN ARABIDOPSIS THALIANA

Jiman Kang, Sohum Mehta and Frank J Turano, Department of Biological Sciences, The George Washington University, Washington DC 20052

Abscisic acid (ABA) is a plant hormone involved in the control of a wide range of physiological processes such as seed germination and development, as well as plant responses to environmental stress. We investigated the possible involvement of AtGLR1.1 in ABA signaling. In the presence of carbon, specially sucrose, but not glucose, mannitol or sorbitol, AtGLR1.1-deficient *Arabidopsis* (*antiAtGLR1.1*) seeds did not germinated. Furthermore, the germination of *antiAtGLR1.1* in the presence of an animal ionotropic glutamate receptor (iGLR) antagonist was inhibited, but germination was restored by fluridone, an inhibitor of ABA biosynthesis. The *antiAtGLR1.1* lines were ABA hypersensitive with respect to root growth and seed germination. Consistent with this result, the stomata of the transgenic plants had smaller openings than similarly treated wild-type plants. At the molecular level, altered expression of genes involved in ABA signaling, such as ABA-biosynthesis (ABA) and ABA-insensitivity (ABI) genes, was observed. These results identify AtGLR1.1 as a regulator of ABA biosynthesis and sensing, which in turn controls seed germination and stomatal opening.

PLANT SUGAR SENSING AND SIGNALING NETWORKS

Jen Sheen, Filip Rolland, Wan-Hsing Cheng, Shuichi Yanagisawa, Qi, Hall, Elena Baena González, Young-Hee Cho, Sang-Dong Yoo, Patricia Leon, Brandon Moore and Li Zhou
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Glucose is a universal nutrient preferred by most organisms and serves essential roles in energy supply, carbon storage, biosyntheses, and carbon skeleton and cell wall formation. The ability to sense glucose signal is of fundamental importance in organisms as diverse as *E. coli*, yeast, flies, mammals, and plants. The significance of glucose as a direct and central signaling molecule in multicellular animals and plants has only been recently recognized. In plants whose life revolves around sugar production through photosynthesis, glucose has emerged as a key regulator of many vital processes, including embryogenesis, germination, seedling development, root, stem and shoot growth, photosynthesis, carbon and nitrogen metabolism, flowering, stress responses, and senescence. We are interested in understanding how plants integrate environmental factors and internal sugar signals to modulate plant growth and development.

Multiple sugar signals and sensors initiate signaling pathways to distinct target genes that affect physiological and developmental programs in plants. To elucidate the fundamental and complex plant glucose signaling networks, we have taken a combination of cellular, genetic, genomic, and proteomic approaches. We have used the simple maize and Arabidopsis mesophyll protoplast system to show that glucose but not sucrose or its metabolites is the signal that mediates global transcription regulation. Using Arabidopsis as a genetic model, we have isolated and characterized *glucose insensitive* (*gin*) and *glucose oversensitive* (*glo*) mutants. Our studies of *gin* and *glo* mutants have revealed extensive and intimate connections between glucose and plant hormone signaling pathways. We have also provided compelling evidence for the uncoupling of glucose signaling and glucose metabolism in controlling gene expression and developmental processes. Many genes involved in carbon and nitrogen metabolism and storage, cell cycle, and stresses are similarly regulated by glucose in yeast, animals and plants. The evolutionarily conserved glucose sensor hexokinase may control the energy budget and resource utilizations through the function of signaling complexes. The flexible and reversible responses to both low and high glucose signals in plant growth promotion and inhibition, respectively, depend on cell types, developmental state, multiple nutrient status, and environmental conditions. The plasticity of plant developmental programs could therefore be attributed to versatile sugar sensing and signaling activities in the plant signal transduction networks.

RECENT REFERENCES

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WHOLE GENOME EXPRESSION ANALYSIS IN PLANTS: MOVING BEYOND ARRAYS

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The complete genome sequence of the model plant Arabidopsis thaliana provides an logical framework on which to begin to construct an integrated analysis of gene expression patterns. Using amplicon arrays encompassing nearly all of the genes represented in the nuclear, chloroplast, and mitochondrial genomes, we have begun to explore evolutionary relationships, position-demendent expression patterns, and the relationship between gene expression and metabolic output. We will present an overview of the technology and its application to address a number of important biological problems.

INSIGHTS INTO FLOODING TOLERANCE OF *SAG12:ipt* ARABIDOPSIS PLANTS THROUGH TRANSCRIPTOME ANALYSES

Tara VanToai[1], Fenglong Liu[2], Linda Moy[2], Le Nguyen Huynh[3], Gary Banowetz[4], and John Quackenbush[2].

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Isopentenyl transferase, the rate limiting enzyme in the cytokinin biosynthesis pathway, is encoded by the ipt gene isolated from *Agrobacterium tumefaciens*. Transgenic *Arabidopsis* plants that express *ipt* under the control of the senescence-specific *SAG12* promoter show normal growth but exhibit a 7- to 10- day delay in plant senescence. The *SAG12:ipt* plants remain greener and produce more biomass and seeds than wild-type (WT) plants under waterlogging and submergence stress. In this study, the temporal patterns of *ipt* expression and cytokinin accumulation were determined at specific time points during the 0 to 5 day flooding period. Whole genome expression profiling using DNA microarrays representing 27,000+ genes encoded in the *Arabidopsis* nuclear, chloroplast and mitochondrial genomes was conducted at the same time points. Under non-stressed conditions, the *SAG12:ipt* plants produce similar levels of cytokinin as WT plants, but after 5 d of waterlogging, the transgenic plants accumulated tenfold more *ipt* transcripts and two to threefold more cytokinin. After 5 d of complete submergence, *ipt* transcript accumulation increased twofold in transgenic plants although no difference in cytokinin accumulation was detected between WT and transgenic plants. The differences in global gene expression between WT and *SAG12*:ipt plants under waterlogging and submergence stressed will be presented.

EST ANALYSIS FOR THE STUDY OF COLD HARDINESS IN BLUEBERRY

Anik L. Dhanaraj*, Lisa J. Rowland, and Janet P. Slovin
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To gain a better understanding of the genetics of cold hardiness in blueberry, a genomics approach based on ESTs (Expressed Sequence Tags) was used to compare genes expressed in blueberry flower buds under cold acclimating and non-acclimating conditions. Two cDNA libraries were constructed using RNA from cold acclimated and non-acclimated flower buds of the blueberry cultivar Bluecrop and about 600 ESTs generated from each of them. Sequences were compared to the publicly available sequences from Genbank, putative functions were assigned, and the cDNAs were categorized into 16 functional groups. A contig analysis was carried out as well to identify redundancies and group homologous cDNAs. cDNAs that were picked from each library the most number of times (4 times or more), presumably representing highly abundant transcripts, were identified. Those that were picked more often from one library than the other library were identified as representing potentially preferentially expressed transcripts. Northern analyses were carried out using a few such cDNAs as probes to verify their preferential expression under cold acclimating or non-acclimating conditions.

IDENTIFICATION IN ARABIDOPSIS OF NOVEL FORMS OF INDUCIBLE RESISTANCE AGAINST PATHOGENS

Terrence Delaney, The Department of Plant Pathology at Cornell University; The Department of Botany and Agricultural Biochemistry at The University of Vermont

Plants employ a number of pathogen-inducible defense systems that aid them in resisting infection and in their recovery from disease. The best known and understood of these is systemic acquired resistance (SAR), which depends upon salicylic acid (SA) accumulation and signaling through the *NIM1/NPR1* gene product. A variety SAR-associated genes are induced during this response, and in addition to encoding likely effectors of resistance, are useful molecular markers that identify plants exhibiting SAR. Part of our research focus is directed toward understanding the regulation of SAR by NIM1/NPR1. Other research is aimed at identifying SAR-independent resistance (SIR) mechanisms, which are uncovered in plants that carry mutations or transgenes that prevent expression of SAR. We recently described the *son1* mutant that shows activation of a novel SIR mechanism that does not require SA signaling, NIM1/NPR1 action or SAR gene product accumulation. The cloned *SON1* gene encodes an F-box protein, which suggests that the resistance phenotype displayed by *son1* plants may be regulated by the targeted ubiquitination/degradation of a positive regulator of SIR. The *son1* mutant phenotype, epistasis analysis of *son1*-mediated resistance, and a model of SON1 action will be presented.

IMPROVED RESISTANCE TO *SCLEROTINIA MINOR* IN TRANSGENIC PEANUT

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Sclerotinia minor is the causal agent of Sclerotinia blight, a highly destructive disease of peanut. There is compelling evidence that oxalic acid is involved in the pathogenicity of *Sclerotinia* (1, 2 and 3). Our objectives were to recover transgenic peanut plants expressing a barley oxalate oxidase gene and to evaluate those plants for resistance to *Sclerotinia minor*. We have regenerated 247 independent transformed lines (27 NC-7, 169 Wilson and 51 Perry). Integration of the transgene was confirmed by Southern analysis and expression by Northern analysis. In addition to its role as a putative disease resistance gene, oxalate oxidase can also be used as a reporter of transgene activity. We have optimized a sensitive, simple and inexpensive enzyme assay that allowed us to measure oxalate oxidase activity in transgenic lines. From the 157 lines assayed so far we have observed a wide range in transgene activity. Two bioassays were also developed. Oxalic acid applied to detached leaflets was used to determine whether the transgene could limit lesion size. Lesion development was significantly reduced in transgenic lines compared to wild type (1 to 10% of wild type). A second leaflet assay examined reduction of lesion size when *Sclerotinia minor* mycelia were applied as an agar plug to the adaxial surface of leaflets in transgenic lines (average reduction in lesion size of 29%, 41% and 17% were observed in cultivars Wilson, NC7 and Perry respectively). The presence of the oxalate oxidase gene successfully limits lesion size in the detached leaf assays and transgenic lines will be tested for resistance to Sclerotinia blight in the field.

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GENETIC APPROACHES TO UNDERSTANDING PRE-mRNA SPLICE SITE SELECTION IN PLANTS

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Exonic splicing enhancers are signals that reside within exons and contribute to the splicing of mRNA precursors by activating splicing at nearby sites. In animals, ESEs are recognized by SR proteins, a family of splicing factors involved in alternative splice site selection.
We have developed an *in vivo* system for the identification and characterization of exonic splicing enhancers (ESEs) in *Arabidopsis thaliana* using vectors designed to display ESE-dependent GUS expression. These vectors contain a strong constitutive promoter and an intron-exon-intron unit from the *SPINDLY* gene. Test sequences are cloned into the center of the exon. Thus far, we have generated a vector containing an exon whose splicing pattern reliably and reproducibly reflects the presence of an ESE (exons lacking the ESE are nearly always skipped while exons with the ESE are nearly always included). The functional ESEs identified so far are GAAGAAGAA, CGATCAACG and TGCTGCTGG. Transition to the high-throughput ESE assays should begin soon.
We are also examining the hypothesis that SR proteins regulate alternative splicing *in vivo* by exploring the phenotypes of loss-of-function mutations in SR protein genes. The complete genome sequence of *Arabidopsis thaliana* indicates 20 SR protein genes. We have obtained T-DNA insertion lines in 16 of these from the Salk Institute Genome Analysis Laboratory. In 8 cases we have been able to identify viable homozygous plants using a genotyping assay. Phenotypes affecting flowering and the growth of roots and shoots have been observed in several of these lines. We are currently testing whether the insertion mutations eliminate RNA from the affected genes, verifying that the observed phenotypes are in fact caused by the T DNA insertions and characterizing these phenotypes in detail. To study the possible effects of these mutations on alternative splicing we have developed a multiplex reverse transcription and PCR assay for genes with alternative splicing that involves exon retention *vs.* exon skipping.
Combining our ESE assay with the SR protein mutations should alsó allow us to determine whether the activity of individual ESEs identified using our assay are dependent upon particular SR proteins and, if so, which ones.

A NOVEL TATA BOX ELEMENT CONTROLS EXPRESSION OF THE PEACH DEHYDRIN GENE, *PPDHN2*, IN RIPE FRUIT AND POTENTIALLY ALTERS THE INTRACELLULAR DISTRIBUTION OF THE COGNATE POLYPEPTIDE

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We have previously identified two dehydrin genes from peach (*Prunus persica*), *Ppdhn1* and *Ppdhn2*. Analysis of the seasonal expression of *Ppdhn1* in peach bark tissues suggests that it is primarily induced in response to cold temperatures and water deficit. Although a strong response to water deficit is observed for *Ppdhn2* in peach bark tissues, induction by cold temperatures is modest by comparison with *Ppdhn1*, suggesting that its primary role might not be associated with temperature stress. To begin to understand how *Ppdhn2* is regulated in response to abiotic stress, we determined and compared the transcription start site (TSS) for *Ppdhn2* in July and January bark and in fully ripe peach fruit. Using RNA ligase-mediated 5′ RACE (5′ RLM-RACE), we identified a single predominant TSS for *Ppdhn2* that was the same in both July and January bark tissue samples, suggesting that seasonal expression is controlled by the same set of transcription factors. RT-PCR data suggested that transcription in developing fruit sampled 7 or 75 days after bloom was initiated at the same site as that of bark, since the predicted PCR products were equivalent in size in these tissues. In contrast, no RT-PCR product was observed with these same primers using RNA from fully ripened fruit (130 days after bloom). 5′ RLM-RACE analysis of RNA from ripe fruit indicated a TSS that was different from the other tissues examined. This site is located approximately 200 bases upstream of the major bark TSS and is apparently driven by a novel TATA box. Furthermore, a 142 b sequence flanked by GT and AG dinucleotides located downstream of the novel TSS is absent from the 5′ RLM-RACE products, indicating that this region of the sequence behaves as an intron. Conceptual translation of the putative mature transcript results in the addition of 34 amino acids to the N-terminus of PpDHN2. Several polypeptide sorting programs predict that the putative protein would be transported to mitochondria. This is the first report describing tissue-specific utilization of different TATA boxes within the same promoter and the first reported suggestion that a dehydrin might be located to mitochondria.

METABOLIC PROFILING OF THE SHORT-TERM *ARABIDOPSIS THALIANA* RESPONSE TO INCREASED CO_2 LEVELS USING GAS CHROMATOGRAPHY- MASS SPECTROMETRY

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The reaction of plant physiology to elevated CO_2 concentrations in the plant growth environment has been the focus of numerous studies, in an effort to understand the effect of potential increase of CO_2 level in the environment on the growth and the product yield of plants. However, the majority of the previous studies monitored the long-term response of the plants after long exposure to high CO_2 levels. In this study, we measured the short-term physiological response of *A. thaliana* (Columbia strain) liquid cultures to increased CO_2 concentration in their growth environment at the metabolic level. Specifically, the experiment involved the growth of two sets of plants for 12 days in Gamborg media under constant light and 23°C. On the 13th day, the two sets of plant liquid cultures were fed air of ambient composition ("control set") and of 1% CO_2 ("perturbed set"), respectively, which were harvested at different time points during the day.

Gas Chromatography – Mass Spectrometry was used to measure the average (over the entire plant) metabolic plant profiles, in an effort to identify the characteristic metabolic fingerprint underlying the reaction of the *A. thaliana* physiology to the imposed CO_2 stress. Metabolic profiling refers to the qualitative and quantitative detection of a variety of low molecular weight intracellular metabolites, including organic acids, amino acids, sugars, sugar alcohols, amines and saccharides, obtained after the break-up of the cellular macromolecules. In this study, we used the methanol extraction protocol described by Roessner et. al. [1], using ribitol as the internal standard. Taking into consideration that enzymatic activity affects and is affected by the concentration of intracellular metabolites, the metabolic profile can provide an extensive picture of the metabolic state of a complex organism. We will present the obtained metabolic profiles in the context of the known *A. thaliana* metabolic network structure and regulation to derive conclusions about the metabolic response of the plant to the external stress. We plan to combine the final conclusions from this analysis with the results from the transcriptional profiling analysis of the same plant liquid cultures (see Dutta et. al.) to identify similarities and differences between the genomic and metabolic short-term reaction of the plant to elevated levels of CO_2 in their growth environment.

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TRANSCRIPTIONAL PROFILING OF THE SHORT-TERM *ARABIDOPSIS THALIANA* RESPONSE TO INCREASED CO_2 LEVELS USING FULL GENOME DNA MICROARRAYS

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Abstract: The reaction of plant physiology to elevated CO_2 concentrations in the plant growth environment has been the focus of numerous studies, in an effort to understand the effect of potential increase of CO_2 level in the environment on the growth and the product yield of plants. However, the majority of the previous studies monitored the long-term response of the plants after long exposure to high CO_2 levels. In this study, we measured the short-term physiological response of *A. thaliana* (Columbia strain) liquid cultures to increased CO_2 concentration in their growth environment at the transcriptional level. Specifically, the experiment involved the growth of two sets of plants for 12 days in Gamborg media under constant light and 23°C. On the 13th day, the two sets of plant liquid cultures were fed air of ambient composition ("control set") and of 1% CO_2 ("perturbed set"), respectively and harvested at different time points during the day.

Full genome DNA microarrays developed by the group of Dr. Quackenbush at TIGR were used to measure the average (over the entire plant) transcriptional plant profiles, in an effort to identify the characteristic gene expression fingerprint underlying the reaction of the *A. thaliana* physiology to the imposed CO_2 stress. The gene expression profiles were analyzed using the TIGR TM4 open-source DNA microarray analysis software. We have identified a few hundred genes that behave differently in the perturbed versus the control plant set. Using the currently available gene annotation, we will present the clustering results in the context of the known *A. thaliana* metabolic network structure and regulation to derive conclusions about the genomic response of the plant to the external stress. We plan to combine the final conclusions from this analysis with the results from the metabolic profiling analysis of the same plant liquid cultures (see Kanani et. al.) to identify similarities and differences between the genomic and metabolic reaction of the plant to elevated levels of CO_2 in their growth environment.

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ANALYSIS OF EXPRESSED SEQUENCE TAGS FROM ROOTS OF RESISTANT SOYBEAN INFECTED BY THE SOYBEAN CYST NEMATODE.

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the most devastating pest of soybean in the United States. The resistance response elicited by SCN in soybean is complex, and genes involved in the response to a large extent are unknown and not well characterized. We constructed cDNA libraries made from mRNA extracted from roots of the resistant soybean cv. Peking at 12 hr, two-to-four days, and six-to-eight days post inoculation with the soybean cyst nematode, population NL1-RHp, similar to race 3. Expressed sequence tag analysis of the libraries provides rapid discovery of genes involved in the response of soybean to the nematode. A total of 3454 cDNA clones were examined from the three libraries, of which 25 cDNAs were derived from nematode RNA. The levels of certain stress-induced genes such as SAM22 and glutathione S transferase (GST8) were elevated in the SCN-infected roots relative to uninoculated roots. Early defense response genes, in particular ascorbate peroxidase and lipoxygenase were abundant in the 12 hr library. By six-to-eight days, the expression of most of those genes was not as abundant, while genes coding for unknown proteins and stress-induced proteins continued to be highly expressed. These ESTs and associated information will be useful to scientists examining gene and protein interactions between nematodes and plants.

INTERACTIONS BETWEEN DEFENSE SIGNALING PATHWAYS IN SOYBEAN PLANTS

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Soybean cyst nematode (SCN) is one of the major pathogens of soybean in the US causing millions of dollars worth of damage each year. The defense response of soybean to SCN is a multigenic trait and varies depending upon the genotypes of soybean and SCN. We looked at the gene expression profile of approximately 6000 genes in nematode (SCN), salicylic acid (SA), jasmonic acid (MeJA) and ethylene (E) treated SCN-resistant soybean plants. Genes that were commonly regulated by two or more treatments were identified. Crosstalk between nematode induced, SA, MeJA and E-activated pathways was observed. The expression of a few genes of these different defense pathways was also determined by qRT-PCR and Northern hybridization to validate the expression results observed from microarray analysis. To test if turning on these pathways was providing any protection against the invading nematode, we induced the SA and JA- pathways by treating the plants with these chemical compounds in an SCN-susceptible soybean cultivar before challenging it with the nematodes. Our results suggest that stimulation of these pathways prior to infection by nematodes causes a reduction in the infection. Overall there appears to be interactions and coordination between the different defense signaling pathways to ward off the invading pathogen

UNDERSTANDING THE FUNCTIONAL SIGNIFICANCE OF CONSERVED SEQUENCE MOTIFS IN THE *ARABIDOPSIS THALIANA* HEXOSE TRANSPORTER, STP1.

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The hexose transporters of *Arabidopsis thaliana* belong to a superfamily of proteins called major facilitator proteins, which have been identified in humans, plants and yeast. These proteins share a common predicted secondary structure which includes 12 transmembrane-spanning alpha-helices(TMD), internal N- and C- termini, a long extracellular loop between TMD 1 and 2, and a large intracellular loop between TMD 6 and 7. Interestingly, in each species studied so far, multiple genes which encode hexose transporters have been identified. In addition, hexose transporters have been found to transport varying substrates with varying kinetics. Therefore, the group of transporters is thought to enable the metabolism of sugars according to organism-specific internal and external environmental conditions. Our hypothesis is that amino acid sequence motifs can be identified which confer the functional diversity seen in this class of proteins. In this preliminary study, we have identified several possible amino acid sequence motifs which may play a role in the function of the *Arabidopsis thaliana* hexose transporter, STP1. We also present prelimary results from a study using site-directed mutagenesis to elucidate the role that these amino acid sequence motifs play in determining the function of hexose transporters.

REGULATION OF GENE EXPRESSION BY HD2-TYPE HISTONE DEACETYLASES

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The four HD2 proteins of *Arabidopsis thaliana* (AtHD2A-D) belong to a unique class of histone deacetylases that is plant specific. Previously, we have demonstrated that one of the members, AtHD2A, can mediate transcriptional repression when targeted to the promoter of a reporter gene (Wu et al., 2000a). Here we report that AtHD2B and AtHD2C can also repress gene expression. AtHD2A and AtHD2C differ from AtHD2B and AtHD2D in the composition of their structural domains. Our data shows that both structural types play a role in the repression of gene transcription. We demonstrate that AtHD2A can mediate gene repression through interactions with transcription factors in plants. By fusing AtHD2A with the DNA-binding domain of the plant transcriptional factor Pti4, the expression of a *GCC* box-containing reporter gene was repressed. We also demonstrated repression of a *GUS* gene with *GAL4* enhancers using transgenic plants that expressed a *GAL4/AtHD2A* fusion gene. Furthermore, the expression of the GAL4/AtHD2A protein using the seed-specific napin promoter (*NAP2*) and the constitutive *tCUP* promoter demonstrated that repression of transgenes could be achieved in a tissue-specific or unrestricted manner. Targeting of HD2 proteins to specific promoters using transcription factor DNA binding domains may therefore provide a new technology for silencing target genes and pathways in plants as well as for assessing the function of unknown transcription factors.

IDENTIFICATION AND CHARACTERIZATION OF INOSITOL 1,3,4-TRISPHOSPHATE 5/6-KINASE AND INOSITOL 1,4,5-TRISPHOSPHATE 3/6-KINASE IN *GLYCINE MAX*

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Phytate, also known as *myo*-inositol hexakisphosphate or Ins(1,2,3,4,5,6)P_6, is the major storage form of phosphorous in plant seeds. Phytate is indigestible by non-ruminant animals such as pigs and poultry, and it chelates cations such as calcium, iron, zinc, and potassium, classifying it as an anti-nutrient. The excretion of unutilized phytate in manure translates to an excess amount of phosphorous runoff which often leads to eutrophication of lakes and ponds. In order to develop low phytate soybeans we would like to elucidate the phytate biosynthetic pathway. Studies conducted in various organisms have demonstrated two potential routes for the phosphorylation of *myo*-inositol. In yeast, a phosphatidylinositol-dependent pathway includes an Ins(1,4,5)P_3 3/6-kinase while in *Zea mays* a phosphatidylinositol-independent pathway utilizes an Ins(1,3,4)P_3 5/6-kinase. In Arabidopsis, genes encoding both kinases have been identified [1,2], and in *Zea mays* a mutation in an Ins(1,3,4)P_3 5/6-kinase has recently been implicated in the *low phytic acid* phenotype [3].
The goal of our current research is to elucidate the pathways for phytic acid biosynthesis in soybean (*Glycine max*). We have identified 38 expressed sequence tag (EST) clones from the soybean public database that have homology with three previously characterized Arabidopsis Ins(1,3,4)P_3 5/6 and Ins(1,4,5)P_3 3/6-kinase genes. Using a combination of sequence comparison tools and molecular techniques we have identified the coding regions for five potential Ins(1,3,4)P_3 5/6-kinase genes and one potential Ins(1,4,5)P_3 3/6-kinase gene. We are inserting the clones into plasmid vectors for expression of tagged proteins in *E. coli*. Once functional proteins have been expressed and purified we will determine their biochemical characteristics and substrate specificity.

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ECTOPIC EXPRESSION OF PHYTASE IN SOYBEAN

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Phytate is a storage compound for phosphorus and carbon (inositol) and makes up 60-80% of the total phosphate in seeds. Soybean meal comprises the majority of the world's protein meal consumption. In non-ruminant animals phytate is indigestible and increases the amount of phosphorus excreted to the environment and the potential for phosphorus pollution. In addition to its storage properties in seeds, phytate is a strong chelator of minerals such as iron and zinc and renders them unavailable in diets of non-ruminant animals. Lowering the phytate content of soybeans will decrease phosphorus pollution and increase the nutrient properties of feed. There have been many attempts to lower the phytate content of seeds through the use of mutagenesis and selection.
Our approach alters the expression of phytase in order to break down phytate as it is produced during seed development. In plants, phosphate and carbon are released from phytate during germination by the enzyme phytase. A construct consisting of a phytase cDNA [1] regulated by a seed specific promoter (from a' ß-conglycinin) [2] was introduced into somatic embryos via particle bombardment. Using this approach we have recovered soybean plants from seven independent transformed lines. Seeds from the T_0 generation were grown and T_1 plants were analyzed for phytase transgene integration by Southern analysis. Of the seven transformants, one was found to have a single integration site. Plants from this line were chosen for further characterization by northern analysis. Northern analysis showed an increase in transgene phytase mRNA expression that occurred in parallel with ß-conglycinin mRNA expression. Developing seeds of T_2 homozygous plants will be examined for phytase activity and phytate content.

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