John Hammond

Mid-Atlantic Plant Molecular Biology Society

Tenth Annual Meeting 1993

Newark, Delaware

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TABLE OF CONTENTS

INTRODUCTION

Welcome to the tenth annual meeting of the **Mid-Atlantic Plant Molecular Biology Society**. The goal of the Society is to assemble scientists from the Mid-Atlantic region of the United States to exchange information and research ideas in plant molecular biology. The Society's annual meeting is designed to be reasonably priced and accessible so as to entice the greatest numbers of students, post-docs, and other scientists to attend and participate in presentations and discussions. In addition, the meeting is designed to be as informal as possible to allow each participant the opportunity to meet the other attendees.

This is our first year out of the immediate Baltimore-Washington area. As a result, we have lost some of those who have typically been able to drive each day from their homes. However, we are expanding our audience and providing a useful forum to new students, post-docs, and researchers.

In addition, we have advertised this year's meeting electronically using the Internet. This is the first time we have used this network to distribute information about our meeting. As a result, we have been able to contact a greater number of researchers, which has opened new opportunities for our Society.

Many people have been involved in the organization and planning of this meeting. We have tried to provide a program that encompasses all areas of plant molecular biology and hope that participants will be stimulated by hearing about subjects that are outside their own immediate interests. Please contact members of the organizing committees if you have thoughts or comments for consideration in the planning of future meetings.

We are pleased to note that Elsevier, publishers of **Plant Science**, have expressed an interest in having the proceedings of this meeting collected and published (as refereed papers) in the journal. We will discuss this opportunity at the business meeting on Friday. We believe that this development is further proof that our Society and especially the program committee, is fulfilling a recognizable need in the area of plant molecular biology.

We wish to thank our sponsors and exhibitors, without whom we could not afford to organize a quality meeting with such an exciting range of invited speakers at such minimal cost to the registrants. Please take the opportunities afforded in the program schedule to visit our exhibitors' displays—the level of interest you show in their products is a critical factor in their willingness to support future meetings.

Last but not least, we thank you for your participation and hope that you will continue to support the efforts of this Society.

Dennis Schaff
John Hammond
Co-Organizers

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GENERAL INFORMATION

Sessions

Registration, plenary sessions, poster sessions, exhibits, and coffee breaks will be held at the University of Delaware, Clayton Hall. Plenary sessions and the keynote address will be held in Room 101B. Poster sessions, exhibits, coffee breaks, and meals will be held in Room 101A. Park in any unreserved spot in the lot adjacent to Clayton Hall.

Lunches, Social Hour/Reception, and Banquet

If you pre-registered, your lunches and banquet are included in the registration price. If you did not pre-register, you could not be included in our count for the catering service, and so meals are not included. However, a restaurant/cafeteria is attached to Clayton Hall.

The lunches, social hour, and banquet will be held in Clayton Hall Room 101A. Vegetarian selections will be available at all meals. The social hour before the banquet will feature snacks and a cash bar. At the banquet, be sure to display the colored card which you should have received with your name tag during the meeting registration indicating your entree selection.

Post-meeting Trip to Longwood Gardens

Longwood Gardens is open from 9:00 a.m.-6:00 p.m. with fireworks and fountains show at 9:15 p.m. Admission for adults is $10 to Longwood Gardens. The fireworks and fountains show is a separate ticket at $15. We will be car pooling. Information will be at the registration desk.

1993 MAPMBS MEETING SCHEDULE

THURSDAY, JULY 15

8:00 a.m. Registration and Poster set-up

8:50 a.m. Opening remarks - Dennis Schaff
Welcome - Costel D. Denson, Vice Provost for Research

GENE REGULATION I
(Moderator: Deborah Loer, USDA-ARS)

9:05 a.m. Rebecca S. Boston, Julie E. Krawetz, and Hank W. Bass (North Carolina State University). Regulation and function of maize ribosome-inactivating proteins.

9:35 a.m. G.D. Babcock and A. Esen (Virginia Polytechnic Institute and State University). Substrate specificity of maize β-glucosidase.

9:55 a.m. Mubarak M. Muthalif and Lisa J. Rowland (University of Maryland, College Park, and USDA/ARS/FL). Are cyclophilins involved in plant dormancy?

10:15 a.m. **COFFEE BREAK, POSTERS (Set-up and Viewing), and EXHIBITORS**

GENE REGULATION I (continued)

11:10 a.m. Carroll P. Vance (USDA/ARS-University of Minnesota). Primary assimilation of nitrogen in alfalfa nodules: Molecular features of the enzymes involved.

11:40 a.m. R. Dickstein, T. Peng, W. Ngo, M.E. Smith, and R. Prusty (Drexel University). ENOD8, a novel early nodule-specific gene, is expressed in empty alfalfa nodules.

12:00 p.m. Ritu Shrivastava (University of Delaware). Nodulation and nitrogen fixation in indigenous Indian Bradyrhizobia symbiotic to *Glycine max* JS-72-44.

12:20 p.m. **LUNCH, EXHIBITORS**

DEVELOPING TECHNOLOGIES

(Moderator: Dennis Schaff, University of Delaware)

1:20 p.m. Paul Gilna, Michael J. Cinkosky, and Gilford M. Keen (Los Alamos). On-line access to GenBank submissions database: the latest advances in community-based access to sequence data submission and maintenance technologies for GenBank.

TRANSFORMATION & TECHNIQUES

(Moderator: Frank Turano, USDA-ARS)

1:50 p.m. Ted M. Klein, A. Luckring, M. Marini, S. McAdams, D. O'Brien, K. Reiter, T. Warner, P. Smith, and T. Jones (DuPont Co.). Maize transformation: An industrial perspective.

2:20 p.m. Dennis A. Schaff and Sunita K. Agarwal (University of Delaware). The APRT selection system.

2:40 p.m. Jhy-Jhu Lin (Life Technologies Inc., GIBCO/BRL). Effects of expression medium on the transformation efficiency of *Agrobacterium tumefaciens* cells using electroporation.

3:00 p.m. **COFFEE BREAK, POSTER VIEWING, and EXHIBITORS**

3:50 p.m. Antoni Rafalski (DuPont Co.). Technology for molecular breeding: RAPD markers, microsatellites, and machines.

4:20 p.m. John C. Wallace, Celia D. Knight, and Wagdy Sewahel (Bucknell University and University of Leeds). Extrachromosomal maintenance of transforming DNA in transformants of the moss, *Physcomitrella patens*.

4:40 p.m. Timothy D. Metz, Anthony M. Shelton, Richard T. Roush, and Elizabeth D. Earle (Cornell University and NY State Ag. Expt. Station). Transgenic broccoli expressing a *Bacillus thuringensis* insecticidal crystal protein: A model system to test resistance management theories.

5:00 p.m. **BREAK**

KEYNOTE ADDRESS
(Introduction: Ben Matthews, USDA-ARS)

5:10 p.m. Sheila McCormick (USDA/ARS - Plant Gene Expression Center). Molecular analysis of gametogenesis in plants.

6:10 p.m. **SOCIAL HOUR**

7:00 p.m. **DINNER**

FRIDAY, JULY 16

PLANT-MICROBE INTERACTIONS
(Moderator: John Hammond, USDA-ARS)

8:00 a.m. Dan Roberts (USDA/ARS/BPDL). Molecular basis of rhizosphere colonization by the plant beneficial bacterium, *Enterobacter cloacae*.

8:30 a.m. James Carrington (Texas A&M University). Replication and movement of a potyvirus that expresses GUS.

9:00 a.m. Peter McGarvey, Marie Tousignant, and J.M. Kaper (USDA/ARS/MPPL). CMV-Ixora: a cucumber mosaic virus defective in satellite RNA replication.

9:20 a.m. Arcady Mushegian, D. Ducasse, R. Richins, J. Wolff, and R.J. Shepherd (University of Kentucky). Control of movement and pathogenesis in caulimoviruses: the case of peanut chlorotic streak.

9:40 a.m. Barbara Valent, Jim Sweigard, Marc Orbach, Seogchan Kang, Leonard Farrall, Anne Walter, and Forrest Chumley (Du Pont Co.). Two cloned genes for host specificity in the rice blast fungus, *Magnaporthe grisea*.

10:10 a.m. **COFFEE BREAK, POSTER VIEWING, and EXHIBITORS**

GENE REGULATION II
(Moderator: Cleo Hughes, USDA-ARS)

11:05 a.m. John C. Watson, Xia Lin, Zhenbiao Yang, and Rajnish Khanna (University of Maryland). Photoregulated expression of protein kinase genes.

11:35 a.m. Sandra H. Russell, Daniel F. Delduco, and Pablo A. Scolnik (DuPont Co.). Elucidating the mode of action of an herbicide—a genetic approach.

11:55 a.m. **BUSINESS MEETING**

12:10 p.m. **LUNCH**

GENE REGULATION II (continued)

1:30 p.m. Michael S. Dobres, Sevnur Mandaci, Dipanwita Maiti, and Albert J. List (Drexel University). An RNA marker for epidermal differentiation in *Pisum sativum*.

2:00 p.m. Mauricio M. Bustos and Maw-Shenq Chern (University of Maryland, Baltimore County). Evidence for differential activation of gene expression by the maturation-specific enhancer UAS1 in embryo and endosperm tissues of tobacco.

2:20 p.m. Gregg Silk and Benjamin F. Matthews (USDA/ARS/PMBL). Cloning and expression of the soybean *dapA* gene encoding dihydrodipicolinate synthase (DS).

2:40 p.m. **CLOSING REMARKS** Thanks for staying on time!

POST-MEETING TOUR OF LONGWOOD GARDENS

1993 MAPMBS POSTER SESSIONS
Thursday 10:15 a.m.-11:10 a.m. & 3:00 p.m.-3:50 p.m.
Friday 10:10 a.m.-11:05 a.m.

Poster (Page)

1 (39) Susan M. Koehler, Gail Matters, and Mark Tucker (USDA/ARS/PMBL and Brown University). Promoter analysis of an ethylene-inducible bean abscission cellulase gene.

2 (40) Kenneth A. Chapman, Ashton Delauney, Jong H. Kim, and Desh Pal S. Verma (Ohio State University). De novo purine biosynthesis in ureide-producing legumes: 5-aminoimidazole ribonucleotide carboxylase and 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide synthetase cDNAs from *Vigna aconitifolia*.

3 (41) Cleo A. Hughes and Benjamin F. Matthews (USDA/ARS/PMBL). Cloning and expression of a soybean cDNA clone encoding asparagine synthetase.

4 (42) Roxanne H. Fisher, Kathy Barton, Jerry D. Cohen, R. Scott Poethig, and Todd J. Cooke (University of Maryland, College Park, University of Pennsylvania, and USDA/ARS/HCQL). Microsurgical and hormonal analyses of an *Arabidopsis* mutant altered in organ elongation.

5 (43) Choirul Muslim and Asim Esen (Virginia Polytechnic Institute & State University). Histochemical localization of rice (*Oryza sativa*) β-glucosidase.

6 (44) Joan S. Gebhardt, Jane M. Weisemann, and Benjamin F. Matthews (USDA/ARS/PMBL). Molecular analysis of the aspartate kinase-homoserine dehydrogenase gene family in soybean.

7 (45) Tomoko Fukasawa-Akada, Shyam K. Dube, Shain-Dow Kung, and John C. Watson (University of Maryland, College Park). Organization and expression of phenylalanine ammonia lyase genes in tobacco (*Nicotiana tabacum*).

8 (46) Sevnur Mandaci, Karen E. Thum, and Michael S. Dobres (Drexel University). Transgenic analysis of gene expression in the plant shoot apex.

9 (47) A. Bruce Cahoon and Fang-Sheng Wu (Virginia Commonwealth University). Plasmolysis facilitates the passage of protein and DNA through the cell wall of intact plant cells.

(48) Xianggan Li and John H. Gallagher (University of Delaware). Foreign gene expression by salt-adapted cells in response to bombardment with the particle inflow gun.

(49) Frank J. Turano and Sona S. Thakkar (USDA/ARS/CSL). Soybean transformation by direct uptake.

(50) Paula Janssen and Sharon J. Keeler (DuPont Co.). Use of SUI, a cytochrome P450 gene from *Streptomyces griseolus,* in a negative selection protocol for plant cells during protoplast transformation.

(51) John Hammond and Kathryn K. Kamo (USDA/ARS/FNCL). Protection conferred by native and chimeric potyvirus coat proteins expressed in transgenic plants.

(52) Rebecca S. Boston, Fan Zhang, and Jeffrey W. Gillikin (North Carolina State Universiy) Interaction of BIP with immature protein bodies in developing endosperm.

(53) K. Xiong and J.J. Fuhrmann (University of Delaware). Distribution of rhizobitoxine in soybean cultivars nodulated by wild-type and isogenic *Bradyrhizobia* differing in toxin production.

REGULATION AND FUNCTION OF MAIZE RIBOSOME-INACTIVATING PROTEINS: Rebecca S. Boston, Julie E. Krawetz and Hank W. Bass, Dept. of Botany, North Carolina State University, Raleigh, NC 27695

Two maize ribosome-inactivating proteins (RIPs) are synthesized as inactive proenzymes (proRIPs) or zymogens. One of these proteins, RIP1, accumulates during seed development under control of the transcriptional activator, Opaque-2. During germination, proRIP1 undergoes a proteolytic processing event that removes a central acidic region and leaves two tightly linked polypeptide chains. The resulting protein produces a diagnostic depurination within a universally conserved loop of mammalian 28S rRNA and exhibits potent toxicity in translational inhibition assays ($ID_{50}=10^{-11}M$). We have shown that maize proRIP1 accumulates coordinately with the major storage proteins of the kernel. By immunodetection and enzymatic activity assays, we have monitored the proteolytic activation that occurs *in vivo* during germination. The timing of activation is coincident with the accumulation of seed proteases responsible for the breakdown of storage proteins suggesting it may function as a general constitutive defense protein during germination. We have also characterized a gene encoding a second RIP (RIP2). This gene is expressed at low levels in a number of different tissues. Like RIP1, RIP2 requires proteolytic activation for enzymatic activity. To better understand the biological role of maize RIPs, we have used mutational analysis to construct a series of clones encoding various domains of proRIP1. These clones have been introduced into *E. coli* expression vectors to allow us to correlate proRIP processing with activity in cell-free *in vitro* translation systems.

b32 water soluble - same pattern of expression as zein (alcohol soluble)
-DNA binding protein, transcription coordinated w/ opaque 2 - missing in opaque 2 lines.

Depurinates A in loop of rRNA - inactivating ribosome
Only known RIP requiring protease activation - others formed as active conformation.

32k form (inactive) detectable to ~ 3 days after germination then ~ 8 + 16k bands detected. Coincides w/ protease activity
Activation by pathogen proteases

~ 1000 fold more RIP necessary to inhibit corn ribosomes than rabbit ribosomes - bacterial ribosomes also resistant to many RIP.

SUBSTRATE SPECIFICITY OF MAIZE β-GLUCOSIDASE

G. D. Babcock and A. Esen; Department of Biology
VA Polytechnic Inst. and State U.
Blacksburg, VA 24061-0406

Maize (Zea mays L.) β-glucosidase (β-glu) is a dimeric protein of identical 60 KD monomers. It is up to 8% of the total protein in young maize shoots, and is located in plastids. Several glycosides were tested as substrates of β-glu. When possible, K_m's and V_{max}'s were determined either by measuring the absorbance of p-nitrophenoxide or by a glucose-oxidase/ peroxidase based assay for glucose production. Various compounds were also tested as inhibitors. When inhibition was observed, K_i's and/or K_i''s were estimated. 4-methyl-umbelliferone-β-D-glucoside was the best artificial substrate (K_m=.143 mM) for which kinetic data was obtained. O-nitrophenyl-β-D-glucoside (K_m=1.64mM), p-nitrophenyl-β-D-glucoside (K_m=.58mM), p-nitrophenyl-β-D-galactoside (K_m=6.32mM) and p-nitrophenyl-β-D-xyloside (K_m=.39 mM), resorufin-β-D-glucoside, and indoxyl-β-D-glucoside were also found to be substrates. No enzymatic hydrolysis of p-nitrophenyl-β-D-glucuronide, phenyl-β-D-glucoside, salicin, arbutin, linamarin, prunasin, methyl-β-D-glucoside, or gentiobiose was observed. Glucose (K_i=170mM), 4-methylumbelliferone (K_i=.973mM), phenyl-β-D-glucoside (K_i=5.68mM), and p-nitrophenol (K_i=1.46mM) showed primarily competitive inhibition. Tryptamine (K_i=3.4 mM, K_i'=15.7mM), salicylate (K_i=60.9 mM, K_i'=119 mM), arbutin (K_i=4.39mM, K_i'=18.8 mM) and salicin (K_i=9.56 mM, K_i'=38.0 mM) showed mixed inhibition. No inhibition by tryptophan or benzoate was observed. Methanolic maize extracts were tested for the presence of β-glu substrates. One major β-glu substrate, tentatively identified as the hydroxamic acid DIMBOA-glc (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one-β-D-glucopyranoside), was found in methanolic maize extracts. The distribution of hydroxamic acid (hx), which occurs as glucoside in intact plants, along coleoptile length was determined. Hxs in K55 and H95 maize shoots were distributed in a manner similar to β-glu, with the highest levels found near the node. In bxbx maize (a mutant deficient in hx), the highest hx levels were found in the mesocotyl region. DIMBOA-glc is a major natural substrate of maize β-glucosidase. Therefore, a major function of the enzyme is to mediate insect and pathogen resistance via the release of the toxin DIMBOA.

DIMBOA correlated to aphid resistance - and inactivates Agrobacterium tumefaciens - problem in transforming maize w/ Agro. - need lines w/ DIMBOA inactivated or to inactivate during transformation

ARE CYCLOPHILINS INVOLVED IN PLANT DORMANCY ?

Mubarack M.Muthalif and Lisa J. Rowland, Dept. of Horticulture, University of Maryland, College Park, MD, 20742 (MMM) and Fruit Laboratory, Beltsville Agricultural Research center, Agricultural Research Service, Beltsville, MD 20705 (MMM and LJR)

Woody perennial plants of temperate zone survive freezing temperatures by entering a state of dormancy. The plants resume growth and buds break in spring, only after sufficient exposures to low temperature ie., satisfaction of chilling requirement. Attempts were made to identify changes in gene expression in dormant floral buds of blueberry in response to chilling unit accumulation. The concentration of 3 polypeptides of 72, 65 and 17 kilodaltons increased dramatically by 10 times in response to chilling. Their concentration decreased dramatically once the buds began to break. These Chilling Responsive Proteins (CRPs) were found to be heat stable. Partial purification was accomplished using Rotofor (Bio-Rad) isoelectric focusing. The polypeptides were found to be basic with isoelectric points of 7-9. Polypeptides were electroblotted onto nitrocellulose, subjected to trypsin digestion, and the resulting peptides were separated by reverse phase HPLC. Several peptides were chosen for amino acid sequencing (Harvard Microchemistry Dept). The sequence of 2 peptides (40 amino acids long) of the 17 kilodalton polypeptide was highly conserved with sequences from a class of proteins called "Cyclophilins". Cyclophilins are important in many fundamental cellular processes, including immune responses and protein folding. The alignment of sequences from Tomato, *Brassica, Arabidopsis,* onion, human and yeast cyclophilins revealed that they share over 80% amino acid sequence similarity with the blueberry sequence. These data identify the 17 kilodalton CRP from blueberry floral buds as a cyclophilin.

Cyclophilins act as chaperonins, among other things

PRIMARY ASSIMILATION OF NITROGEN IN ALFALFA NODULES: MOLECULAR FEATURES OF THE ENZYMES INVOLVED. Carroll P. Vance, USDA-ARS, Plant Science Research, Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108

The primary assimilation of nitrogen (N) in alfalfa root nodules involves complex intermingling with carbon (C) metabolism. Integrated functioning of both cytosolic and organelle targeted enzymes is required to link N assimilation with C metabolism. To understand how N and C metabolism are controlled in root nodules requires fundamental knowledge of how the plant genes involved are regulated. While significant progress has been made in understanding the regulation of glutamine synthetase (GS), much less is known about the genes controlling other enzymatic steps in this process. To that end we have isolated, purified and characterized the root nodules enzymes aspartate aminotransferase (AAT), phosphoenolpyruvate carboxylase (PEPC) and glutamate synthase (NADH-GOGAT). Moreover the cDNAs encoding these crucial enzymes were isolated and characterized. While the most prominent forms of GS associated with N assimilation in nodules is located in the cytosol, AAT and NADH-GOGAT appears to be organelle targeted. The deduced amino acid sequence and immunogold labeling show that nodule enhanced AAT is localized to amyloplasts. Comparison of the deduced amino acid sequence of nodule enhanced NADH-GOGAT to the N-terminal sequence of the processed protein indicated that NADH-GOGAT has a 101 amino acid presequence. However, it is unclear as to which organelle NADH-GOGAT is targeted. Cytosolic phosphoenolpyruvate carboxylase (PEPC), which can be expressed in legume root nodules to levels comparable to those detected in C4 leaves, provides a substantial amount of carbon for malate, aspartate and asparagine biosynthesis. Protein immunoblots, enzyme assays and RNA blots showed that AAT, PEPC, and NADH-GOGAT proteins, activities, and mRNAs were enhanced some 15-fold during the development of effective alfalfa nodules. By comparison, the expression of AAT and PEPC mRNAs was reduced by 65% in ineffective nodules and little to no AAT and PEPC activity and protein was detected. NADH-GOGAT was different from AAT and PEPC in that expression had an absolute requirement for a factor(s) related to effective nodules. The data suggests that NADH-GOGAT plays a key role in regulating N assimilation. Moreover, plastids in nodules play a major role not only in C metabolism but also in N metabolism.

ENOD8, A NOVEL EARLY NODULE-SPECIFIC GENE, IS EXPRESSED IN EMPTY ALFALFA NODULES. R. DICKSTEIN, T. PENG, W. NGO, M. E. SMITH, and R. PRUSTY, Department of Bioscience and Biotechnology, Drexel University, Philadelphia, PA (USA)

We have discovered a new nodule-specific gene from alfalfa: ENOD8. It is expressed early in nodule development and in empty nodules elicited by exopolysaccharide deficient *Rhizobium meliloti*. Empty nodules are devoid of infection threads and intracellular rhizobia, and can be elicited by early rhizobium nodulation signal(s). Thus, ENOD8 is expressed in a developmental pathway triggered as a result of *Rhizobium* nodulation signal transduction. Because of its expression pattern, we predict that the role of the ENOD8 gene product is in nodule organogenesis.

An ENOD8 cDNA was sequenced and found to encode a novel product. Its deduced polypeptide sequence was found to be similar to the non proline-rich domains of the putative polypeptides encoded by a class of anther-specific genes from *Arabidopsis thaliana* and *Brassica napus*. Recent RNA blotting experiments showed that an ENOD8-hybridizing transcript is present in alfalfa flower buds. This transcript is larger than that of nodule-specific ENOD8.

NODULATION AND NITROGEN FIXATION IN INDIGENOUS INDIAN BRADYRHIZOBIA SYMBIOTIC TO GLYCINE MAX JS-72-44

Ritu Shrivastava, 301 Worrilow Hall, Animal Science Department, University of Delaware, Newark DE 19717

In view of our fast depleting energy resources Biological Nitrogen Fixation (BNF) is of paramount importance. As a renewable energy source BNF has been incorporated as a major issue in India's biotechnology program. Survey, isolation and testing of Bradyrhizobium strains being used as biofertilizer has been taken up at a world wide basis by different agencies as NifTal and ICRISAT.

These Institutes recommend specific strains for particular regions. But it is quite likely that their ability to produce effective symbiotic nitrogen fixation might change under varied ecological conditions. Hence the present work is an attempt to solve out this problem by isolating and screening Indian Bradyrhizobium strains and evaluating them for nodulation, leghaemoglobin content, acetylene reduction and yield.

A comparative study of native strain Rj(R)5 with two exotic strains of B.japonicum Rj123 and Rj 138 showed higher grain yield by 4.5% and 50.8% respectively. Other parameters like exopolysaccharide secretion alone and in response to salt stress, glucose consumption (explanta), and synthesis of leghaemoglobin and nitrogenase enzyme activity were always compared with that obtained from exotic species. All the activities were found to be higher in indigenous strain. Further our observations show correlations between the exopolysaccharide (EPS) secretion and the nitrogenase activity in all the strains. However, a negative correlation ($r = +0.697$) between the nodule size and nitrogenase activity was observed during the present investigation. Considering the above performance it could be concluded that native strains show potential to be the future strain in India's expanding Biofertilizer industry.

ON-LINE ACCESS TO GENBANK SUBMISSIONS DATABASE: THE LATEST ADVANCES IN COMMUNITY-BASED ACCESS TO SEQUENCE DATA SUBMISSION AND MAINTENANCE TECHNOLOGIES FOR GENBANK.

Michael J. Cinkosky, Paul Gilna*, Gifford M. Keen.
Theoretical Biology and Biophysics Group, MS K710, Los Alamos National Laboratory, Los Alamos, NM 87545

The mission of the GenBank submissions project at LANL is to become a means of direct electronic communication for sequence data between researchers; in much the same way as a scientific journal publishes and communicates a scientific report, the appearance of your data in GenBank constitutes an parallel form of communication and publication, only in a much more direct, rapid and structured form.

As the needs of the biological community for rapid dissemination of high quality data evolve, it is becoming increasingly important that researchers assume greater responsibility for the way their data appear in GenBank. Our goal is to provide the community with better direct access to this communication channel so that you may exercise this responsibility. This presentation will discuss versions of our in-house database access tool, the Annotators WorkBench (AWB), which allow data depositors to access the submissions database directly.

There are presently two versions of this tool available: AWB (version 3.0) has a graphic-user-interface (GUI) based, point and click style interface and is presently available to run on an OpenWindows or X-Windows capable machine. AWB (version 2.x) is as ascii-terminal based version that will run on most other platforms.

Through the use of AWB, users may thus deposit, annotate and release data directly in GenBank without recourse to intermediate tools and using exactly the same tools as used here at LANL. Users may return at any time and update or add to their data. A manual covering both versions of AWB is available via anonymous FTP from genome.lanl.gov.

Users who wish to establish an account that will allow them to directly deposit, annotate, and maintain their own data may contact us at:

off-site@genome.lanl.gov

This work is funded by the National Center for Biotechnology Information.

TRANSFORMATION OF MAIZE BY PARTICLE BOMBARDMENT.

T. M. Klein, A. Luckring, M. Marini, S. McAdams, D. O'Brien, K. Reiter, T. Warner, P. Smith, T. Jones. DuPont Agricultural Products, Experimental Station 402/4250, Wilmington, DE, 19880.

The improvement of maize by molecular techniques is dependent on efficient methods for gene transfer. Particle bombardment has become the method of choice for maize transformation in a number of labs. Some physical parameters that are related to components of the gene gun have been modified and these modifications will be discussed. Biological factors also affect the process and some of these will be highlighted (i.e., the nature of the target tissue and the use of osmotic protection).

Embryogenic callus is bombarded with genes that can confer resistance to the herbicides glufosinate or chlorsulfuron. Transgenic clones can be recovered within 8 weeks after bombardment and mature seed can be harvested from regenerated plants after an additional 4 to 6 months. From 5 to 50 independent transformants can be recovered from one experiment, depending on the selection system used. Currently maize transformation is almost exclusively carried out in industrial labs. The feasibility of transferring this technology to academic labs will be discussed.

Suspension cultures - difficult to maintain. Limited to some lines Technically demanding.

0.5 M sorbitol/mannitol Equal sorbitol + mannitol - 0.25 each)
Humidity influences -- reduce time between membrane preparation and use - store in desiccator.
Waffle irons cover better - Donna Cup) stopping screen better than plate.

? low expressors methylated high expressors not methylated.

Grow 2-7 days before transferring to selection medium then subculture 2-3 week intervals regenerate 8-10 plants / clone.

bar selection ~ 90% regeneration, ~ 40% - seed.

THE APRT SELECTION SYSTEM.

Dennis A. Schaff and Sunita K. Agarwal. Department of Plant and Soil Sciences, College of Agricultural Sciences, University of Delaware, Newark, DE 19717-1303.

The APRT system has the advantage over resistance to phytotoxic chemicals (antibiotics or herbicides) in having both positive forward and backward/reverse selection, which can be used in the selection of transformed plants. APRT (adenine phosphoribosyltransferase; EC 2.4.2.7) has a simple selection system with positive selection for both the functional APRT enzyme (APRT+) and non-functional APRT enzyme (APRT-). APRT is in the adenine salvage pathway. APRT converts adenine to AMP using 5'-phosphoribosyl-1-pyrophosphate (PRPP). APRT provides the only known mechanism whereby free adenine can be reclaimed. The APRT system works because there are two pathways to produce AMP: 1) the purine *de novo* pathway in which IMP (inosine 5'-monophosphate) is converted to AMP; and 2) the adenine salvage pathway by APRT.

APRT does not show a high degree of specificity for the exact structure of adenine and can also act on cytokinins and adenine analogues like 6-methlypurine, 2,6-diaminopurine, and 2-fluoroadenine. APRT can utilize these adenine analogues as substrates and convert them to their nucleotide forms, which are toxic. Plants that lack APRT activity survive in the presence of these analogous. The amount of adenine analogue used for selecting APRT- plants is such that it kills all APRT+ (wild-type) plants. The APRT system can function as a conditional auxotroph for AMP. To select for the APRT+ phenotype, growth media can be supplemented with adenine, azaserine, and alanosine (AAA medium). The *de novo* pathway for AMP synthesis would be blocked by azaserine and alanosine . Only the APRT+ plants can utilize adenine to synthesize AMP and survive. The APRT- plants can not produce AMP and will die. We produced transgenic APRT- *Arabidopsis* plants with an antisence construct.

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Effects of expression medium on the transformation efficiency of *Agrobacterium tumefaciens* cells using electroporation.

Jhy-Jhu Lin, Life Technologies Inc., (GIBCO/BRL), Molecular Biology Research and Development, Grovemont Circle, Gaithersburg, MD 20877.

The introduction of recombinant plasmid DNAs into *Agrobacterium tumefaciens* LBA4404 cells was achieved by electroporation using a field strength of 16.7 kv/cm. The transformation efficiency was $>5x10^6$ cfu/μg. Different expression media such as LB broth, LB broth + 1% mannitol, S.O.C. broth, M9 broth, and YM broth have been examined for the optimal expression of *A. tumefaciens* LBA4404 cells after electroporation. Among these media, YM broth is the best medium for expression of *A. tumefaciens* cells. The difference in transformation efficiency between YM broth and LB broth was about 40 fold. Different expression times were also investigated to optimize the transformation efficiency of *A. tumefaciens* cells. With a three hour expression time, the transformation efficiency was consistently two fold higher than with a one hour expression period. However, the amount of viable cells after three hours of expression was the same as after one hour expression.

YM broth 2-3 X yield of SOC broth

Yeast extract	0.04%
Mannitol	1.0%
NaCl	1.7 mM
$MgSO_4 \cdot 7H_2O$	

TECHNOLOGY FOR MOLECULAR BREEDING: RAPD MARKERS, MICROSATELLITES, AND MACHINES.

Antoni Rafalski, Agricultural Products Department, DuPont Co. P.O.Box 80402, Wilmington DE 19880, Tel. (302) 695-4348.

A genetic map is but a tool and a starting point It enables one to focus on a small segment of the genome for the purpose of molecular analysis, physical mapping, chromosome walking and cloning the genes of interest. It provides anchor points for assembling sets of contiguous clones. The availability of large YAC, P1 and other large insert libraries has simplified these tasks, but progress in the construction of publicly available index maps using highly polymorphic, PCR-based genetic markers is still needed. Di- and trinucleotide simple repeat based microsatellite markers in particular, due to their abundance and high informativeness are expected to be of great importance in future mapping efforts in plants. A genetic map also facilitates the construction of genetic diagnostic tools that will revolutionize the practice of plant breeding. The science of mapping traits, including those of agronomic interest, is established. Many markers systems, including RFLPs, RAPDs and Microsatellite Repeats are available. However, the technology of genetic diagnostics in plant breeding is in its infancy. The sample throughput and cost requirements are very different from those of medical DNA diagnostics. Automation of the DNA isolation process, of RAPD- or PCR-based allele identification and of the data handling, including sample identity preservation, is necessary. Progress in the development of molecular breeding technologies will be reviewed.

RAPDs behave as dominant marker - will not distinguish AA from A
Bulk segregant analysis - R. Michelmore et al - populations differ
in presence/absence of individual trait (- RANDY/KAREL). Unique band → desired trait
choice of enzyme - stoffel fragment more reproducible - but different
banding patterns from amplitaq
Microsatellites — up to 27 alleles mapped in soybean by microsatellite
repeats - sequence tagged sites.

EXTRACHROMOSOMAL MAINTENANCE OF TRANSFORMING DNA IN TRANSFORMANTS OF THE MOSS, *Physcomitrella patens*: John C. Wallace, Celia D. Knight, and Wagdy Sewahel, Dept. of Biology, Bucknell University, Lewisburg, PA 17837, and Dept of Genetics, University of Leeds, Leeds LS2 9JT, UK.

The moss *Physcomitrella patens* represents, in many ways, an excellent model system for the study of fundamental processes of plant development (Cove, 1992). When *Physcomitrella* is transformed using a particle bombardment technique, three classes of antibiotic-resistant transformants appear: those that express the transgene for only up to a few weeks time (transients); those that will express the gene permanently only if kept under selection pressure (unstables); and those that continue expression regardless of culture conditions (stables). Our hypothesis is that the unstable class of transformants maintains and replicates the transgene extrachromosomally. In support of this model, southern analysis of the unstables has shown the presence of plasmid-sized molecules containing the transgene in uncut genomic DNA; these can be rescued by direct transformation into *E. coli*. The rescued plasmids appear to be identical to those originally used in the transformation, i.e. no moss sequences were acquired. Transformation with the transgene alone (CaMV promoter and neomycin phosphotransferase coding sequence), purified away from the pUC vector sequence, can also produce unstable transformants, so if there is an origin of replication it is not that used by bacteria. Finally, cotransformation of two plasmids conferring resistance to different antibiotics often results in transformed moss resistant to both. Surprisingly, in unstable transformants of this type, selection for maintenance of one of the plasmids also results in maintenance of the other. This system has great potential as a tool for the cloning of developmentally important genes.

TRANSGENIC BROCCOLI EXPRESSING A *BACILLUS THURINGIENSIS* INSECTICIDAL CRYSTAL PROTEIN: A MODEL SYSTEM TO TEST RESISTANCE MANAGEMENT THEORIES. Timothy D. Metz[1], Anthony M. Shelton[2], Richard T. Roush[3] & Elizabeth D. Earle[1]. [1]Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853; [2]Dept. of Entomology, New York State Agricultural Experiment Station, Geneva, NY 14456; [3]Dept. of Entomology, Cornell University, Ithaca, NY 14853.

Bacillus thuringiensis insecticidal crystal protein (*Bt* ICP) genes are being genetically incorporated into many crop plants in order to improve insect control. Unfortunately, the benefits of these genetically-transformed plants may not be realized due to the development of insecticide resistance. Diamondback moth, an important pest of crucifers worldwide, has already developed resistance to *Bt* sprays in the field, and there is concern that other insects may also develop resistance to *Bt* ICPs in transgenic plants. Principles of managing resistance to plant-incorporated insecticides must be developed if they are to have a commercially viable future. We are using the only case of field-evolved *Bt* resistance as a model system to study different options for resistance management. We report the production of transgenic broccoli expressing a *Bt* ICP to be used in this study. Flowering stalk explants of broccoli were inoculated with an *Agrobacterium tumefaciens* strain containing the *NPTII* gene and a *Bt cryIA(c)* gene optimized for plant expression. Culturing the explants on shoot induction medium containing 2 mg/L benzyladenine and 25 mg/L kanamycin allowed selective regeneration and growth of transformed shoots. Transformed shoots rooted on hormone-free medium containing 25 mg/L kanamycin whereas untransformed controls did not. As a further test of transformation, leaf pieces from putative transformants were cultured on medium containing 50 mg/L kanamycin. Leaf pieces from transformants remained green, producing callus and roots while controls and escapes bleached within a week. A total of 164 kanamycin-resistant plants were obtained from 5 broccoli lines. The overall transformation efficiency was 6.5%. Initial screening of transformants for *Bt* ICP expression was conducted either on intact plants or on detached leaves using 5-10 1st instar larvae of a *Bt*-susceptible diamondback moth strain. Insect mortality and leaf damage were scored after 3, 4 and 5 days; 101 of 145 kanamycin-resistant plants provided 100% insect mortality. Southern blots done on some of these plants confirm the presence of the *Bt* gene. Selected plants that gave 100% mortality of susceptible larvae were also assayed with 1st instar larvae from a strain of diamondback moth that had developed resistance to *Bt* in the field and with F_1 hybrids from a cross between the resistant and susceptible strains. *Bt*-resistant larvae showed >50% survival on all plants tested, whereas all F_1 larvae died. The ability of these transgenic plants to kill susceptible larvae while serving as a suitable host for resistant larvae makes them an excellent model for testing the various *Bt* resistance management strategies that are currently available.

300 mg/l Timentin to kill Agro bacteria, 7 days.
1st week
then 200 mg/l + Kan.
Source? Effectiveness?
Smith Kline Beecham veterinary strain
write for copy
2 wk transfers
NO longer than 3 wks
powder @ RT stock @ -20°

MOLECULAR ANALYSIS OF MALE GAMETOGENESIS IN PLANTS

Sheila McCormick, Plant Gene Expression Center
USDA/ARS/UC-Berkeley, 800 Buchanan St., Albany,CA 94710

The events that end with the formation of a pollen grain begin with meiosis and involve an intricate and tightly controlled set of structural and molecular changes, requiring gene expression in both the gametophytic and sporophytic tissues of the anther. We want to understand the regulatory circuits that control differential gene expression in pollen, to determine the roles that pollen proteins play during pollen maturation and germination, and to understand the contribution of sporophytic gene expression during pollen development.

We characterized several gametophytically-expressed genes from tomato (LAT52, LAT56 and LAT59). We used both transient and stable expression assays to define cis-acting sequences of the promoters that are important for pollen expression, and are now examining protein interactions with these cis elements towards the isolation of the genes that encode these proteins. We are also screening Arabidopsis for mutations in genes encoding such trans-acting factors. The LAT56 and LAT59 proteins are similar in sequence to ragweed allergens and to pectate lyases, while the LAT52 protein shows some similarity to proteinase inhibitors. We are using immunological and transgenic plant analyses towards determining the function of these proteins. For example, antisense experiments with LAT52 suggest that this protein is required for normal pollen germination. Lastly, we are using map-based cloning to isolate and characterize genes that act sporophytically to influence pollen development (male sterile genes).

Very little knowledge of meiotic-specific genes.
Pollen promoters effective in lily pollen? - who?
? pollen transformation → transgenic plants?
Common boxes act as general enhancers - effect on truncated 3
No silencers found in pollen-specific promoters, minimal promoters pollen-specific. No common sequences in minimal promoters.
Some homologies to promoters in other species — especially tobacco
5' leaders not contributing to pollen specificity - but shorter leaders had higher level expression.

MOLECULAR BASIS OF RHIZOSPHERE COLONIZATION BY THE PLANT-BENEFICIAL BACTERIUM, ENTEROBACTER CLOACAE: D.P. Roberts; USDA/ARS, Beltsville, MD.

The goal of the research program is to understand how populations of the plant-beneficial bacterium Enterobacter cloacae are established and maintained in association with the subterranean portions of plants. Currently, we are investigating the nutritional basis of spermosphere and rhizosphere colonization by E. cloacae. A transposon mutant library of E. cloacae strain 501R3 was constructed with mini-Tn5 Km and screened for mutants no longer capable of growth on various carbohydrates and soluble organic acids. A variety of mutants with deficiencies in carbohydrate and amino acid utilization were isolated. The impact of these nutritional deficiencies on proliferation of E. cloacae in plant spermosphere and rhizosphere is being investigated. In addition, a spatial analysis technique has been developed to determine the impact of these mutations on rhizosphere colonization by E. cloacae. This spatial analysis technique determines the location of the mutants in the rhizosphere and the extent of the root system colonized by the mutants. The gene(s) affected by transposon insertion will be determined by DNA sequencing and subsequent biochemical testing. Hopefully, this strategy will allow us to determine the assemblage of Enterobacter cloacae genes and traits important to spermosphere and rhizosphere colonization.

REPLICATION AND MOVEMENT OF A POTYVIRUS THAT EXPRESSES GUS. James C. Carrington, Department of Biology, Texas A&M University, College Station, TX

A "tagged" strain of tobacco etch potyvirus (TEV) that expresses the gene for ß-glucuronidase (GUS) was generated for studies of viral replication and movement. Using sensitive *in situ* histochemical and *in vitro* fluorometric assays for GUS activity, a series of TEV-GUS mutants containing defects in the capsid protein region was analyzed in protoplasts and plants. Mutants containing single or double amino acid substitutions, or deletions of the N-terminal domain, were able to replicate in protoplasts as well as wild-type TEV-GUS. These mutants, however, were debilitated in cell-to-cell movement even though some mutants were able to form virions, suggesting distinct functions of the capsid protein in assembly and transport. Cell-to-cell and long-distance movement functions were rescued in transgenic plants expressing TEV capsid protein, although virus was not detected in these plants. Additional transgenic complementation assays revealed that the capsid protein N-terminal domain is necessary for potentiating movement, but not assembly. Progress on mapping other movement functions in the TEV genome will also be discussed.

9.30

CMV-IXORA: A CUCUMBER MOSAIC VIRUS DEFECTIVE IN SATELLITE RNA REPLICATION

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Molecular Plant Pathology Lab, ARS, USDA
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The satellite RNAs of cucumber mosaic virus (CMV) are small subviral pathogens with no appreciable primary sequence similarity to their associated "helper" virus. CMV satellite RNAs have the ability to modulate the disease symptoms induced by their helper virus and thereby produce either a more or less severe form of disease. Satellite variants that induce new symptoms have been studied extensivly and the nucleotide sequences necessary for the induction of tomato necrosis and tobacco chlorosis have been determined. The CMV strain Ixora (CMV-Ix) is unique in that it does not support the accumulation of several necrogenic and nonnecrogenic satellite RNAs. One satellite variant that CMV-Ix does support is T-CARNA 5. T-CARNA 5, however, does not induce tomato necrosis in combination with CMV-Ix even though the satellite's sequence is typical of necrosis inducing satellites and T-CARNA 5 will induce necrosis in combination with other CMV strains. We have used electroporation of tomato protoplasts to show that the failure of CMV satellites to accumulate is due to a failure of CMV-Ix to support the replication of the satellite. Pseudorecombinant infections between CMV-Ix and CMV-1 genomic RNAs indicate that the genetic component responsible for the defect in replication lies on RNA 1 of CMV-Ix. In addition, we have cloned and sequenced the complete genome of CMV-Ix and report the results of our analysis to date.

CMV-IX RNA in pseudorecombinants correlates w/ lack of satellite replication

RNA 1 of RNA 3 CMV-IX does not allow tomato necrosis w/ T satell

CONTROL OF MOVEMENT AND PATHOGENESIS IN CAULIMOVIRUSES: THE CASE OF PEANUT CHLOROTIC STREAK.

A.Mushegian, D.Ducasse, R.Richins, J.Wolff and R.J.Shepherd.
Plant Pathology, University of Kentucky, Lexington KY 40546

Peanut chlorotic streak virus (PClSV) is a caulimovirus which infects plants from 7 families, e.g. solanaceous and legumes. Like other plant pararetroviruses, PClSV expresses most of its genes from a genome-length polycistronic transcript. Translation of downstream cistrons from this mRNA is mediated by protein encoded by ORFVI. ORF V product contains an array of protease-reverse transcriptase-RNAse H domains and presumably is virus replicase. Product of ORF IV is the major virus capsid protein. Product of caulimovirus ORFI has been implied in virus cell-to-cell movement. Functions of four other ORFs, called VII, A, B and C, remained unknown.

To confirm that ORF I controls cell-to-cell movement of PClSV, insertion, deletion and a point mutation in a putative core domain of ORF I were introduced. Quantitative assay of virus replication in single cells was developed, based on immunocapturing of progeny followed by competitive PCR. One per 10^4 of epidermal cells became infected with ORF I mutants, to produce ca. 3.10^4 virions per cell. Complementation of an RNA virus movement by PClSV was shown, suggesting that caulimovirus is able to translocate its RNA from cell to cell. A recombinant virus was constructed where ORF I of PClSV was replaced by ORFI from another caulimovirus, CaMV, to study shifts in host range.

New restriction sites were inserted between ORFs VII, I, A, B and C of cloned PClSV genome without effect on virus infectivity. Deletions and insertions revealed that ORFs A and C are essential for replication. The primer for minus strand DNA synthesis, $tRNA^{Met}$, binds to virus RNA within ORF A coding sequence, and an extra copy of this primer-binding site was inactive when inserted at different location; thus, part of ORF A is required in cis for virus replication. ORF C may be a functional equivalent of CaMV ORF III, a gene for minor capsid protein. Function of gene B is under investigation.

Deletion of 432 bp ORF VII increased virus reproduction and severity of infection. More young cells, including apical meristems, were dying upon infection with the deletion mutant. Evidence is presented that this is due to decrease in genome size, rather than to distortion of a cis-element in or protein product of ORFVII.

High infectivity of cloned DNA, ease of rub inoculation and broad host range of PClSV promise a venue for turning it into a useful vector for gene expression in planta. Replacement of PClSV ORF VII and evaluation of stability of inserted genes are underway.

MOLECULAR CHARACTERIZATION OF AVIRULENCE GENES FROM THE RICE BLAST FUNGUS.

Barbara Valent*, Jim Sweigard, Marc Orbach[1], Seogchan Kang, Leonard Farrall, Anne Walter, and Forrest Chumley. DuPont Science and Engineering Laboratories, Wilmington, DE 19880-0402. USA

We are characterizing cloned host specificity genes from Magnaporthe grisea. The avirulence gene Avr2-YAMO prevents strains of the rice pathogen from infecting rice cultivar Yashiro-mochi. A second gene, Pwl2, prevents diverse strains of the fungus from infecting a second species of host plant, weeping lovegrass. Thus, this host species specificity gene has a mode of action similar to that of classical avirulence genes. Progress will be reported toward cloning a third avirulence gene, Avr1-TSUY, which prevents strains of the rice pathogen from infecting rice cultivar Tsuyuake. M. grisea strains carrying Avr2-YAMO, Pwl2, or Avr1-TSUY frequently give rise to mutants that have gained the ability to infect Yashiro-mochi, weeping lovegrass, or Tsuyuake, respectively. Genetic instability of some host specificity genes appears to be a feature of the chromosomal location of the genes. The Avr2-YAMO gene resides within 2 kb of the tip of a chromosome. Independent spontaneous mutation events that occur at the Avr2-YAMO locus include deletions, point mutations and insertions. Several independent spontaneous mutants that had gained the ability to infect weeping lovegrass have deletions of Pwl2 and surrounding sequences. We previously predicted by genetic analysis that pathogens of grasses other than rice contain avirulence genes that function in preventing infection of specific rice cultivars. We have now demonstrated that pathogens of Pennisetum spp. and pathogens of Digitaria spp. contain alleles of Avr2-YAMO that function in preventing infection of rice cultivar Yashiro-mochi when transformed into a virulent strain of the rice pathogen. Pwl2 identifies an interesting M. grisea gene family. Members of this gene family that function in preventing fungal strains from infecting weeping lovegrass have been isolated from pathogens of Pennisetum spp., of Digitaria spp., and of Eleusine spp.

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PHOTOREGULATED EXPRESSION OF PROTEIN KINASE GENES:
John C. Watson, Xia Lin, Zhenbiao Yang, and Rajnish Khanna; Department of Botany, Maryland Agricultural Experiment Station, and Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742-5815

As a first step toward understanding the role protein kinases play in photoregulated leaf development, we are investigating a set of genes encoding putative protein kinases in the garden pea. We previously isolated a suite of partial cDNA clones that encode distinct forms of protein kinase homologs [Lin *et al.*, (1991) *Proc. Nat. Acad. Sci. USA* 88: 6951-6955]. These partial cDNAs, called PsPK1 through PsPK5, are each encoded by a single copy nuclear gene and correspond to rare class mRNAs. The deduced amino acid sequences show that each has structural features typical of members of the protein-serine/threonine kinase family involved in signal transduction. We are determining full-length cDNA sequences to understand more fully the structure of the putative PsPK kinases. Thus far, we have sequenced cDNA clones encoding the entire PsPK5 mRNA [Lin and Watson (1992) *Plant Physiol.* 100: 1072-1074] and the 3′ one-third of the PsPK3 mRNA. The high degree of similarity between PsPK5 and known kinases is restricted primarily to the conserved catalytic core found in all eukaryotic protein kinases.

We found previously that the PsPK mRNAs accumulate differentially during the greening of etiolated pea seedlings (Lin *et al.*, 1991). Exposure of etiolated seedlings to continuous white light causes PsPK3 and PsPK5 mRNAs to decline within 24 hours in apical buds. In contrast, PsPK4 RNA increases over a period of days in light, while PsPK1 and PsPK2 transcripts show little change. We have since examined in detail the kinetics of mRNA decline after transfer to light. PsPK5 mRNA declines detectably even within 20 minutes, reaching its minimum level within 3-6 hours. PsPK3 mRNA declines more slowly. After seedlings given 24 hours of white light are placed back in darkness for an additional 24 hours, PsPK5 mRNA reaccumulates but PsPK3 mRNA does not. This indicates that light does not permanently switch off PsPK5 gene expression. In examining the organ-specificity of PsPK gene expression, we find that PsPK5 mRNA is detectable only in buds and hooks but not stems or root tips of dark-grown seedlings. In contrast, PsPK3 mRNA is present throughout the seedling, being highest in hooks and root tips. In fact, PsPK3 and PsPK5 mRNA levels are higher in hooks than in buds. We find this very interesting because the hook region is the most photoresponsive portion of an etiolated pea seedling and contains the most phytochrome. We are in the midst of determining what photoreceptor regulates PsPK3 and PsPK5 expression. The rapid light-regulated expression of the PsPK5 gene and the similarity of the PsPK5 polypeptide with known second messenger-dependent kinases suggests to us that PsPK5 may be involved in signal transduction during the early phases of photoregulated development.

ELUCIDATING THE MODE OF ACTION OF AN HERBICIDE - A GENETIC APPROACH: Sandra H. Russell, Daniel F. Delduco, and Pablo A. Scolnik. DuPont, Science and Engineering Laboratories, P.O. Box 80402, Wilmington, DE, USA 19880-0402.

Cinmethylin is an herbicide that affects the growth and development of plant meristems. Exposure of *Arabidopsis* seedlings to cinmethylin results in a decrease of root growth and an increase in lateral root formation. Shoot growth is largely unaffected. At high concentrations of cinmethylin, the shoot meristem is affected and callus-like nodules form on the short root. We have isolated 60 mutants that show resistance to cinmethylin, as assayed by root growth. Dominant, semi-dominant, and recessive mutations have been found. The recessive mutants belong to at least four complementation groups. Although most mutants exhibit a fairly normal phenotype, five co-dominant resistant mutants exhibit bleaching of the shoot when exposed to cinmethylin. This bleaching only occurs in those plants homozygous for the resistance mutation. Results from phenotypic mapping suggest that the resistance mutation of three mutants is located on chromosome 1. RAPD mapping will be discussed.

"putant" – putative mutants

AN RNA MARKER FOR EPIDERMAL DIFFERENTIATION IN PISUM SATIVUM

Michael S. Dobres, Sevnur Mandaci, Dipanwita Maiti, Albert J. List.
Department of Bioscience and Biotechnology, Drexel University,
Philadelphia, PA 19104

We describe a novel pattern of tissue and position dependent accumulation for transcripts encoding a lectin-like protein (*Blec*) in pea (*Pisum sativum L.*,). This pattern indicates a regulatory difference between the protodermis of the apical meristem and the derived epidermal cells of surrounding shoot apex: *Blec* RNA is absent or present at very low levels in protodermal cells of the apical meristem but is highly expressed in the derived epidermal cells of leaf primordia flanking the apical meristem. A similar pattern is seen during floral development: transcripts are undetectable in the protodermis of the floral dome and floral organ primordia, but are first detected during organ formation and expansion. This gene-regulatory difference between the protodermis and the derived epidermis is consistent with the hypothesis that the apical meristem functions as a unit distinct from the surrounding shoot apex. To further analyse the mechanism of transcript accumulation during epidermal differentiation we are currently analysing the expression of the *Blec4* gene in transgenic alfalfa.

Evidence for differential activation of gene expression by the maturation-specific enhancer UAS1 in embryo and endosperm tissues of tobacco.

Mauricio M. Bustos and Maw-Shenq Chern. Department of Biological Sciences, UMBC, 5401 Wilkens ave., Baltimore, MD 21228. Fax: (410) 455-3875.

Seed maturation involves extensive changes in gene expression in embryonic and endosperm tissues of dicot and monocot seeds. We are using a biochemical approach to understand the molecular basis of such regulation in the large seeded legume *P. vulgaris* (common bean) and in tobacco. The phaseolin UAS1 enhancer-like element (-295 to -64, Bustos et al., 1991) regulates maturation-specific activation of homologous and heterologous promoters in tobacco embryo and endosperm tissues. Histochemical detection of GUS expression in whole-mounted seeds shows that UAS1 activity occurs earlier in the embryo than in the endosperm. A 3'-end segment of UAS1 (52 bp) activates expression only in the endosperm indicating the existence of partially separate domains within UAS1 for embryo (Emb) and endosperm (End) activation of gene transcription. Using site-specific mutagenesis and gel retardation analysis we have demonstrated sequence-specific binding of bean nuclear proteins at five locations throughout UAS1. Different nucleotide motifs within the putative Emb and End domains form characteristic protein/DNA complexes with distinct electrophoretic mobilities. One bean factor that recognizes a G box-like motif within the End domain interacts with the DNA double helix in nearly the same way as a cloned Opaque-2 protein that activates transcription of α-zein genes in maize endosperm. Opaque-2 belongs to a large family of transcription factors with a conserved bZIP DNA binding domain. Based on this observation we designed a PCR-based strategy to isolate cDNA sequences coding for bZIP proteins expressed in immature bean cotyledons. Multiple PCR-cDNAs were obtained and their nucleotide and deduced aminoacid sequences were compared to those of other plant bZIP genes. The protein products of these cDNAs will be tested for specific binding to UAS1 to determine whether they encode factors found in nuclear protein extracts. We are also using the cDNAs as probes to isolate cognate genes from a bean genomic DNA library.

Bustos, M.M., Begum, D., Kalkan, A F., Battraw, M.J. and Hall, T.C. (1991) *EMBO J.*, 10, 1469-1479.

Cloning and Expression of the Soybean dapA Gene Encoding Dihydrodipicolinate Synthase (DS)

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In plants, the first committed step in the pathway to lysine synthesis is the condensation of aspartate B-semialdehyde and pyruvate, catalyzed by the enzyme dihydrodipicolinate synthase (DS). DS activity undergoes feedback inhibition by lysine and may control lysine synthesis. Cloning of the soybean dapA gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the dapA gene may be an important way of increasing lysine synthesis in soybeans and other crop plants. The dapA gene has not been previously cloned from dicots. We have cloned the dapA gene of soybean (G. max var. Century) using PCR.

Portions of the soybean dapA gene were amplified and cloned using degenerate PCR primers based on the sequences of monocot dapA genes. dapA sequences were amplified from soybean genomic DNA and cDNA. Inverse PCR was used to clone additional sequences within the dapA open reading frame. 3' RACE PCR was used to amplify the 3' end of the dapA gene. The sequence encoding the 5' end of the mature DS protein was obtained from dapA clones from a soybean genomic DNA library in lambda GEM11. PCR primers corresponding to the 5' and 3' ends of the region encoding the mature DS protein were used to amplify the gene from cDNA. The gene was cloned into pUC18 for expression in E. coli. The DS enzyme encoded by the pUC18 clone is feedback inhibited by lysine like the wild type soybean DS enzyme. This is the first report of the cloning and expression of a dicot gene encoding DS.

PROMOTER ANALYSIS OF AN ETHYLENE-INDUCIBLE BEAN ABSCISSION CELLULASE GENE

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Abscission, the process by which plants shed their organs, is accomplished by enzymes which degrade the cell walls and middle lamella to form the fracture plane. Ethylene-induced bean leaf abscission correlates with the *de novo* accumulation of a pI 9.5 cellulase in the abscission zone. We are analyzing promoter deletions of the bean abscission cellulase (BAC) gene fused to the reporter genes β-glucuronidase (GUS) and luciferase to identify cis-acting regulatory sequences responsible for its ethylene-inducible and abscission zone-specific expression. Ethylene-inducible leaf abscission in tomato also correlates with an increase in cellulase activity. Tomato was transformed via *A. tumefaciens* with promoter deletion constructs fused to GUS, and transformed regenerates were selected. Original regenerates and their progeny were screened for incorporation of the appropriate GUS construct by PCR and Southern Blot hybridization. GUS expression was measured using fluorescent and histochemical enzyme assays and Northern blot analysis. A promoter construct containing 210 bp of sequence 5' to the transcription start site is sufficient to drive low levels of GUS expression in leaf abscission zones as early as 24 hours following ethylene induction. Expression in adjacent stems is several fold lower. Expression is inhibited by silver thiosulfate, an inhibitor of ethylene responses. Transient expression of the promoter/reporter gene constructs following particle gun bombardment into bean abscission zone explants indicates that sequences upstream of the 210 bp promoter enhance mRNA expression.

De Novo Purine Biosynthesis in Ureide-Producing Legumes: 5-aminoimidazole ribonucleotide carboxylase and 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide synthetase cDNAs from Vigna aconitifolia

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ABSTRACT

Root nodules of many tropical legumes export fixed nitrogen in the form of urieides that are produced by oxidation of **de novo** synthesized purines. In order to investigate the regulation of **de novo** purine biosynthesis in these nodules, we have isolated cDNA clones encoding 5-aminoimidazole ribonucleotide (AIR) carboxylase and 5-aminoimidazole-4-N-succinocarboxamide ribonucleotide (SAICAR) synthetase from a mothbean (Vigna aconitifolia) library by functional complementation of **purE** and **purC** mutations respectively in Escherichia coli. Sequence analysis of the isolated cDNA clones revealed that the two enzymes are separate proteins in mothbean, unlike in animals where both activities are associated with a single bifunctional polypeptide. As is the case in yeast, the mothbean AIR carboxylase has a N-terminal domain homologous to the eubacterial **purK** gene product. Data is presented indicating that the **PurK**-like domain facilitates the binding of CO_2 and is dispensable in the presence of high CO_2 concentrations. Since translation of the isolated cDNA clone in E. coli apparently generates a truncated polypeptide lacking at least 140 Nterminal amino acids, this part of the N-terminal domain of the enzyme may not be essential for CO_2-binding.

CLONING AND EXPRESSION OF A SOYBEAN cDNA CLONE ENCODING ASPARAGINE SYNTHETASE

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Asparagine plays an important role in plant growth and development because of its involvement as a nitrogen transport compound in higher plants. Asparagine synthesis is mediated by the enzyme asparagine synthetase (AS). AS has not been characterized extensively because of its extreme instability *in vitro*. Therefore, expression of a soybean AS gene in *E. coli* would enable the purification of the AS protein for characterization and antibody production. The region corresponding to the predicted mature soybean AS protein was amplified by the polymerase chain reaction (PCR). The 5' oligonucleotide primer contained the proposed glutamine binding site with an *Eco*RI site at the extreme 5' end. The inverse oligonucleotide corresponded to a region 3' of the stop codon along with a *Bam*HI site. These primers were designed to place the SAS2 gene in-frame with the *lac*Z gene of the pUC18 vector. Plasmid DNA from positive colonies was isolated and used to transform an AS deficient *E. coli* strain. Complementation experiments revealed that the soybean gene was expressed functionally in *E. coli*. This was also confirmed in preliminary AS assays using HPLC which directly measured the synthesis of asparagine.

MICROSURGICAL AND HORMONAL ANALYSES OF AN ARABIDOPSIS MUTANT ALTERED IN ORGAN ELONGATION.
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We have isolated a seedling-lethal mutant called gordo following EMS mutagenesis of Arabidopsis seeds. gordo segregates as a nuclear recessive. If grown on enriched medium, all the multicellular organs of this mutant exhibit radial expansion instead of uniaxial elongation and its stunted inflorescence fails to set seed. Wild-type and gordo embryos were dissected out of developing seeds in order to perform microsurgical and hormonal studies. The roots of gordo torpedo embryos surgically separated from the apex elongate three times as much as those roots which remain attached to the apex. Wild-type heart embryos treated with napthalene acetic acid mimic the gordo phenotype, but other auxins and auxin antagonists have no significant effect on the length-to-width ratios of wild-type and gordo embryos. The free auxin levels (ng/g f.w.) of gordo seedlings are more than five times the levels of free auxin found in wild-type seedlings. The ethylene levels (nmole/plant) of gordo seedlings are three times those found in wild-type seedlings. These results indicate that the mutation in gordo may lie in a gene encoding an auxin conjugating enzyme, leading to the higher levels of free auxin present in this loss-of-function mutant. The higher auxin levels may, in turn, induce the higher levels of ethylene which may cause the radial expansion of the mutlicellular organs in the mutant. This work was supported by USDA-CRGO-89-37261-4791 and USDA-CRGO-91-37304-6655.

HISTOCHEMICAL LOCALIZATION OF RICE (*Oryza sativa L*) β-GLUCOSIDASE

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β-glucosidase catalyzes the hydrolysis of β-D-glucosidic bonds between glucose and an aryl or alkyl aglycone. Its function may be in the activation of growth hormones and defense against phytopatogens and insects. The enzyme in plants is localized in the cell wall, in the cytosol, or inside an organelle in the cell depending on taxa. The purpose of this study was to determine tissue and subcellular location of the enzyme in rice using substrates such as 6-bromo-2-naphtyl β-D-glucopyranoside (6-BNPG) and 5-bromo-4-chloro-3-indolyl β-D-glucopyranoside (X-glu) which yield colored insoluble products. Coleoptiles of 5-6 day-old germinated seeds were used for free-hand sectioning, and as sources of protoplasts and plastids. The sections, protoplasts, and plastids were incubated with 6-BNPG and X-glu and observed under the light microscope. The development of bright brown color for 6-BNPG staining and blue for X-glu in the cells indicated the presence and location of β-glucosidase.

Results show that at the tissue level there is intense staining in the epidermis and bundle sheeth and less intense staining in the cortex. At the subcellular level, the enzyme is localized in the plastids. This localization is confirmed in the stained protoplast and plastid preparations. The addition of δ-gluconolactone (a specific competitive β-glucosidase inhibitor) to the incubation medium with the dye prevented the color formation, indicating that the enzyme catalyzing the hydrolysis of substrates is β-glucosidase. In order to confirm these results further, the supernatant of the lysed plastids was tested for enzyme activity using para-nitrophenyl β-D-glucopyranoside (pNPG) as substrate. These data also indicate that the rice β-glucosidase is localized in the plastid.

MOLECULAR ANALYSIS OF THE ASPARTATE KINASE-HOMOSERINE DEHYDROGENASE GENE FAMILY IN SOYBEAN

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Aspartate kinase (AK) controls the first step common to the synthesis of the essential amino acids lysine, threonine, isoleucine, and methionine. As the first enzyme in the pathway, AK potentially exerts a key role in regulating the entry of aspartate into the pathway. The ATP-dependent phosphorylation of aspartate to β-aspartyl phosphate is catalyzed by AK. β-aspartyl phosphate is reduced to aspartate semialdehyde which may be used as a substrate by either dihydrodipicolinate synthase (DS), leading to lysine biosynthesis, or homoserine dehydrogenase (HSDH), leading to threonine, isoleucine, and methionine biosynthesis. Higher plants commonly contain at least two forms of AK that are differentially feedback inhibited by the amino acid endproducts lysine or threonine. HSDH is feedback inhibited by threonine.

The cloning and sequencing of the plant genes that code for the enzymes of the asparate pathway have begun only recently. Two partial cDNAs encoding one bifunctional protein possessing both AK and HSDH activities have been isolated from soybean cDNA libraries. The 5' end of the cDNA encodes a chloroplast transit peptide consistent with the biochemical localization of these enzymatic activities. Differential screening of genomic libraries and Southern blots suggested the presence of additional genes encoding AK or HSDH activities. Genomic clones representing the cDNA and a second probable bifunctional AK-HSDH have been identified. Sequence analysis of these clones has demonstrated a high amount of similarity between the two gene copies within the AK coding regions and the chloroplast transit peptides. Southern blot hybridizations suggested the presence of a third AK gene in the soybean genome. A portion of this third tentative AK gene was amplified from size-fractionated restricted genomic DNA. Sequence analysis of this fragment has demonstrated the presence of AK coding sequences similar to the two bifunctional AK-HSDH genes. It is not known whether this third AK gene also contains HSDH coding sequences.

ORGANIZATION AND EXPRESSION OF PHENYLALANINE AMMONIA-LYASE GENES IN TOBACCO (*NICOTIANA TABACUM*): Tomoko Fukasawa-Akada,[1,2] Shyam K. Dube,[2] & Shain-Dow Kung[2,1] John C. Watson[1,2,3]; [1]Department of Botany, [2]Center for Agricultural Biotechnology, and [3]Maryland Agricultural Experiment Experiment Station, University of Maryland, College Park, MD 20742

Phenylalanine ammonia-lyase (PAL) catalyzes the first reaction in the general phenylpropanoid pathway leading to the production of flavonoids, coumarins, lignins and other phenolic compounds. The significance of these compounds in plants, as flower pigments, UV-B protectants, IAA-transport inhibitors, cell wall components, and phytoalexins is well documented. To investigate the regulation of PAL biosynthesis, we previously have isolated a PAL gene from a tobacco genomic library and determined the complete nucleotide sequence (gPAL1). Using various parts of gPAL1 as probes, genomic Southern blot analysis indicated the presence of a small family of PAL genes in tobacco genome (*pal1* through *pal4*) with high similarity between *pal1* and *pal2*. To gain further insight into the sequence relationships among the four genes, and to study the expression of individual PAL genes, a suite of cDNA clones, called the cPAL series, were isolated from a tobacco leaf cDNA library. So far, we have identified three distinct types of PAL cDNA clones. Based on their sequences and hybridization profiles, it appears that cPAL1 corresponds to *pal1* whereas cPAL3 and cPAL4 share high sequence similarity with each other and correspond to *pal3* and *pal4*, respectively. Based on these analyses, we can divide the PAL gene family into two distinct subfamilies, one consisting of *pal1* and *pal2* and another of *pal3* and *pal4*. Comparative genomic blot analyses show that the *N. tomentosiformis* genome appears to contain only *pal1* and *pal3*, whereas the *N. sylvestris* genome contains only *pal2* and *pal4*. Since *N. tomentosiformis* and *N. sylvestris* are believed to be the progenitor species of *N. tabacum*, we conclude that *pal1* and *pal3* (or *pal2* and *pal4*) diverged prior to the evolution of *N. tabacum*. The expression of the PAL gene family was examined using RNA gel blot analysis. Under high stringency hybridization conditions, a gPAL1 probe primarily detects transcripts from *pal1* and *pal2* while a cPAL3 probe primarily detects transcripts from *pal3* and *pal4*. With both probes, PAL transcript levels were significantly higher in flowers and roots than in leaves and stems of mature plants, suggesting that the two subfamilies exhibit similar expression profiles in mature organs of tobacco.

TRANSGENIC ANALYSIS OF GENE EXPRESSION IN THE PLANT SHOOT APEX

46

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The goal of this project is to investigate the regulation of genes specifically expressed in the growing tip of plants. As a model system we are studying a family of lectin-like genes in pea (Blec) that are highly expressed in epidermal tissue of the actively growing shoot apex. We are interested in identifying the portion of the gene responsible for the observed epidermal tissue specificity.

As a first step, we have isolated, mapped and sequenced a genomic clone containing the bud lectin- like (Blec) gene from pea. Sequence analysis demonstrated that the Blec gene is transcriptionally active and so represents an expressed member of the Blec gene family. Computer analysis of 5' flanking sequences of the Blec gene reveals some conserved sequences that might function as gene regulatory elements. For functional analysis of the Blec gene, we are using a transgenic alfalfa system. The Blec genomic clone was introduced into the genome of alfalfa plants via Agrobacterium-mediated transformation. Our preliminary RNA analysis shows that pea Blec gene is correctly expressed in transgenic alfalfa. This demonstrates that the regulatory signals necessary for Blec gene expression are conserved between different legume species. Further experimentation will attempt to map these regulatory sequence.

PLASMOLYSIS FACILITATES THE PASSAGE OF PROTEIN AND DNA THROUGH THE CELL WALL OF INTACT PLANT CELLS:

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Plasmolysis was used as a tool to facilitate the passage of large fluorescent-labelled macromolecules of calf thymus DNA, bovine serum albumin, and c-phycoerythrin through the cell wall of onion epidermal cells and petunia suspension cells. BSA was found to pass through the cell wall of living onion and petunia cells at frequencies of ≈20% and ≈5%, respectively, without binding to the plant cell wall. DNA and c-phycoerythrin appeared to first non-specifically bind to the cell wall before moving through the wall into the space created by plasmolysis.

The recovery rate and the relative volume loss of plasmolyzed onion epidermal cells were studied using sucrose, mannitol, $CaCl_2$, and NaCl as solutes. It was observed that plasmolyzed cells were able to remain viable for several days. However, lysis of cells did occur during the loss of protoplast volume under severe plasmolysis and gain of protoplast volume during recovery. Under plasmolysis conditions which produced similar relative loss of protoplast volume, sugar solutes (mannitol and sucrose) caused less loss of viability than the salt solutes ($CaCl_2$ and NaCl).

We believe that since plasmolysis can facilitate the passage of large macromolecules across the plant cell wall and plasmolysis conditions can be optimized such that minimal viability loss can accompany maximum plasmolysis, this procedure, if accompanied by a membrane permeabilization step, may be a useful tool for introduction of macromolecules into intact plant cells.

FOREIGN GENE EXPRESSION BY SALT-ADAPTED CELLS IN RESPONSE TO BOMBARDMENT WITH THE PARTICLE INFLOW GUN

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A Particle Inflow Gun was constructed to successfully bombard 0, 85, 170, and 255 mM NaCl-adapted suspension cells of salt-tolerant plant, *Kosteletzkya virginica* with the GUS gene. The cells adapted at 85 mM NaCl had similar GUS transfer efficiency to the 0 mM NaCl control with about 800 blue loci per bombardment, while expression was less in the 170 mM and 255 mM NaCl-adapted cells. Either increasing bombardment pressure to 240 PSI or cutting the distance from the cells to the particle filter holder to 9 cm enhanced the GUS expression in cells of all salinity treatments. The size of the blue loci was different among various salt treatments, with larger blue spots occurring at the higher salinity treatment. An ABA responsive EM promoter was induced to express the GUS gene with 10^{-4} M ABA or with salts in the post-bombardment medium in both control and NaCl-adapted cell lines, but the number and size of blue loci were smaller than that when the CaMV35S promoter was used. Low GUS gene expression in higher NaCl-adapted cells might be due to reduced DNA uptake or to some physiological limitation, although the cells grow vigorously in the medium with 0, 85, and 170 mM NaCl, and there is slow growth at 255 mM NaCl salinity. Stable transformed callus has been achieved using this PIG bombardment method.

SOYBEAN TRANSFORMATION BY DIRECT UPTAKE

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Numerous techniques to transfer genes into plants have been developed and refined over the last decade. These techniques have tremendously advanced our understanding of gene regulation in plants. Moreover, these techniques have made the potential and promise of crop improvement a decade ago a reality today. Unfortunately some important agronomic crops, including soybean and maize, are not particularly amenable to conventional transformation techniques. However, these crops have been successfully transformed, but the successes have been limited to a handful of researchers with tremendous financial backing and large pools of human resources. The rapid advancement of scientific knowledge and improvement of these crops, via molecular techniques, is dependent on the development of a simple and reliable gene transfer system.

We have focused on the development of a non-tissue culture dependent gene transfer system in soybean. Dry soybean (*Glycine max* L. Merr. cv. Williams) seeds were imbibed in sterile water or vector DNA (200ug/ml) for three hours with aeration. The vector, pBI 121 (Promega), is a 13 kbp plasmid which contains a GUS cassette with the CaMV 35S promoter and NOS terminator in the binary vector pBIN 19 with the NPT II gene (conferring geneticin resistance). Seeds were sterilized and grown on 1 x MS salts 0.8% phytagar with geneticin (25 ug/ml) for 4 to 5 weeks. Developing plants were maintained in a growth chamber at 27°C under cool white lamps (300 umol m-2 s-1) with a 8 hr/16 hr dark/light cycle.

Transformed plants (gen^r) were transferred to 1 x MS for one week (to enhance root development), then transferred to soil. After 20-30 days, the day and night length was changed to 12 hr/12 hr to induce flowering and seed development. At the present time, we have putative transformants at several stages of development; 3-5 week-old (selected on geneticin), 6-12 week-old (selected on geneticin for 5-7 weeks and transferred to soil). Most of the older plants (12 weeks) have already flowered and have set seed. We have initiated Southern blot analysis on some of these plants but the experiments have not been completed. Preliminary results show that vector DNA is present in soybean genomic DNA extracts up to 2 weeks after imbibition. Southern blot analysis will also be used to determine if the DNA has been integrated into the soybean genome.

Use of SUI, a cytochrome P450 gene from *Streptomyces griseolus* in a negative selection protocol for plant cells during protoplast transformation.

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Targeting of transgenes to homologous loci in plant or animal genomes occurs at a very low level relative to non-homologous, random integration events. Negative selectable markers have been used to aid in the enrichment of transformed mammalian cells with homologous gene replacement events (Capecchi, Mario R. 1989, Science 244:1288-1292). Negative selections for plant cells may be useful in enriching for gene targeting events via homologous recombination during plant transformation. In the presence of the *Streptomyces griseolus* SU1 gene product, cytochrome P450, the non-phytotoxic sulfonlyurea R7402 is catabolized in an N-dealkylation reaction to form an herbicidal product (O'Keefe et al. 1992, Biochemical Society Transactions, 20:357-362). We have investigated the expression of the SU1 cytochrome P450 as a negative selection for transformation of plant protoplasts.

Protoplasts were isolated from wild type tobacco leaves, from stably transformed tobacco plants expressing the cytochrome P450 along with a kanamycin resistance marker and from plants expressing the kanamycin resistance marker with an alternate transgene marker. Protoplasts were mixed to simulate transformation situations and plated onto media containing Kanamycin, R7402 or both. DNA from calli recovered from these selections were assayed via PCR for the presence of transgene markers. We were able to select against P450 expressing cells using 1 -2 ppb R7402 applied at 1 week post protoplast isolation and recover non-P450 expressing, kanamycin resistant cells from the mix. Current efforts are aimed at confirming the utility of this negative selection protocol in tobacco protoplast transformation.

PROTECTION CONFERRED BY NATIVE AND CHIMERIC POTYVIRUS COAT PROTEINS EXPRESSED IN TRANSGENIC PLANTS. John Hammond and Kathryn K. Kamo, Florist and Nursery Crops Lab.,USDA-ARS, Beltsville Agricultural Research Center, Beltsville, MD 20705.

Nicotiana benthamiana plants transformed with a) the coat protein (CP) gene of bean yellow mosaic virus (BYMV), b) BYMV CP with 14 N-terminal amino acids from nopaline synthase (nos), and c) chimeric genes with the N-terminal domains of BYMV and C-terminal domains of a pepper isolate of potato virus Y (PVY) or of zucchini yellow mosaic virus (ZYMV), were challenged with BYMV and other potyviruses.

Some transformants of each construct were at least partially resistant to initial BYMV infection (BYMV and nos-BYMV CP) and/or replication (recovering from symptoms and detectable virus in upper growth).

Some BYMV/PVY chimera plants also recovered from PVY infection. One transformant each of the BYMV CP and the BYMV/PVY chimera also conferred some resistance to turnip mosaic virus (TuMV); some plants were not infected, some showed a delay in symptom expression, and some recovered from symptoms.

BYMV, PVY, TuMV, pepper mottle virus and tobacco etch virus were purified from transformants expressing various CP constructs. In each case the transgene CP was found to be incorporated into virus particles. The level of transgene CP incorporated was not correlated with the degree of resistance, suggesting that re-coating of incoming viral RNA is not an important mechanism of resistance to potyviruses.

Resistance to initial infection and to replication or transport appear to occur via distinct mechanisms that may be affected by tissue specificity of CP expression.

INTERACTION OF BIP WITH IMMATURE PROTEIN BODIES IN DEVELOPING ENDOSPERM: Rebecca S. Boston, Fan Zhang and Jeffrey W. Gillikin, Department of Botany, North Carolina State University, Raleigh, NC 27695

The maize homolog of the immunoglobulin binding protein, BiP, is a 75 kDa polypeptide that is overproduced in the endosperm of the seed-specific regulatory mutants, *floury-2* (*fl2*), *Mucronate* (*Mc*) and *Defective endosperm-B30* (*De*-B30*). Immunocytochemical localization studies of mutant endosperm tissue demonstrate that BiP is present in the ER as well as at the periphery of protein bodies, the major storage organelles of endosperm. In addition to harboring high levels of BiP, protein bodies in *fl2*, *Mc* and *De*-B30* mutants have altered morphology. The alteration of protein body morphology is accompanied by an abnormal distribution of storage proteins (α-, β-, and γ-zeins) within the protein bodies and qualitative differences in the electrophoretic mobility of zeins. The association of BiP with protein bodies can be disrupted with ATP but little release occurs with AMP-PNP (an ATP analog) suggesting a requirement for ATP hydrolysis. BiP can also be chemically cross-linked to protein bodies with disuccinimidyl tartarate (DST), demonstrating its close association with polypeptides within this storage organelle. The high levels of BiP, the abnormal spatial distribution of the zeins and the qualitative differences in zein electrophoretic migration suggest a normal cellular response to improper assembly of storage proteins. Similar increases in BiP can be induced in suspension cultures by treatment with tunicamycin or glucose depletion. These data, taken together with the ATP-dependent release data and cross-linking studies, support a role for BiP as a molecular chaperone in protein body formation.

DISTRIBUTION OF RHIZOBITOXINE IN SOYBEAN CULTIVARS NODULATED BY WILD-TYPE AND ISOGENIC BRADYRHIZOBIA DIFFERING IN TOXIN PRODUCTION: K. Xiong and J. J. Fuhrmann, Department of Plant and Soil Sciences, University of Delaware, Newark, Delaware, 19717-1303.

Certain strains of Bradyrhizobium japonicum produce the phytotoxin rhizobitoxine (RT) which causes a distinctive chlorosis of newly-formed leaves of susceptible soybean (Glycine max (L.) Merr.) genotypes. Although the chlorosis was first reported in the 1950s, the physiological and ecological significance of RT production is uncertain. Early work revealed that soybean genotypes differ in their susceptibility to RT^+ strains. However, it has not been resolved whether this variation results from differences in RT production by bradyrhizobia when associated with contrasting host genotypes, variation in RT metabolism among soybean cultivars, or a combination of these or other mechanisms. Therefore, we conducted greenhouse experiments to determine the response of six soybean cultivars to nodulation by five strains of bradyrhizobia: USDA 61 and USDA 94 (RT^+ wild-type strains), USDA 110 (RT^- wild-type strain), and two Tn5-induced mutants of USDA 61 exhibiting either absent (RX 18E) or enhanced (RX 17G) levels of RT production. Plants were grown in horticultural vermiculite, watered with an N-free nutrient solution, harvested approximately 45 days after planting, rated for RT symptoms, and analyzed for shoot, root, and nodule biomass and RT concentrations. Concentrations of RT were typically highest in the root nodules and lowest in root tissue and were affected by both the nodulating strain and associated host genotype. In general, foliar chlorosis ratings were better correlated with shoot RT concentrations than with nodular concentrations. No RT was detected for USDA 110 and RX 18E for any of the cultivars examined. The relative order of RT production among the RT^+ strains was generally USDA 94 > RX 17G > USDA 61. When nodulated by either USDA 61 or RX 17G, cultivars rated as RT-resistant (based on visual chlorosis rating) tended to have lower nodular RT concentrations than did plants rated as RT-sensitive. No such trend was evident for plants nodulated by USDA 94 which exhibited uniformly high RT concentrations in nodules. These results indicate that mechanisms of RT resistance in soybean may differ depending on the particular cultivar-strain combination under consideration and may include differences in RT production in nodules, RT export from the nodules, and RT degradation during and following transport to the shoot.

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