MOLECULAR STRATEGIES FOR CROP IMPROVEMENT

Organizers: Charles Arntzen, James Peacock and Marc Van Montagu

April 16-22, 1990

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Poster Sessions

Transformation of Recalcitrant Crops

R 001 TRANSGENIC RICE PLANTS

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Routine production of fertile transgenic rice plants has become possible by direct gene transfer by electroporation of protoplasts using the Hm^r gene as a selectable marker(1). Also, co-transformation has been established as a simple method to introduce nonselectable genes into rice.

To examine regulated expression of monocot genes in transgenic rice plants, the promoters of maize Adh-1 and wheat histone H3 were fused with the GUS gene, and these constructs were introduced into rice protoplasts. Analysis of cell-specificity in expression of these promoters in different tissues of transgenic plants by GUS histochemical assay indicated that these monocot promoters are correctly expressed in specific cell types of transgenic rice plants. As another example of monocot genes, maize transposons, Activator (Ac)/ Dissociation (Ds) were also introduced into rice plants and it was shown that Ac and Ds, when trans-activated by Ac, traspose in transgenic rice plants. These studies show that rice can be used as a model plant for studies in gene regulation of monocot genes, and that gene transfer can be applied for improvement of this important crop.

1. K.Shimamoto et al. 1989 Nature 338:274-276

Identifying New Genes: Insertion Mutants and Gene Tagging

R 002 REGULATORY GENES ISOLATED BY TRANSPOSON TAGGING AND CROSS-HYBRIDIZATION, Stephen L. Dellaporta, Thomas Brutnell, Gabriella Consonni, Jychian Chen, Alison DeLong, Roger Krueger, Giuseppe Gavazzi, Maria A. Moreno, Chiara Tonelli, Department of Biology, Yale University, New Haven, CT 06511 and Department of Genetics, University of Milan, Milan, Italy.

A non-targeted method for efficient transposon tagging and gene cloning has been developed for maize using the mobile element *Activator* (*Ac*). The strategy involves the ability to select kernel progeny in F1 populations containing single *Ac* elements transposed from particular donor sites. These transpositions events result in an array of both dominant and recessive mutant phenotypes. Molecular clones for several loci including *R*, *P*, and *tassel-seed* have been isolated by *Ac* insertional mutagenesis. Other related genes in both maize and other plant species have been isolated by cross-hybridization to these clones. We will report on the fine structure genetic and molecular analysis of these genes by sequential insertion mutagenesis with *Ac*, cDNA sequencing, and RNA expression studies.

R 003 POST-TRANSCRIPTIONAL REGULATION OF GENE EXPRESSION, Virginia Walbot, Peter Christie, Daniel Gallie, Kenneth Luehrsen, Juli Nash, and Letizia Pitto, Department of Biological Sciences, Stanford University, Stanford CA 94305-5020

Although the primary step in gene expression is transcription, the final level of protein production depends on transcript processing, mRNA export from the nucleus, the translatability of the message, and mRNA half-life. Our recent work concerning the role of mRNA leaders and 3'-untranslated regions will be described, as will the impact of stress treatments on mRNA function. Progress in elucidating the requirements for intron processing in maize will also be presented. In prior work (1) we established that the presence of an intron can stimulate gene expression, apparently at the post-transcriptional level. Additional data support this model.

1. Callis, J., M. Fromm and V. Walbot. 1987. Genes & Development 1: 1183.

Use of Positional Information in Identifying Agronomically Important Genes

R 004 PHYSICAL MAPPING OF THE *ARABIDOPSIS* GENOME AND ITS APPLICATIONS, Brian M. Hauge, Jerome Giraudat, Susan Hanley, Inwan Hwang, Takauki Kohchi and Howard M. Goodman. Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston MA.

We are engaged in a project to construct an integrated physical/RFLP map of the *Arabidopsis thaliana* genome. The first stage of the physical mapping project involves the linking of random clones using the "fingerprinting" strategy of Coulson et.al., (*Proc. Natn. Acad. Sci.* 83, 7821-, 1986). We have reached the practical limit of this approach by analyzing approximately 18,000 cosmid clones, thereby achieving a sampling redundancy of on the order of 10. The 18,000 clones fall into some 800 clusters (contigs) representing about 95% of the genome. We are currently undertaking several approaches to bridge the remaining gaps: multi-enzyme fingerprinting of end-clones to enhance statistical detection of overlaps; selecting linking clones by hybridization with end-probes; and using YACs as probes to bridge regions which are poorly cloned in cosmids. Our RFLP map which currently contains 153 markers, provides the contact points for alignment of the genetic and physical maps.

One of our major objectives for engaging in this project is to simplify the cloning of genes where only the locus and not the product of the gene is known. We are using the maps to clone genes involved in the synthesis and response to the hormones GA and ABA. For the two loci (*ga-2* and *abi-3*) which we have carefully examined, we are able to localize the genes to individual cosmids.

R 005 **USE OF POSITIONAL INFORMATION IN CLONING GENES INVOLVED IN MULTIGENIC TRAITS.** Tim Helentjaris & Mark Walton, Molecular Biology Group, Ceres/NPI, Salt Lake City, Utah 84108. Restriction Fragment Length Polymorphisms (RFLPs) have provided a powerful tool for the dissection of multigenic phenotypes into individual genetic loci. RFLPs allow researchers to determine how many major genes are involved in complex phenotypes, what is their relative impact, what is their gene action, and what is their approximate genomic location. Comparison of results from several studies suggests that for many of the most important agronomic traits, there exist major loci that contribute significantly and are relatively influential across both genotypes and environments. What is the potential for using this information to: 1) clone these loci and 2) subsequently improve these traits by genetic engineering? Current strategies are unable to rely solely upon positional information to clone loci of these types but combination of these results with other types of information should result in productive approaches. Once obtained, these clones should allow researchers to gain considerably more insight into the process of the culmination of several gene actions to create a complex phenotype. Results to date also suggest that significant impact on these phenotypes could be accomplished through altering one or small numbers of genes through genetic engineering.

R 006 MOLECULAR STRATEGIES FOR CROP IMPROVEMENT, USING GENETIC LINKAGE TO DNA MARKERS. Andrew H. Paterson*[1,7], Joseph W. DeVerna[2], John D. Hewitt[3], Daniel Zamir[4], Susan Damon[5], Eric S. Lander[6], and Steven D. Tanksley[7]. [1]E. I. duPont de Nemours, Wilmington, DE 19880 and Univ. Delaware, Newark, DE 19717; [2]Campbells Inst. Res. Tech., Davis, CA 95616; [3]Northrup-King Res., Gilroy, CA 95021; [4]Hebrew Univ. of Jerusalem, Rehovot, ISR 76-100; [5]U. C. Davis, Davis, CA 95616; [6]Whitehead Inst., Cambridge MA 02142; and [7]Cornell University, Ithaca, NY 14850.

DNA markers provide a medium for information exchange between classical plant breeding and molecular biology, in an integrated approach to crop improvement. Many agriculturally important traits are influenced by numerous genes, with unknown products, and requiring field assays which are subject to environmental fluctuations. Genetic complexity and environmental variation hinder the rate of gain possible through classical plant breeding. Further, lack of known gene products or simple assays for gene function hinder cloning and study of the genetic factors influencing complex traits. Using DNA markers at 10-20 cM intervals, phenotypic variation in complex traits can be assigned to particular chromosomal regions of an organism, called 'quantitative trait loci' (QTLs). By studying recombinants within these chromosomal regions, QTLs might be mapped to genomic regions of 1 cM or less, which could conceivably be cloned intact. Ultimately, identification of genes associated with quantitative differences in agriculturally important traits may permit classical plant breeding to utilize the tools of molecular biology, toward improvement of quantitative traits. These objectives may be facilitated by the observation that QTLs appear to occur at some common locations in different species. Information transfer between plant breeding and molecular biology, through DNA markers, may create new "integrated strategies" for crop improvement.

Crop Improvement to Overcome Production Limitations

R 007 NOVEL APPROACHES TO CONFER RESISTANCE TO VIRUS DISEASES IN TRANSGENIC PLANTS, Roger N. Beachy[1], Gregg Clark[1], Ali Nejidat[1], C. Michael Deom[1], Ron Gafny[1], Patricia Moore[1], Schmuel Wolf[2], and William B. Lucas[2], [1]Department of Biology, Washington University, St. Louis, MO 63130; [2]Botany Department, University of California, Davis, CA 95616.

In the past 7-8 years considerable effort has been expended to produce transgenic plants that resist virus infection and disease development. While limited success was achieved by chimeric genes that express RNA sequences complementary to viral RNA (i.e., antisense RNA), expression of genes which encode viral capsid proteins has been highly effective. Thus far "coat protein (CP) mediated protection" has been applied to provide resistance to eight different viruses in a number of different plants and is expected to be generally applicable to control plant virus diseases.

Although CP mediated resistance is widely used, the molecular and cellular basis for resistance is not well understood. We have continued to explore the nature of resistance to the tobamoviruses and potyviruses using both transgenic plants and novel transient gene expression approaches. The status of this research will be summarized.

We have also initiated research to characterize the nature of the interaction(s) of the 30 kDa "Movement Protein" of TMV with susceptible and resistant tobacco cultivars. The TMV MP is responsible for modifying the function of plasmodesmata in susceptible tobacco cultivars, thereby enabling the infectious agent to move from cell-to-cell and throughout the plant. Infection in resistant tobacco cultivars is limited to the necrotic local lesion and surrounding areas. Independent assays indicated that the MP does not modify the plasmodesmata to the same extent in the resistant host except under specifically defined conditions. We propose that the inability of the MP to modify plasmodesmata is due to the product of the resistance gene. Furthermore, it may be possible to identify the resistance gene based upon the characteristics and function of the MP. The results of experiments to define the role of the MP and the resistance gene will be reported.

R 008 PROGRESS IN THE DEVELOPMENT OF INSECT RESISTANT CROPS, D.A. Fischhoff, F.J. Perlak, R.L. Fuchs, S.C. MacIntosh, W. R. Deaton, X. Delannay and S.R. Sims, Monsanto Agricultural Company, St. Louis, MO 63198. The development of genetically engineered insect resistant crop plants has been a major goal of agricultural biotechnology. Our primary approach to achieve this goal has been the expression in plants of the insecticidal proteins of *Bacillus thuringiensis* (*B.t.*). These proteins are ideal candidates for genetic improvement of crops because they are potent single protein insecticides with a high degree of specificity for several agronomically important insects, and these proteins have no activity against non-target organisms. Early work demonstrated that lepidopteran-active *B.t.* genes could be expressed in plants at insecticidal levels if the genes were truncated to encode only the active N-terminal half of the protein. Tomato plants containing these truncated genes have been field tested in multiple locations for three years, and they have shown excellent control of relatively sensitive pests such as tobacco hornworm (*Manduca sexta*) and promising, but incomplete, control of less sensitive insects such as tomato fruitworm (*Heliothis zea*). These plants showed essentially complete control of tomato pinworm (*Keiferia lycopersicella*), an insect that is extremely difficult to control with chemical insecticides or with microbial preparations of *B.t.* A major hurdle in achieving commercially viable control of less sensitive lepidopterans which are major pests in many crops has been the increase of the insecticidal potency of the transgenic plants. Biochemical approaches to this problem have included the isolation of genes encoding proteins with higher intrinsic specific activities against target pests and the discovery of enhancers of *B.t.* activity. Serine protease inhibitors coexpressed at low levels with *B.t.* proteins enhance the insecticidal activity of the plants by three to ten fold. The most significant increase in efficacy has come through increased plant expression of *B.t.* genes. This has been achieved most dramatically through the specific modification of the *B.t.* coding sequence. Increases in *B.t.* protein expression of up to 500 fold, up to 0.1% of total soluble protein, have been achieved with modified coding sequences in tomato, tobacco and cotton. Cotton plants expressing such levels of *B.t.* protein show a high degree of protection from boll damage by *H. zea* in greenhouse tests performed under conditions of high insect pressure. Similar increases in plant expression have been achieved with modified coding sequences for the coleopteran-active *B.t.* gene in tomato and potato.

R 009 ENGINEERED MALE STERILITY OF CROPS, C. Mariani, M. De Beuckeleer, W. De Greef, V. Gosselé, R.B. Goldberg*, J. Leemans, Plant Genetic Systems N.V., J. Plateaustraat 22, 9000 Ghent, Belgium, *UCLA, Dept. of Biology, Los Angeles, CA 90024

We will present the engineering of a dominant nuclear gene encoding male sterility.

A number of genes have been characterized that are expressed exclusively during early anther development of tobacco. Their expression is limited to the tapetum cells. We have used the 5' regulatory region of such a gene to target the expression of a ribonuclease to the tapetum cells. This results in a male sterile phenotype. This nuclear male sterility gene has been introduced into several crops (Solanaceae and Brassicaecae), linked to the herbicide resistance gene bar.

We will discuss how such male sterility can be used for efficient hybrid seed production.

Designing Crops for Resistance to Environmental Stress

R 011 SALT TOLERANCE - EXPRESSION OF A DEVELOPMENTAL PROGRAM, Hans J. Bohnert, John C. Cushman, E. Jay DeRocher, Gabriele Meyer and Daniel M. Vernon, Department of Biochemistry, The University of Arizona, Tucson, AZ 85721.

Many plants which tolerate drought and high salt have evolved the ability to express a limited number of biochemical pathways that confer advantages under such stresses. One of these pathways, Crassulacean Acid Metabolism (CAM), is found in several plant families in individual species which usually inhabit arid hot climates. Our plant model, the common ice plant, can be induced to express CAM in response to environmental stresses. Stress perception leads to a number of physiological changes. At least part of the adaptive response chain occurs at the level of gene transcription, which may be regulated by common stress response elements and proteins. The expression of CAM, which is a long-term process, must, however, be preceeded or be concomitant with the expression of salt tolerance for the plants to survive. Several aspects of the whole plant response to salt stress are being studied: (1) We measured the complexity of the response by direct genomic screening of changes in gene expression. From the frequency of changes observed by screening a representative percentage of the low copy number sequences of the ice plant genome (390,000 Kbp) we estimate that, as the plants experience salt stress, the transcription of roughly 100 genes is enhanced or induced, while transcription of approximately the same number of genes is repressed. (2) We are in the process of identifying genes that are differentially expressed at different times after salt stress, and we have isolated transcripts which appear to accumulate faster than mRNAs for known CAM enzymes. (3) The stress-enhanced expression of one important CAM enzyme, PEPcase, has been examined in-depth. Part of the increase in this enzyme is due to enhanced transcription of one gene. Studies of PEPcase expression have shown that the ability to respond to stress is a function of plant maturation. Systems for both transcriptional activation of genes and systems adjusting mRNA half-life and translation have been shown to change over development. This work is supported by USDA, NSF, and Arizona Agricultural Experiment Station.

R 012 IDENTIFYING GENES FOR DROUGHT TOLERANCE, John E. Mullet, Robert Creelman, Hugh Mason, Jennifer Jones and Felix Guerrero, Texas A&M University, College Station, Texas 77843-2128

Plants employ a diverse set of strategies to deal with dry environments and periods of water deficit. These range from alterations in life cycles to escape drought, to changes in morphology which help avoid severe water deficit (*i.e.*, thick cuticle, deep roots, vascular and stomatal systems which meter water and improve water use efficiency). We are attempting to identify the genetic basis of some of these traits in sorghum using RFLPs. Plants also adjust to intermittent water deficit by increasing root to shoot ratios, altering levels of plant growth regulators such as ABA and through osmotic adjustment. Furthermore, in response to severe dessication, specific proteins accumulate which may confer dessication tolerance (*i.e.*, Close *et al.*, 1989; Pla *et al.*, 1989; Dure *et al.*, 1989; Mundy and Chua, 1988). Our lab has identified several genes which are rapidly induced in plants exposed to mild water deficit. One of these genes encodes a protein homologous to cysteine proteases and a second gene encodes a protein homologous to aldehyde dehydrogenases. We have also studied the molecular basis of differential stem vs. root growth inhibition in soybean seedlings exposed to -0.3 MPa soil. Stem growth inhibition is accompanied by decreased polysomes, increased ABA and changes in poly(A)RNA populations. The role of ABA in the differential growth response will be discussed.

R 013 TRANSFORMATION OF THE CRYOBEHAVIOR OF THE PLASMA MEMBRANE BY COLD ACCLIMATION, Peter L. Steponkus, Department of Agronomy, Cornell University, Ithaca, NY 14853

Disruption of the plasma membrane is a primary cause of freezing injury in winter cereals; and under conditions that preclude intracellular ice formation, destabilization of the plasma membrane is a consequence of freeze-induced osmotic stresses and cell dehydration. However, the mechanism of injury depends on the magnitude of the osmotic stress and the extent of cell dehydration. When suspensions of protoplasts isolated from non-acclimated leaves of winter rye (*Secale cereale* L. cv. Puma) are frozen to temperatures over the range of 0 to -5°C, the osmolality of the unfrozen portion of the suspending medium increases from 0.5 to 2.7 and osmotic contraction results in endocytotic vesiculation of the plasma membrane. Sufficiently large area reductions are irreversible, and the protoplasts lyse during osmotic expansion following thawing of the suspending medium before regaining their initial volume/surface area. When frozen to -10°C, the osmolality of the unfrozen portion of the suspending medium is 5.37 (with an osmotic potential of -12MPa), and >90% of the osmotically active water is removed from the protoplasts. Under these conditions the semipermeable characteristics of the plasma membrane are disrupted such that the protoplasts are osmotically unresponsive during melting of the suspending medium. This form of injury is a consequence of several alterations in the ultrastructure of the plasma membrane, including the formation of aparticulate domains and lamellar-to-hexagonal$_{II}$ phase transitions in the plasma membrane and subtending lamellae. These changes, which are manifestations of demixing of the membrane components, are predicted by a theory of bilayer interactions at low hydration levels. In excess water, the close approach of adjacent bilayers is minimized by strongly repulsive hydration forces. However, osmotic stresses of sufficient magnitude result in the removal of water from adjacent bilayers. Upon close approach, demixing of the membrane components and lamellar-to-H_{II} phase transitions are possible.

Cold acclimation increases the tolerance of the plasma membrane to osmotic excursions and decreases the propensity for dehydration-induced lamellar-to-H_{II} phase transitions. In protoplasts isolated from cold-acclimated rye leaves, freeze-induced osmotic contraction results in the formation of exocytotic extrusions of the plasma membrane, which is readily reversible, and the protoplasts regain their initial volume/surface area when returned to isotonic conditions. Also, dehydration-induced lamellar-to-H_{II} phase transitions are not observed after severe dehydration. In both cases, the differential cryobehavior is a consequence of alterations in the lipid composition of the plasma membrane - as evidenced by the fact that liposomes prepared from plasma membrane lipid extracts of non-acclimated and cold-acclimated rye leaves also exhibit the same differential behavior. The plasma membrane lipid composition of non-acclimated and cold-acclimated leaves differs only in the proportions of the various lipid species without any species unique to either. Thus, the differential cryobehavior of the plasma membrane is a consequence of altered lipid-lipid interactions resulting from the altered proportions of the different lipid species. However, although the plasma membrane contains >120 different molecular species of lipids, apparently only an increase in the proportion of the di-unsaturated species of phosphatidylcholine is responsible for the differential cryobehavior. Selective enrichment of the plasma membrane of non-acclimated protoplasts with dilinoleoylphosphatidylcholine by a protoplast-liposome fusion procedure transforms the cryobehavior of the plasma membrane such that exocytotic extrusions are formed during osmotic contraction and lamellar-to-H_{II} phase transitions do not occur under conditions of severe dehydration.

Designing Crops for Biological Stress Resistances

R 014 MOLECULAR RESPONSE TO MICROBIAL ATTACK, Christopher J. Lamb, Desmond J. Bradley, Yonatan Elkind, Jan Kooter, Michael A. Lawton, and Richard A. Dixon*
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Induction of phytoalexins, lytic enzymes and wall reinforcement involves transcriptional activation of defense genes, in some cases within 2-3 minutes of an elicitation signal. Rapid response genes include those encoding chitinase and phenylpropanoid biosynthetic enzymes for production of phytoalexins and lignin monomers. Genes encoding cell wall hydroxyproline-rich glycoproteins are induced more slowly and at a distance from the initial perturbation, in response to several distinct endogenous intercellular stress signals. Recent studies to characterize *cis*-acting nucleotide sequences and *trans*-acting factors involved in these complex patterns of defense gene activation will be described.

Many defense genes undergo developmental as well as environmental regulation and recent findings on the tissue- and cell-type-specific expression of defense genes and the molecular mechanisms underlying the interplay between developmental programs and environmental stimuli will be considered. Moreover, expression of a bean phenylalanine ammonia-lyase (PAL) gene in transgenic tobacco under the control of a chimeric CaMV 35S - PAL promoter causes abnormal development. The phenotypes involve both inhibition of the synthesis of bulk phenylpropanoid products such as lignin and flavonoid pigments and also hormonal-like effects suggesting dysfunction of novel signal systems based on phenylpropanoid compounds.

We have recently observed the apparent disappearance of two specific cell wall structural proteins in response to fungal elicitor or glutathione. This response, which reflects the insolubilization of these major wall proteins by H_2O_2-mediated oxidative cross-linking, is initiated within 1 to 2 minutes of elicitor addition, and complete within 5 minutes. We propose that this protein cross-linking, which presumably toughens the cell wall, is a novel, ultra-rapid defense mechanism.

R 015 MOLECULAR MARKERS AND THE ANALYSIS OF LETTUCE DOWNY MILDEW. Richard W. Michelmore, Department of Vegetable Crops, University of California, Davis, CA 95616.

The genetic basis of specificity in the gene-for-gene interaction between lettuce (*Lactuca sativa*) and its obligate, fungal parasite, *Bremia lactucae* are being determined utilizing parallel studies of host and pathogen. Detailed genetic maps are being developed for both organisms utilizing restriction fragment length polymorphism (RFLP) markers to locate disease resistance genes (*Dm*) in *L. sativa* and matching avirulence genes (*Avr*) in *B. lactucae*. At least 13 dominant *Dm* genes are clustered in four linkage groups while the *Avr* genes are not tightly linked. Over 170 markers have been mapped in *L. sativa* and over 90 in *B. lactucae*. Both organisms seem to have at least a total of 2000cM in their genomes. Genes for resistance to several other pathogens are also being mapped relative to the clusters of *Dm* genes. Maps generated from several crosses are being compared to identify changes in chromosome structure and frequencies of recombination. RFLP markers are also being used to analyze levels of variation in different regions of the genome. Regions containing clusters of *Dm* genes now are being saturated with markers by a variety of techniques including the use of near-isogenic lines and preparative pulsed-field gel electrophoresis. Long-range restriction maps are being generated by pulsed-field gel electrophoresis to determine the relationships between physical and genetic distance near to *Dm* genes as a prerequisite for chromosome walking. Ultimately, *Dm* and *Avr* genes will be cloned by chromosome walking from linked RFLP markers using a variety of genomic libraries, including yeast artificial chromosome libraries.

R 016 GENE-FOR-GENE INTERACTIONS SPECIFYING DISEASE RESISTANCE IN *ARABIDOPSIS-PSEUDOMONAS* INTERACTIONS. Maureen Whalen, Roger Innes, Andrew Bent and Brian Staskawicz. Department of Plant Pathology, 147 Hilgard Hall, University of California, Berkeley, CA. 94720.

We are currently developing *Arabidopsis thaliana* as a model host plant to study the molecular basis of specificity and disease resistance in response to the bacterial pathogens *Pseudomonas syringae* pv. *maculicola* and pv. *tomato*. A collection of diverse geographical strains of *P.s.*pv. *maculicola* and pv. *tomato* were inoculated into over thirty ecotypes of *Arabidopsis thaliana* and six strains were identified that were virulent pathogens on most ecotypes of *Arabidopsis*. Initial results also suggest that natural variation may exist in *Arabidopsis* for resistance to the various strains tested. We have also successfully cloned and identified several putative avirulence genes from two avirulent strains of *P.s.* pv. *tomato*. This was accomplished by constructing genomic cosmid libraries of DNA from the *P.s.tomato* avirulent strains 1065 and T1 and conjugating cosmid clones into the virulent DC3000 strain. The exconjugants were inoculated into the Col-O ecotype and scored for avirulence. We are currently screening a collection of *Arabidopsis* ecotypes for ones on which the exconjugants containing the avirulence gene remain virulent. Our initial screen has identified several potential candidates. These ecotypes are currently being crossed with the resistant ecotype Col-O and segregating progeny will be scored for inheritance of resistance. If this proves successful we plan to map the genetic position of this locus as a prelude to genomic walking and/or transposon tagging.

Plant Quality Improvement: Metabolism in Oil Crops

R 017 STRATEGY OF SEED OIL IMPROVEMENT AND MOLECULAR BIOLOGY OF OIL BODIES, Anthony Huang, Dept. of Botany and Plant Sciences, University of California, Riverside, CA 92521

The current status of seed oil improvement via breeding and genetic engineering will be outlined. The metabolism of oils in seeds during seed maturation and germination will be described in terms of the potential and limitation in altering the metabolic pathway by classical breeding and modern technology. Examples of ongoing projects on seed oil improvement via genetic engineering will be discussed.

The second part of the lecture will deal with the cell biology of oil bodies in seeds during seed maturation and germination. Also, the molecular biology of the abundant proteins (oleosins) localized on the surface of the oil bodies in diverse seeds will be described.

R 018 REPROGRAMMING LEVELS OF FATTY ACID SYNTHESIS ENZYMES IN DEVELOPING EMBRYOS OF RAPESEED, Vic C. Knauf, Jean C. Kridl, Donna E. Scherer, Deborah S. Knutzon, Sharon E. Radke, Brian Sugimoto, Susan Foxall-Van Aken and Gregory A. Thompson, Calgene, Inc., 1920 Fifth St., Davis, CA 95616. Although additional elongation steps may take place in the endoplasmic reticulum, the primary site of fatty acid biosynthesis (FAS) in developing embryos of rapeseed is in the proplastid organelle. At least 11 proteins directly involved in FAS appear to be soluble and encoded by separate nuclear genes. Our interests include 1) the initial steps of committment of acetyl-CoA to FAS and the condensation of malonyl-ACP with growing acyl chains, and 2) latter steps which direct palmityl-ACP to proplastid lipid synthesis, to triglyceride synthesis, or further modification to stearoyl-ACP and oleoyl-ACP. Our progress in the modulation of specific enzyme levels in transgenic plant tissue by using cloned genes in conjunction with embryo-specific promoters in sense and antisense configurations will be described.

R 019 ACYL CARRIER PROTEIN AS A PROBE OF THE ORGANIZATION AND REGULATION OF PLANT FATTY ACID SYNTHESIS, John B. Ohlrogge[1], Alenka Hlousek-Radojcic[1], Jan G. Jaworski[2], Martha A. Post-Beittenmiller[1], and Katherine M. Schmid[1]. 1. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824; 2. Department of Chemistry, Miami University, Oxford, OH 45056.

Acyl chains are attached to acyl carrier protein (ACP) as thioesters during plastid fatty acid synthesis (FAS), Δ9-desaturation and transfer to glycerol-3-phosphate. Our lab has been investigating several aspects of ACP biochemistry and molecular biology. 1) Plants have multiple genes for ACP which encode distinct isoforms that differ in their amino acid sequence (≈70 % identity), organ-specific expression, and Km values with enzymes of plant fatty acid metabolism. We have found that both spinach and *Arabidopsis* possess ACP genes which are expressed in all organs examined (seeds, leaves, and roots) and additional ACP genes which are expressed primarily in leaves. 2) We have transformed tobacco with a spinach ACP-I gene behind the SSU promoter and this has resulted in a two to four fold increase in ACP concentration in tobacco leaves. Significant levels of spinach acyl-ACP-I were detected demonstrating that the spinach protein is biologically active in tobacco FAS. Lipid analysis of the transformed plants indicated that the increased ACP levels caused no significant alterations in leaf lipid biosynthesis. 3) In an effort to identify rate limiting steps in FAS, we have developed methods to separate and identify pools of acyl-ACP substrates, intermediates and products of the FAS pathway. Pools of malonyl-ACP were found to be very low suggesting that steps leading to its formation may determine the rate of plant fatty acid synthesis.

R 020 THE DEVELOPMENT OF NEW SPECIALTY OILS BY GENETIC ENGINEERING. Chris Somerville, John Shanklin, Bertrand Lemieux, Sue Gibson, Ellen Kearns, Frank Van de Loo and Ted Underhill[¶], MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824 and [¶]NRC Plant Biotechnology Institute, 110 Gymnasium Road, Saskatoon, Saskatchewan, Canada.

The field crops which are commonly grown in North America for production of vegetable oil, produce oil which is of less than ideal composition for some food and non-food applications. Conventional breeding approaches coupled in some cases with mutagenesis has resulted in some very valuable modifications of oil quality in a number of crop species. However, on the basis of our understanding of plant lipid metabolism, there are certain desirable qualities which are considered unlikely to be achieved by conventional breeding. In some cases it may be possible to achieve these desired modifications by introducing cloned genes for key enzymes involved in fatty acid synthesis or modification into crop species. A related goal is the production of new specialty crops which produce oil with novel qualities. For example, castor oil has many non-food industrial uses and is significantly more valuable than sunflower or soybean oil. The essential difference between the major constituent of castor oil and that produced by certain varieties of sunflower is the presence of a single hydroxyl group on the fatty acid. It may, therefore, be worthwhile to genetically engineer a species like sunflower so that it produces an oil equivalent to castor oil. Similarly, there are numerous other opportunities to transfer genes involved in fatty acid modification from species with poor agronomic properties to species which have been adapted to North American agricultural practices. Some of the problems associated with achieving these goals will be discussed. In addition, progress in the use of mutants of *Arabidopsis* for identifying many of the genes which regulate fatty acid desaturation and elongation will be presented.

Gene Regulation in Transgenic Plants

R 021 GENES THAT REGULATE THE PROGRESSION OF THE CELL CYCLE IN HIGHER PLANTS, Michael A. Lawton, Salk Institute for Biological Studies, La Jolla CA 92037.

The machinery governing the progression of the cell division cycle is highly conserved in eukaryotes. In fission yeast commitment to cell division is dependent upon the activity of the p34 protein kinase which is encoded by the *cdc2* gene. During each cell cycle p34 associates with cyclins, which are synthesized *de novo* during each progressive cycle, and with a second protein, corresponding to the product of the *suc1* gene, to form the maturation promoting factor (MPF). In yeast, the activity of MPF is regulated by the opposing actions of the *cdc25* and *wee1* genes, while studies in *Drosophila* have implicated a *cdc25* homolog called *string* in the regulation of asynchronous cell division during embryogenesis. As a first step in understanding how the spatial and temporal pattern of cell division is regulated in plants we have isolated molecular clones encoding homologs of the *cdc2* protein kinase. Since this gene product forms the heart of the regulatory engine governing cell division it represents a likely target for the action of plant growth regulators. We are also attempting to isolate homologs for the *cdc25* gene as these may play an important role in regulating the developmental expression of plant p34 activity.

Regulatory and Public Perception Issues Related to Agricultural Biotechnology

R 022 **SCIENTISTS DEBATE IN THE PUBLIC ARENA OR WE NEVER GET TO PLAY IN OUR HOME COURT!** Jerry Caulder, Mycogen Corporation, 5451 Oberlin Drive, San Diego, CA 92121. The issue of how to allow science and its related technology the freedom necessary to be developed to their fullest potential for the common good, while at the same time assuring a skeptical public that their safety is being looked after, is certainly not a new one. The contributions that commercial biotechnology can make to agriculture and a host of other areas are unquestionably enormous. Yet the public's understanding of the underlying science and its grasp of biotechnology's capabilities and limitations seems not to have kept pace with the industry's development. In an era when science and technology, and their practitioners, are both admired and mistrusted, this neglect augurs poorly for the public policy decisions now at the forefront. The scientist, when debating the pseudoscientist, is in a Catch 22 situation -- the scientist can never be certain and yet the pseudoscientist is never uncertain. This allows the pseudoscientist to force the scientist into a position of absolutes or into proving a negative. Many organizations and individuals have sought to latch onto scientific evidence to defend their attempts in recent years to rationalize a system of societal controls. Yet science cannot provide sufficient certainty to dispel peoples' beliefs and probably will not be able to do so for many decades. Those who are concerned about correcting widespread misperceptions about biotechnology should begin by stressing three fundamental facts: (1) Today's biotechnology is only one point on a long continuum of scientific inquiry, (2) The techniques of biotechnology are largely extensions of processes that occur continually in nature; and (3) Biotechnology does not describe a single procedure or process but encompasses a diversity of means for using living matter to develop useful products. Scientists with an informed media must demystify biotechnology for the public at large to ensure that the public accepts it. It is crucial that scientists and informed lay people emphasize to the public that genetic engineering is as natural as plant breeding. Without public acceptance, support and encouragement, the applications of biotechnology to agriculture may be regulated out of existence.

R 023 REGULATORY AND PUBLIC PERCEPTION ISSUES RELATED TO AGRICULTURAL BIOTECHNOLOGY, Dr. Robert T. Fraley, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

The field testing of genetically modified plants containing foreign genes by several research groups over the last two years represents an important step in the commercialization of plant biotechnology research. The "success" of such field tests, while quite important, has tended to overshadow other key needs in the commercialization process. The presentations in this session will overview the R&D process and also focus on issues such as 1) proposed registration requirements for genetically modified plants, 2) proprietary protection needs and concerns for genetically modified plants, and 3) communicating to the public about the benefits and issues surrounding agricultural biotechnology products. Technical success is only a small part of the challenge facing the future of agricultural biotechnology. Balanced, science-based regulatory requirements and adequate proprietary protection to encourage R&D are critical needs. Establishing public confidence through open and effective communications and dealing effectively with misperceptions may be the greatest challenge we face. For the first time, the UCLA conference will include a session which addresses these important issues. This should represent an excellent opportunity to gain an in depth understanding of these complex issues and to discuss critical issues with experts in each of these areas.

R 024 IFBC STRATEGIES FOR ASSURING THE SAFETY OF FOODS PRODUCED BY GENETIC MODIFICATION, R.L. Hall, International Food Biotechnology Council, Washington, D.C. 20036. The IFBC has proposed a series of procedures and criteria for evaluating and assuring the safety of food plants and microorganisms produced by genetic modification. These rely primarily upon data drawn from three sources: 1) knowledge of, and confidence in, the genetic background of a product and the procedures of genetic modification employed in its development, 2) data on composition of the product, and 3) toxicological evaluation based on testing in animals. While demanding in total, the proposed system is flexible, and permits extensive trade-offs among the three data sources. The paper will describe the power and limitations of each source and general guidelines for allowing informed judgment to choose the reliance to be placed on each.

R 025 RIGHTS IN BIOTECHNOLOGICAL DEVELOPMENTS, Donald J. Quigg, Neuman, Williams, Anderson & Olson, Crystal Plaza One - Suite 903, 2001 Jefferson Davis Highway, Arlington, Virginia 22202.

What rights pertain to biotechnological developments? To Animal rights! To Plant Rights! To User Rights! To Inventors' Rights! Obviously, some rights overlap other rights. How much should we or can we afford to vacillate in that consideration?

According to the Constitution, Article 1, Section 8, it was apparently the intention of the founding fathers that the old adage should apply to inventors: "To Each His Own!" The Constitution clearly states "The Congress shall have the power ... To promote the Progress of Science ..., by securing for limited Times to ... Inventors the exclusive Right to their respective ... Discoveries.

We will try to differentiate between the various rights and try to evaluate their strengths and weaknesses. The paper will address the definition of a patent and the right it conveys. It will address the stepwise approach that Congress and the Courts have taken in addressing protection of increasingly technical subject matter.

This year marks the bicentennial year of the patent system in the United States. The system has served us well. Should we whittle away at its underpinnings based on allegations that it is depriving those indigents who need the inventions and that exclusive patentees are getting rich? Who will give the inventor the incentive to risk capital if the exclusiveness is removed? Think carefully about the "dog, his bone and the reflecting brook."

Plant Quality Improvement: Grain and Tuber Development

R 026 IMPROVEMENT OF WHEAT GRAIN QUALITY, Richard Flavell, John Innes Institute, Colney Lane, Norwich, Norfolk, NR4 7UH, United Kingdom.

Wheat seeds are used as an animal feedstuff and in a wide range of products in the human food industry. Most research has focused on breadmaking quality because it has been shown that good quality is dependent upon a minimum percentage weight of protein and the particular composition of the protein. Genetic and biochemical analyses of the complex array of seed storage proteins have classified them into groups encoded by complex genetic loci. The protein complex particularly responsible for the all-important visco-elastic property of dough is made from the high molecular weight (HMW) and low molecular weight (LMW) glutenins. The former are encoded by three loci with two copies of the gene at each locus. Allelic variation at each of the loci have been exploited in plant breeding programmes. The sequences of proteins from two particular alleles that confer very different breadmaking qualities differ by twelve amino acids [1]. The two proteins differ in conformation. The conformational differences are due to amino acid differences in the 'C' terminal segment of the protein [2]. These and other results suggest how an "ideal" HMW glutenin might be designed to produce a stronger protein matrix in dough useful to the human food and other industries.

To insert new alleles by genetic engineering demands a transformation procedure and the regeneration of whole plants. Recent progress on this for wheat will be described. Insertion of the wheat genes into tobacco and analysis of gene expression has revealed a single short region upstream from the transcription start site that determines endosperm-specific expression of the gene in tobacco and presumably wheat seeds. Inclusion of such a segment of DNA should ensure appropriate expression of an introduced gene. Analyses of natural wheat variants differing in the number of copies of HMW glutenin genes suggests that an increase in the number of HMW glutenin genes can improve dough strength. There is therefore reason to believe that addition of extra active genes of the right structure by transformation into wheat will modify seed structure and dough quality.

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[2] Flavell, R.B., Goldsbrough, A.P., Robert, L., Schnick, D. and Thompson, R.D. (1989). Genetic variation in wheat HMW glutenin subunits and the molecular basis of breadmaking quality. Bio/Technology, in press.

R 027 MOLECULAR AND GENETIC ASPECTS OF GENES IMPORTANT IN STARCH BIOSYNTHESIS, L. C. Hannah, J. Bae, J. Baier, M. Bhave, M. Clancy, M. Giroux, L. Ingham, and J. Shaw, Vegetable Crops Department, University of Florida, Gainesville, FL 32611. Starch synthetic genes of plants not only control a constituent of the plant which is important agriculturally, their analysis has contributed to the understanding of gene structure, regulation and evolution. For example, two genes in maize encode sucrose synthase, an enzyme whose physiological function in the endosperm is the catabolism of sucrose for starch synthesis. Although the two genes are expressed differently, comparison of their sequences strongly suggests that they arose via a gene duplication followed by divergence. Exonic but no intronic sequences show much similarity. The last intron of shrunken-1 (Sh1), the gene expressed predominantly in the endosperm does not have a counterpart in the second gene. Sequence analysis suggests that this intron arose via an internal duplication within the gene. Incorporation of the large first intron of Sh1 into 35S promoter - CAT constructs leads to a 10 to 100 fold increase in CAT activity in transient assays involving protoplasts of monocots. The physiological significance of this to Sh1 expression in the endosperm remains to be elucidated.

A step occurring later in the path to starch, ADP-glucose pyrophosphorylase, is under the control of two genes, shrunken-2 (Sh2) and brittle-2 (Bt2). We have recently cloned and sequenced these genes. It would appear that each of these complementary genes encodes a subunit for this enzyme and that the genes probably arose via a gene duplication. Many of the spontaneous Sh2 mutations and one transposable element-induced mutant appear to alter the splicing of the mature transcript. Expression of Sh2 and Bt2 is coordinated in that mutation at either gene leads to elevated steady-state transcript levels of the other gene. Furthermore, mutation in another enzyme step late in starch synthesis also increases Sh2 and Bt2 transcripts.

R 028 MANIPULATION OF STARCH IN POTATOES BY NEW MUTANTS AND ANTISENSE RNA.

Richard G.F. Visser, Will J. Feenstra* and Evert Jacobsen, Department of Plant Breeding, Agricultural University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands and *Department of Genetics, University of Groningen, Biological Centre, Kerklaan 30, 9751 NN Haren, The Netherlands.

Starch is the major storage carbohydrate in potato and consists of two components, amylose and amylopectin. Starch synthases and branching enzymes are directly involved in the formation of amylose and amylopectin, respectively.
Starch as such or in modified form is widely used in the food, paper and textile industries. The properties of starches from different sources vary considerably and the chemical and physical characteristics are typical to the biological origin. Manipulation of the activities of the different starch synthases and branching enzymes can yield starches in which not only the amylose/amylopectin ratio is altered, but also total starch content is changed.
In potato, we have tried to alter starch composition by two approaches; mutation induction and genetical engineering by using the so-called antisense technique.
A mutant was obtained (2n=1x=12) after a mutagenic treatment of monoploid leaf tissue which was defective for one of the starch synthases and therefore lacked amylose. Although this recessive amylose-free (amf) mutant was obtained it proved not possible to obtain a double mutant, in this monoploid amf background, lacking both starch synthase and branching enzyme activity.
The other approach, using antisense genes of granule bound starch synthase, yielded different classes of transgenic plants with varying synthase activities and amylose content.
The main problem in potato is that it is a vegetatively propagated polyploid crop, which makes selection and breeding of recessive mutants, such as the amf mutant, difficult and time-consuming. The antisense technique would circumvent all these problems since an antisence gene acts as a dominant suppressor gene thus making it possible to mimic mutant phenotypes directly in tetraploid varieties.
Results regarding both approaches will be presented and (dis)advantages of both approaches will be discussed.

Plant Quality Improvement: Post-Harvest Physiology Traits

R 029 THE ENZYMATIC POLYMERIZATION OF ISOPENTENYL-PP INTO ISOPRENOID RUBBER: STRATEGIES FOR INCREASING SYNTHESIS.

C.R. Benedict, G.A. Greenblatt and K.V. Venkatachalam, Department of Biochemistry & Biophysics, Texas A&M University, College Station, TX 77843

Rubber transferase is a new enzyme bound to rubber particles from Parthenium argentatum which catalyzes the formation of ^{14}C-polyisoprene from IPP and an allylic-PP initiator. Chromatography of the ^{14}C-polyisoprene on linear columns of 1x10^{6}Å to 500Å Ultrastyragel shows it is composed of different size rubber chains with a weight average mol wt of 1x10^6. The enzyme catalyzes chain initiation and the polymerization of 1000's of IPP monomers into a high mol wt rubber polymer. The transferase has been solubilized from the particles and purified. The purified preparation is composed primarily of a 52kD component which binds to Concanavalin A on lectin blots. ^{14}C-Photolabile substrate analogs are being used to identify the 52kD component as bound rubber polymerase. The amino acid sequences of CNBr fragments of the 52kD polypeptide are being determined for the synthesis of PCR fragments and the isolation of the transferase gene. The bound rubber transferase catalyzes the synthesis of a rubber polymer with a mol wt and a *trans, cis* configuration of double bonds similar to natural rubber. The bound rubber transferase is different from the soluble rubber transferase recently isolated by Genentec workers which catalyzes only chain elongation of rubber chains. The bound rubber transferase occupies a branch point in the isoprenoid pathway utilizing one GPP or FPP initiator for the synthesis of a single rubber chain. Crude stem homogenates are being treated with anti-bound rubber transferase IgG to determine if the bound transferase accounts for the total synthesis of rubber polymer in the stems.

Treating seedlings of P. argentatum with a substituted tertiary amine increases bound rubber transferase activity 12-fold in mature plants. Immunoblots of proteins from the rubber particles shows the 52kD polypeptide increases with increasing bound rubber transferase activity. ^{13}C-NMR analysis of ground stems shows treating plants with different concentrations of the amine increases the rubber content 7 to 33%. The mode of action of the amine in stimulating rubber synthesis may be analogous to the action of the substituted amines in other tissues where the synthesis of isoprenoid compounds is increased by enhancing the expression of the genes coding for the synthesis of enzymes in the isoprenoid pathway.

R 030 MOLECULAR REGULATION OF CELL WALL STRUCTURE IN RIPENING FRUIT,
Alan B. Bennett, Coralie Lashbrook, Dean DellaPenna, Robert L. Fischer and Jim Giovannoni, Dept. of Vegetable Crops, University of California, Davis, CA 95616 (ABB, CL, DD)and Dept. of Plant Biology, University of California, Berkeley, 94720 (RLF, JG).

Ripening-associated softening is an important determinant of fruit quality. Aside from the obvious contribution of texture to fruit quality, the timing and extent of softening determines the timing of harvest which in turn affects other quality parameters such as flavor, color and nutritional value. In addition fruit softening is associated with increased pathogen susceptibility and eventual storage life of the fruit. Thus, control of fruit softening provides an attractive means to enhance multiple components of fruit quality and longevity.

Degradation of cell wall polymers contributes to ripening-associated fruit softening. In tomato, and a number of other fruit, degradation of pectins and hemicellulose are the major cell wall structural changes associated with fruit ripening and softening. In other fruit, including strawberries and peppers, pectin degradation does not occur. Thus, the major cell wall structural change in these fruit appears to involve the degradation of hemicellulosic components.

To determine the biochemical basis of fruit softening we have studied the physiological functions of the pectin-degrading enzyme, polygalacturonase, in transgenic fruit of a ripening-impaired mutant (*rin*) that normally fails to accumulate polygalacturonase. These experiments indicate that transgenic expression of polygalacturonase in *rin* fruit parallels its expression in wild-type fruit and results in degradation of cell wall pectins. We have shown that this activity contributes to enhanced susceptibility of the transgenic tomato fruit to a common postharvest pathogen but does not lead to fruit softening. Thus, the action of polygalacturonase is sufficient to degrade cell wall pectins and plays an important role in ripening-induced pathogen susceptibility but is not sufficient to induce fruit softening.

Because hemicellulose degradation has been identified as another structural component of the cell wall likely to be important in fruit softening we have initiated experiments to clone genes encoding hemicellulose-degrading enzymes and test their function in the processes of fruit softening and fruit ripening. The enzymic basis of hemicellulose degradation and our progress in cloning tomato hemicellulases will be discussed.

The data presently available confirm the expectations that textural changes accompanying fruit ripening are brought about by multiple enzymes acting to modify discrete components of the fruit cell wall. This suggests that only limited effects on the softening process are likely to be achieved by controlling the expression of single cell wall hydrolase genes.

R 031 CONTROL OF ETHYLENE BIOSYNTHESIS, Hans Kende, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824

Ethylene is involved in the regulation of many developmental plant processes, e.g. seed germination, fruit ripening and vegetative growth, and it is also formed in response to environmental and biotic stress. Hence, ethylene biosynthesis is tightly controlled in plant tissues. There are also well-documented examples for positive and negative feedback control of ethylene biosynthesis. Ethylene is formed from carbon atoms 3 and 4 of methionine via S-adenosylmethionine (AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC). In most instances, the limiting step in ethylene biosynthesis is the conversion of AdoMet to ACC mediated by the enzyme ACC synthase. The ACC-dependent ethylene-forming enzyme (EFE) is usually constitutively expressed. ACC synthase has been isolated from a number of organism and has been characterized using monoclonal antibodies. In tomato extracts, it is a protein of 50 kDa molecular mass (1). In vitro translation studies indicate that ACC synthase in tomatoes is synthesized as a polypeptide of 56 kDa. Radiolabeling coupled with immunological methods showed that the enzyme is de novo synthesized in response to induction by wounding. The enzyme is turned over very rapidly and is irreversibly inactivated by its substrate. The latter is based on covalent binding of a part of the methionine moiety to the enzyme (2). One of the interesting questions awaiting to be answered concerns the mechanism by which chemical and environmental stimuli induce ACC-synthase activity. Are there multiple genes each with a regulatory sequence specific for one class of effectors or are there only one or two genes with multiple regulatory elements? The recent cloning of ACC synthase from zucchini (3) will help to resolve this problem and may also enable one to control ethylene production by genetic engineering. In contrast to ACC synthase, much less is known about EFE. This activity does not withstand tissue homogenization. It has been found to be associated with plant vacuoles, and evidence indicates that it is bound to the tonoplast. Its functioning requires membrane integrity and, apparently, a transmembrane ion gradient.

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R 032 ENZYMOLOGY AND MOLECULAR BIOLOGY OF TROPANE ALKALOID BIOSYNTHESIS. Yasuyuki Yamada, Takashi Hashimoto, Jun Matsuda, Souichi Okabe, Yasuhiro Amano and Asuka Hayashi, Research Center for Cell and Tissue Culture, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Roots of several solanaceous plants synthesize tropane alkaloids such as hyoscyamine and scopolamine. Many of the plants that produce these alkaloids have been used since ancient times for their medicinal, hallucinogenic and poisonous properties. Now it has become possible to purify alkaloid biosynthetic enzymes from fast-growing root cultures and to clone their genes.

Hyoscyamine 6β-hydroxylase (H6H; EC 1.14.11.11) catalyzs the 6β-hydroxylation of [S]-hyoscyamine in the biosynthetic pathway leading to scopolamine. The hydroxylase is a 2-oxoglutarate-dependent dioxygenase which requires dioxygen, 2-oxoglutarate, ferrous ion and ascorbate for catalysis [1]. Besides hydroxylation of hyoscyamine, H6H epoxidizes 6,7-dehydrohyoscyamine (a synthetic alkaloid) to scopolamine, and also dehydrogenates, at a low rate, 6β-hydroxyhyoscyamine to scopolamine [2,3]. We purified H6H to homogeneity from cultured roots of Hyoscyamus niger. Immunohistochemical studies using monoclonal antibodies raised against purified H6H indicated that scopolamine is synthesized specifically in the cells near phloem in the roots. Oligonucleotide probes based on the internal amino acid sequences of H6H were used to screen a cDNA library from cultured H. niger roots. All the known amino acid sequences were found in a hybridization positive clone. The β-galactosidase fusion protein made from this clone was recognized by H6H-specific monoclonal antibodies. Northern hybridization showed that H6H mRNA is abundant in cultured roots, and present in plant roots but absent in leaves, stems and cultured cells of H. niger.

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Plant Transformation, Identifying Plant Genes, and Gene Mapping

R 100 ISOLATION AND CHARACTERISATION OF cDNA CLONES FOR ADPG PYROPHOSPHORYLASE AND WAXY LOCUS IN WHEAT. Charles C. Ainsworth and Joanna R. Clark, Department of Biochemistry and Biological Sciences, Wye College, University of London, Ashford, Kent TN25 5AH, UK

A cDNA library from 20 dpa mRNA from hexaploid wheat grain (cv. Chinese Spring) was constructed on λgt10. The library was screened with heterologous probes for ADPG pyrophosphorylase and waxy locus. A full-length (2.3kb) waxy locus cDNA clone and several partial ADPG pyrophosphorylase clones were isolated. The waxy locus clone and two ADPG pyrophosphorylase clones were subcloned into pUC19 and sequenced. The waxy locus clone shows strong homology with published maize and barley sequences. Southern analysis of deletion stocks of Chinese Spring wheat shows that the waxy locus is organised as a triplicate set of single copy genes on the short arms of chromosomes 7A and 7D and the long arm of chromosome 4A. Two ADPG pyrophosphorylase cDNA clones have been sequenced and exhibit strong homology with a rice cDNA [1] but much less homology with another published wheat endosperm cDNA sequence [2]. Waxy locus and ADPG pyrophosphorylase transcripts accumulate to high levels during mid grain filling which parallels enzyme levels and rates of starch synthesis.

1. Preiss, J. *et al.* (1987)
2. Olive, M.R. *et al.* (1989)

R 101 THE 5' LEADER EXON AND INTRON SEQUENCES FROM TWO SOYBEAN ACTIN GENES IN TRANSGENIC PLANTS, Yong-qiang An, Wm. Vance Baird*, Leslie Pearson# and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, GA 30602. *Horticulture Department, Clemson University, SC 29634-0375. #Westvaco Forest Science Laboratory, P.O. Box 1950, Summerville, SC 29484.

Actin is ubiquitous in all eukaryotes and with exception of yeast is encoded by a multigene families in all plants, animals and protists in which it has been examined. Actins also are associated with a lot of diverse important biological functions. Despite the general lack of intron conservation in animal actin genes, two soybean actin genes have an 5' untranslated leader exon with structure similarity to that found in vertebrate actin genes. All functional higher plant actin genes sequenced so far contain a potential intron acceptor site in the 5' untranslated region 10 to 13 nucleotides upstream from the initiator ATG. This conservation implies that the leader intron and exon sequences may have important biological fuctions.

Soybean contains three highly divergent classes of actin, which have not shared a common ancestor for 300-600 million years. A detailed analysis on the SAc6 and SAc7, representives of the kappa and mu classes of actins, have revealed leader introns of 1400 and 800 nt. respectively. The upstream sequences and leader exon sequences of the soybean genes contain many unusual features including (CT) repeats and long stretches of pyrimdine-rich DNA. We are making several reporter gene fusion constructs in order to assay functions of the leader intron and the leader exons in transgenic plants.

R 102 BARLEY CHLOROPLAST DNA-BINDING PROTEINS: ISOLATION OF THE BARLEY CHLOROPLAST NUCLEOID, Brian J. Baumgartner and John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843

A procedure for the rapid isolation of the membrane bound DNA-protein complex (nucleoid) from barley primary leaf plastids has been devised. The nucleoid fractions were enriched 5-fold in transcription and 7-fold in DNA synthesis specific activity, respectively. Lithium dodecyl sulfate/polyacrylamide gel electrophoresis showed that many polypeptides unique to the fraction were present. Proteins with DNA-binding activity were analyzed by in situ gel DNA-binding assays. In this assay the proteins are renatured in situ after electrophoresis and the gels are soaked with a radiolabeled DNA fragment. DNA-binding proteins are then detected by autoradiography of dried gels. As with the LDS PAGE gels, several polypeptides with DNA-binding activity were observed which were only present in the DNA-protein complex. Trypsin and DNAse I pretreatments of isolated intact chloroplasts did not influence DNA binding; similar treatments with osmotically lysed plastids however reduced or abolished DNA binding. These data show that the DNA-binding proteins were protected by the intact outer plastid envelope and thus were localized within the chloroplast. Properties of the DNA-binding proteins of barley plastid soluble, membrane, and nucleoid protein fractions are presented.

R 103 MOLECULAR ANALYSIS OF A TRANSPOSON-INDUCED DELETION IN TRANSGENIC TOMATOES, Francois J. Belzile and John I Yoder, Department of Vegetable Crops, University of California, Davis, Davis, CA, 95616. The maize transposable element *Ac* has been known to cause chromosomal rearrangements in maize. We transformed *Ac* into tomato and examined self progeny of primary transformants by Southern hybridization. A group of six siblings shared a single, transposed *Ac* at the same location. One of the siblings showed evidence of a deletion in the plant sequences to one side of the element. This deletion (~1kb) has been restriction mapped and clones of the deleted and undeleted sequence are being examined.

R 104 CLONING DIFFERENTIALLY EXPRESSED RARE MESSAGE, Madan K. Bhattacharyya, Cathie Martin and T.H.N. Ellis. John Innes Institute, IPSR, Colney Lane, Norwich, NR4 7UH, England.
Differential screening has been one of the major methods applied in cloning plant genes. The method would be very useful if common messages could be partially substracted before screening, especially when the particular genes sought are expressed at a very low levels. Magnetic beads such as Dynabeads M-280, which are conjugated to streptavidin, have been applied recently in PCR-sequencing and purification of transcription factor. The present study explores the possibility of using Dynabeads as a solid support for single-stranded cDNA to which complementary cDNA or mRNA can be hybridized. In this way the rarely expressed messages can be enriched before a differential screeninng procedure.

R 105 IDENTIFICATION OF THE HIGH LYSIN MAIZE FORMS ACCORDING TO ASPARTATE AMINOTRANSFERASE FEATURES. Valentina S. Bilchuck, Nadezhda P. Kotsyubinskaya, Yuriy V. Donchenko. Department of Molecular biology, Research Institute of Biology, State University, Dniepropetrovsk 320625, USSR.
High lysin opaque-2 (o2) maize mutants are characterized by the variability of the quantitative and qualitative protein content and the nitrogen metabolic enzyme activity. The redistribution of protein fractions in o2 maize is accompanied with the considerable change of asparagine and glutamine amino acid quantitative content in the grain. We have determined that the activity of aspartate amino transferases in the leaves was decreazed while it was increased in grain during maturation period. The most significant differences of the o2 mutant enzime activity were registered at days 29-36 after pollination. The simplest criterion of the o2 gene identification is the maize endosperm structure. However, it is not a reliable one. We recommend to use aspartate aminotransferases for this purpose. This is a very simple and reliable method of grain material estimation. The change in the aspartate aminotransferases activity is accompanied with the redistribution of the enzime isoforms and is a feature determined genetically.

R 106 TRANSIENT EXPRESSION OF CHIMAERIC GENES IN CASSAVA USING HIGH VELOCITY MICROPROJECTILES, Didier Bogusz, Claudine Franche, Christian Schopke, Claude Fauquet and Roger N. Beachy, Department of Biology, CB 1137, Washington University, St Louis, Mo 63130, USA.

The objective of the International Cassava-Trans Program is to produce transgenic cassava plants resistant to cassava viruses using the coat protein mediated protection strategy. Due to the fact that cassava is extremely difficult to transform and to regenerate, an alternative and rapid method to evaluate the potential of each construct, has been developed. Transient expression of chimaeric genes in cassava using high velocity microprojectiles was established. The ß-glucuronidase (GUS) gene was used as a reporter gene system to study the activity of several promoters in cassava. Chimaeric genes were delivered by bombarding young cassava leaves with tungsten particles coated with DNA. The biolistic device used was driven by air pressure. Two days after particle delivery, expression of the reporter gene was determined. Comparison of the activity of the cauliflower mosaic virus 35S promoter, two enhanced cauliflower mosaic virus 35S promoters and the Arabidopsis ubiquitine 1 promoter will be presented.

R 107 CLONING OF THE DIHYDROFLAVANOL-4-REDUCTASE GENE IN *LYCOPERSICON*, Monica Bongue, Sharman O'Neill, John Yoder, Department of Vegetable Crops, University of California, Davis CA 95616

Dihydroflavanol-4-reductase, (DHFR), is an important enzyme in the anthocyanin biosynthesis pathway. This enzyme is encoded by the *pallida* gene in *Antirrhinum majus*, and the *DFR* gene in petunia. When the *pallida* genomic clone from *A. majus* was used as a heterologous probe on a Southern blot of *L. esculentum* and *L pennellii* DNA, two distantly related tomato species, a restriction fragment length polymorphism was observed. Segregation of the RFLP in the F2 progeny of this interspecific cross suggests that in tomato this enzyme is encoded by a single locus. A cDNA library prepared from tomato hypocotyl tissue was screened with the petunia *DFR* cDNA clone. Three putative positive cDNA clones of about 1.7 kb were obtained and are currently being sequenced.

R 108 **CHITINASE EXPRESSION IN TRANSGENIC TOBACCO PLANTS: INCREASED PROTECTION AGAINST A SOIL-BORNE FUNGAL PATHOGEN,** Richard Broglie, Karen Broglie, Mark Holliday and Ilan Chet, Agricultural Products Dept., E.I. DuPont de Nemours & Co. (Inc.), Wilmington, DE. 19880

The process by which plants protect themselves against potentially pathogenic microorganisms is dependent upon the timely accumulation of a number of host synthesized proteins that are produced in response to pathogen attack. Among these proteins is the lytic enzyme chitinase which catalyzes the hydrolysis of chitin, a β-1,4-linked homopolymer of *N*-acetyl-D-glucosamine. Although no substrate for this enzyme has been found in plants, chitin is a ubiquitous component of many phytopathogenic fungi. For this reason it has been suggested that the function of chitinase in plants is to provide protection against chitin-containing pathogens. Recent studies of the induction kinetics of plant defense transcripts indicate that the timing of the appearance of the proteins may play an important role in resistance to fungal attack. In healthy, uninfected plants, chitinase is present at low, basal levels. However, treatment with ethylene, oligosaccharide elicitors, or infection with fungi results in an induction of chitinase mRNA levels and an increase in enzyme activity. DNA sequence elements situated immediately upstream of the chitinase coding region are responsible for ethylene- and elicitor-regulated expression. In an attempt to enhance fungal resistance in plants, we modified the expression pattern of a bean endochitinase gene by replacing the inducible native promoter with the highly active CaMV 35S promoter. Transgenic plants containing this gene were found to exhibit higher basal levels of chitinase and increased resistance to *Rhizoctonia solani*, the causative agent of seedling damping-off and root rot disease, when compared to plants not containing the modified chitinase gene. The resistant phenotype is manifested both by an increase in the rate of survival of plants grown in infested soil and a reduction in root damage caused by fungal attack.

R 109 USE OF AN INTERSPECIFIC CROSS FOR DEVELOPMENT OF A SATURATED RFLP MAP OF RICE, Mathilde Causse, Gerard Second* and Steve D. Tanksley, Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853 and *: perm. adress ORSTOM, Montpellier, France.
Our laboratory recently reported the development of an RFLP linkage map of rice based on a cross between two distantly related cultivars of *O.sativa* (McCouch *et al.* 1988) Using this cross, 230 markers were placed into 18 linkage groups corresponding to the 12 rice chromosomes. To complete this map and reduce the number of linkage groups to 12, we have begun analyzing a backcross population derived from a cross between cultivated rice and a wild rice species, *O.longistaminata*. High levels of restriction fragment length polymorphism between these species make them ideal candidates for use in developing a saturated RFLP mapping. A previous study at the isozyme level had shown that, in spite of the strong reproductive barrier that separate these species, the backcross to the cultivated rice gives relatively normal segregations (Causse et Ghesquiere, unpubl.). We report here the development of an RFLP map based on this cross. In general, recombination is less in this interspecific backcross than in the previous reported intraspecific cross. In the near term this is an advantage since previously independantly segregating linkage groups can now be combined into single, complete linkage groups. Currently, we are attempting to develop an *in vitro* propagation system which may allow prolonged maintenance of this backcross population for continued RFLP mapping. Once a high density map is completed in rice (ca. 1000 markers) it can be used both for breeding purposes and for chromosome walking to genes targeted for cloning.

R 110 TRANSIENT EXPRESSION OF FOREIGN CHIMERIC GENES IN HYBRID LARCH (LARIX EUROLEPIS) PROTOPLASTS, Pierre J. Charest and Krystyna Klimaszewska. Molecular Genetics and Tissue Culture group, Petawawa National Forestry Institute, Forestry Canada, Chalk River, Ontario, Canada, K0J 1J0.
Protoplasts isolated from an embryogenic cell line of hybrid Larch (Larix eurolepis) were electroporated with either a square wave pulse generator or a decay pulse generator with different gene constructions. Expressions of chloramphenicol acetyltransferase, neomycin phosphotransferase and b-glucoronidase genes were detected in transient assays. The genes were hooked up to either the CAMV 35S promoter or the NOS promoter. Expression was lower with the NOS promoter. The highest levels of expression of the chimeric genes were obtained with the square wave pulse generator and the CAT assay appeared the most sensitive reporter gene. The b-glucoronidase gene expression was detected in situ by reaction with X-glu or by fluorescence by reaction with MUG; however, an endogenous background level of reaction was observed with non-electroporated protoplasts. These results indicated the possibility of using genes designed for crop plants in Conifer genetic engineering.

R 111 MICROPROJECTILE MEDIATED DELIVERY OF MARKER GENES AND THEIR EXPRESSION IN IN VITRO CULTURES AND IMMATURE ZYGOTIC EMBRYOS OF WHEAT AND BARLEY, R.N. Chibbar, K.K. Kartha, N. Leung, F. Georges, K. Caswell, J. Qureshi, C. Mallard and E. Kendall, Plant Biotechnology Institute, NRCC, Saskatoon, Sask. Canada.
Immature zygotic embryos of cereals can give rise to full plants either through precocious germination or via the development of embryogenic calli. This makes them ideal target tissues for the delivery of genes through the microprojectile bombardment technique, for the development of transgenic cereals. In the present investigations tungsten particles (0.73 μ) coated with different vectors harboring marker genes were delivered in to barley cell suspension cultures, callus cultures or immature zygotic embryos of wheat and barley using the Biolistics (Dupont) gun. Chloramphenicol acetyl transferase (CAT) activity was observed in barley cells and immature zygotic embryos after 20 hours of delivery. However, for the expression of CAT activity the presence of Adh1 intron1 was required. In the case of wheat, CAT enzymatic activity was very low and hard to detect. However, using CAT specific antibodies we were able to detect CAT gene expression in wheat immature embryos also. Further, it was also observed that wheat extracts contained an inhibitor of CAT activity which could not be inactivated by treatment at 65°C, unlike barley or other plants. In wheat embryos we could also observe the expression of β-glucuronidase (GUS) activity based on the histochemical staining. This expression of GUS gene activity did not require the presence of Adh1 intron1 as was observed for expression of CAT gene. These and further efforts on the stable transformation of cereals will be discussed.

R 112 HISTOLOGICAL ANALYSIS OF ß-GLUCURONIDASE EXPRESSION IN REGENERATING TRANSFORMED EXPLANTS OF *VITIS VINIFERA* L. Sheila M. Colby, Adrian M. Juncosa, and Carole P. Meredith. Department of Viticulture and Enology, University of California, Davis, CA 95616.

Although plant regeneration and *Agrobacterium* infection are routine, grape has proven recalcitrant to regeneration of fully transformed plants by this method. Two histological techniques were used together in a unique approach to understand problems encountered in the development of an *Agrobacterium*-mediated transformation system for grape and to help devise strategies for success. First, the anatomy of direct shoot organogenesis from in vitro-grown grape leaves was studied by light microscopy. Regenerating petiole stubs of leaf explants were fixed at three day intervals and were sectioned longitudinally to determine the developmental sequence of direct shoot organogenesis. Patterns of shoot meristem initiation were studied to determine their relevance to *Agrobacterium*-mediated transformation. The results of these studies provided the anatomical details necessary to interpret ß-glucuronidase (GUS) expression patterns in regenerating petiole stubs of leaf explants which were cocultivated with a disarmed *A. tumefaciens* strain carrying GUS and kanamycin resistance genes. After four-weeks of culture on shoot-inducing medium containing kanamycin, histochemical analysis of GUS expression in regenerating explants revealed sharply-defined blue regions. Several patterns of GUS expression in regenerating explants will be presented with their possible implications for *Agrobacterium*-mediated transformation.

R 113 REGENERATION AND TRANSFORMATION OF CUCUMBER

C.M.Colijn-Hooymans, H.Hakkert, J.Bergervoet, J.Custers and J.J.M.Dons
Center of plant breeding research, P.O.BOX 16, 6700 AA Wageningen, The Netherlands

Cucumber is one of the most important glasshouse crops in The Netherlands. Regarding its regeneration and transformation ability cucumber is a recalcitrant crop and a lot of research was needed to develop a good regeneration procedure for cucumber. By using cotyledon explants as starting material for we developed a reproducible regeneration procedure. The method we have developed now is very attractive for transformation experiments because:
-The procedure is very simple,reproducible and proceeds without a callus phase.
-From explant to regenerated plants takes only 6 weeks.
-From each explant 5 regenerated plants could be obtained on the average.
-A low number of somaclonal variants was found (less than 10 %).
For Agrobacterium transformation of cucumber we first started with in vivo transformation on stems of young cucumber plants with different wildtype strains of Agrobacterium tumefaciens Those experiments showed that the strongest tumor reaction was observed with the nopaline strains (e.g.C58). Furthermore we tested the cotyledon regeneration system for the sensitivity for kanamycin. At a concentration of 50 mg/l kanamycin no bud formation on the cotyldon explants was found. Transformation experiments of cotyledon explants were performed with C58 Agrobacterium strains with different binary vector systems. The regeneration procedure and the results of the transformation experiments will be presented.

R 114 SITE-SPECIFIC RECOMBINATION IN PLANT CELLS MEDIATED BY BACTERIOPHAGE P1 RECOMBINASE. Emily Dale and David Ow, Plant Gene Expression Center, USDA/ARS/U.C. Berkeley 800 Buchanan St., Albany, CA 94710.

A site-specific recombination system has many potential uses for rearranging genetic material in higher eucaryotic cells, for example: the control of gene expression by deletion or inversion of DNA segments, the clustering of transgenic constructs via site-specific integration, and the generation of chromosomal translocations. We describe a first step towards the application of a site-specific recombination system in plant cells. By use of a transient assay, we demonstrate that the bacteriophage P1 *cre* gene when expressed in tobacco cells produces a functional recombinase which recognizes its target sites, *loxP*, and mediates reciprocal genetic crossovers at these sites. When the *loxP* sites are present in *cis* to one other, and arranged in either direct or inverted orientations, we detected Cre/*lox*-specific deletion and inversion events, respectively. The placement of *loxP* sites in *trans* resulted in the co-integration of the substrates by Cre-mediated intermolecular recombination. These results provide the basis for the further developement of the Cre/*lox* recombination system as a tool for manipulating DNA in plant cells. Our current work, which is directed towards the use of the Cre/*lox* system as a means to control gene expression and to target DNA sequences into the plant genome, will be presented.

R 115 A BIFUNCTIONAL GENE FUSION BETWEEN NEOMYCIN PHOSPHO-TRANSFERASE AND β-GLUCURONIDASE: A BROAD SPECTRUM GENETIC MARKER FOR PLANTS, Raju S.S. Datla*, Joe K. Hammerlindl, Lawrence E. Pelcher, Gopalan Selvaraj and William L. Crosby, Plant Biotechnology Institute, NRC Canada, 110 Gymnasium Road, Saskatoon, SK, Canada S7N-0W9. Neomycin phospho-transferase and β-Glucuronidase genes are the most widely used selectable and biochemical markers for studies involving plant transformation and development. We have generated a novel gene fusion between these two genes to combine their ideal features. The fusion gene when expressed in E. coli and N. tabacum produced a fusion peptide with catalytic properties of both the enzymes. The fusion gene has been demonstrated as a selectable marker to isolate transgenic plants, as a sensitive marker for biochemical assays and also as a marker for histochemical localization. Potential uses for this bifunctional fusion gene are discussed.

R 116 TRANSGENIC FERTILE INDICA RICE PLANTS REGENERATED FROM PROTOPLASTS.
Swapan K. Datta, Alex Peterhans, Karabi Datta and Ingo Potrykus. Swiss Federal Institute of Technology (ETH), Institute for Plant Sciences, CH-8092 Zürich, Switzerland, FAX (1) 252 9613.
Indica-type rice (Oryza sativa var.Indica) is the most important crop plant in the world. It would be desirable to make it accessible to improvement via gene-technology. This requires methods for gene transfer. We have established plant regeneration from protoplasts isolated from a microspore-derived suspension culture of Chinsurah Boro II. Direct gene transfer into protoplasts was by PEG (6000 MW, 40%) treatment. The marker gene was hygromycin phospho-transferase II under 35S promoter and terminator. Selection for resistent clones began 14 days after culture initiation and was at 25 ug/ml hygromycin B. 77 green plantlets were regenerated from hygromycin-resistent clones; 24 plants were raised to maturity. 10 of those have been analysed by Southern so far; 9 of those have the marker gene stably integrated into the genome. Sexual offspring obtained are under molecular analysis. The data on the integration of the marker gene and its transmission to progeny will be presented.

R 117 A TRANSIENT ASSAY TO TEST HETEROLOGOUS PROMOTER ACTIVITY IN Picea glauca (WHITE SPRUCE) USING ELECTRICAL DISCHARGE PARTICLE ACCELERATION, David D. Ellis[1], B.H. McCown[1], D.R. Russell[2] and D.E. McCabe[2]. [1]University of Wisconsin-Madison, Department of Horticulture, Madison, WI 53706 and [2]Agracetus, Middleton, WI 53562.
An important component of the successful genetic engineering of trees is the regulation of expression of introduced genes into forest trees. To date, few studies have focused on the controlled expression of foreign genes in perennial crops. Transient assays have been used to analyze promoter activity, function and regions important for regulated control of plant genes in herbaceous plants. Using the biolistic method, electrical discharge particle acceleration, we studied inducible heterologous promoter activity in Picea glauca (white spruce) embryos and seedlings. Promoters tested include: an arabidopsis and soybean ribulose-1,5-bisphosphate small subunit (rbcS) promoter, a maize phosphoenolpyruvate carboxylase (PEP) promoter, a soybean heat shock promoter, a maize alcohol dehydrogenase (ADH) promoter, and a soybean auxin inducible promoter. The first three promoters are light inducible, while the other three are inducible by a 1 hr 42°C heat shock, flooding, or a 3 hr high auxin (25 uM 2,4-D) treatment. All promoters were used to drive the B-glucuronidase marker gene and expression was compared to a 35s Cauliflower Mosaic Virus (CaMV) promoter. Expression in white spruce embryos was detected from all promoters tested, yet inducible GUS expression was observed only with the heat shock promoter. This method allows testing not only of promoter activity but also the testing of tissue specific expression.

R 118 CHARACTERISTICS AND USES OF CHROMOSOME BREAKAGE INDUCED BY THE MAIZE *AC/DS* SYSTEM IN TRANSGENIC TOBACCO AND TOMATO. J. English, H. Dooner*, and J. Jones, Sainsbury Laboratory, John Innes Institute, Colney Lane, Norwich, NR4 7UH, UK and * DNAP, 6701 San Pablo Avenue, Oakland, CA, 94608, USA.

Chromosome breakage was the first manifestation of transposable element activity detected by Barbara McClintock. When the element *Dissociation* was located on a chromosome arm proximal to dominant marker genes, the loss of these genes was observed dependent on the presence of *Activator* in the genome. More recently, two structures correlated with chromosome breakage have been characterized: "Double *Ds*" and "Fractured *Ac*", which are complex rearrangements of *Ds* and *Ac*.

To find out more about the structural requirements for chromosome breakage, a number of constructions resembling "Double *Ds*" and "Fractured *Ac*" have been introduced into tobacco and tomato. The chromosome-breaking activity of these structures will be monitored genetically by the loss of marker genes included on the introduced T-DNA in tobacco and tomato, and also by the loss of endogenous marker genes in tomato. Cytological and molecular characterization of the resulting structures will be undertaken.

When chromosome breakage occurs, it will make the following approaches possible:

(i) We will be able to observe the aberrant transposition events that lead to chromosome breakage and hopefully gain insight into the mechanism of transposition of *Ac*.

(ii) A chromosome breakage-fusion-bridge cycle may result in the genomic stress required to induce native transposable elements to move.

(iii) "Targeted" chromosome breakage may be used to address questions such as whether or not certain phenotypes are cell-autonomous.

Preliminary results, including studies on tobacco families segregating for putative chromosome-breaking structures, will be presented.

R 119 **Long Range Restriction Mapping in Lactuca sativa**

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A long range restriction map is being developed for Lactuca sativa in genomic regions containing disease resistance genes. Long range gene mapping involves the generation of large DNA fragments by digestion with one of several rare-cutting restriction enzymes, fractionation of the fragments by pulsed field gel electrophoresis, and Southern analysis with DNA probes. RFLP markers linked to genes determining resistance to downy mildew and procedures for isolating and fractionating high molecular weight DNA have recently been developed for lettuce. To provide complete and accurate data it is desirable to build overlapping maps based on a number of physically linked single copy probes. To obtain further probes we are developing procedures for using PFGE preparatively to enrich for sequences physically linked to existing RFLP markers. DNA fractionated by PFGE is directionally cloned into a lambda vector to create libraries enriched for the ends of large fragments. This approach will allow us to identify markers with a defined location on physical maps. The long range maps will be used to estimate the relationship between physical distance and recombination frequency. This information is a prerequisite for chromosome walking as a means of cloning disease resistance genes.

R 120 OPINES INFLUENCE *VIR* GENE INDUCTION IN AGROBACTERIUM TUMEFACIENS, Stanton B. Gelvin, K. Veluthambi, M. Krishnan, Department of Biology, Purdue University, West Lafayette, IN 47907 Certain opines, such as octopine, nopaline, leucinopine, and succinamopine, can potentiate the induction of the *vir* genes of the *Agrobacterium tumefaciens* Ti-plasmid by acetosyringone. This hyperinduction is dependent upon acetosyringone and the genes *virA* and *virG*. These opines enhance the double-stranded cleavage of the T-DNA borders, the accumulation of T-strands within the bacterium, and the transformation of recalcitrant species such as cotton. In the presence of tobacco protoplasts, the induction of the gene *pinF* is lower in bacteria harboring a mutation in the octopine synthase (*ocs*) gene than in bacteria harboring a wild-type *ocs* gene. Exogenous addition of low concentrations of octopine to the protoplast inducing mixture increases the induction of the *pinF* gene in the *ocs-* strain to the level seen in the *ocs+* strain. However, addition of high concentrations of octopine (greater than 200 μM) to either of these strains, in the presence of protoplasts, inhibits *pinF* induction. We hypothesize that low concentrations of opines enhance the transformation process during the early stages of tumorigenesis. When a tumor is formed, however, the higher concentrations of opines repress *vir* gene induction.

R 121 TOWARDS AN INTEGRATED LINKAGE MAP OF COMMON BEAN (Phaseolus vulgaris L.). Paul Gepts*, Shree Singh#, Rubens Nodari*, Belén Garrido*, and Epimaki Koinange*, *Department of Agronomy and Range Science, University of California, Davis, CA95616, and #Centro Internacional de Agricultura Tropical, Apartado aéreo 6713, Cali, Colombia, SA. Our goal is to replace the current rudimentary linkage map of common bean by an integrated map combining molecular markers and genes for qualitative and quantitative morpho-agronomic traits. We have developed four recombinant inbred populations between parents with contrasting evolutionary origins (Mesoamerican vs. Andean) in order to maximize intra-specific polymorphisms at the molecular level. In addition, the parents differ for multiple agronomic traits such as growth habit, tolerance to biotic and abiotic stresses, photoperiod sensitivity and flavonoid metabolism. Two genomic libraries (PstI and EcoRI-BamHI) have been established. As expected, the PstI library contains a higher proportion of single copy sequences than the EcoRI-BamHI library (80 vs. 20 %). Unexpectedly, however, the former library revealed a much higher level of polymorphism than the latter (80-90 % vs. 0 %). We are investigating the causes of this marked discrepancy. Our map will be used to: 1) investigate genome evolution in Phaseolus sp.; 2) identify important genes such as the I^+ gene for Bean Common Mosaic Virus resistance and the fin gene for determinacy; 3) devise appropriate breeding strategies; and 4) assist in marker-based selection.

Abstract Withdrawn

R 123 RESTORATION OF DELETIONS IN THE CHLOROPLAST GENOME OF WHEAT POLLEN ALBINO PLANTS: A MODEL SYSTEM FOR CHLOROPLAST TRANSFORMATION. I. Holme, R. Zemetra, H. Daniell. Department of Biological Sciences, University of Idaho, Moscow, ID 83843. One of the major problems in chloroplast transformation is the selection process due to the large number of chloroplast genomes per cell. An easily detectable selection system would therefore be of great value in optimizing the recovery of transformants. Wheat albino plants derived from anther culture have deletion(s) in their plastid genome (Day and Ellis, Cell, 39, 359). Restoration of deletion(s) in the ct genome of albino plants using the biolistic process with the chloroplast genome fragment(s) from green plants should transform to photosynthetic competency of regenerated tissue. Visible selection of the transformants without using antibiotics or herbicides pressure would be an ideal method. Smilar restoration experiments have been successful in Chlamydomonas non-photosynthetic mutants requiring acetate due to deletions in the atpB gene (Boynton et al, Science 240, 1534). Our recent analysis of restriction fragments of the ct genome from albino and green plants indicated interesting differences. Corresponding changes were also evident in ^{35}S methionine labeled thylakoid and stromal proteins obtained after in organello translation of plastids from albino and green plants. Deletions in ct DNA fragments of albino plants are now being localized by Southern blots. The deleted fragment(s) will be subcloned from wild type cp DNA and bombarded into the albino plant tissue by the biolistic process.

R 124 INTRINSIC GUS-LIKE ACTIVITIES IN SEED PLANTS, Ching-yeh Hu, Paula P. Chee, Robert H. Chesney, James H. Zhou, Paul D. Miller and W. Timothy O'Brien, Biology Department, Wm. Paterson College, Wayne, NJ 074370 USA (C-y.H., R.C., W.T.O.); Molecular Biology Division, The Upjohn Co., Kalamazoo, MI 49001 (P.P.C.); Department of Genetics, Iowa State University, Ames, IA 50011 (J.H.Z.); and DNA Plant Technology Corp., Cinnaminson, NJ 08077 (P.M.)

Fifty-two species of seed plants, covering some Gymnosperms and all the key groups of Angiosperms, were surveyed for their intrinsic beta-glucuronidase(GUS)-like activities. Histochemical and fluorometric assays indicated that, with few exceptions, intrinsic GUS-like activities of various degrees of intensities were detected in certain part(s) of the fruit walls, seed coats, endosperms or, especially, the embryos of a large variety of diverse seed plants. The vegetative organs of seedlings and mature plants usually lack such activities. Intrinsic GUS-like activity in seeds usually diminish rapidly during the germination process. Most of the intrinsic GUS-like activities of the immature embryos of soybean and string bean disappeared after one to a few day's *in vitro* culturing. ELISA tests indicated that the enzyme(s) responsible for these intrinsic GUS-like activities does not appear to be antigenically similar to *E. coli* beta-glucuronidase.

R 125 TRANSFORMATION OF PEA USING BINARY AND COINTEGRATE VECTORS OF AGROBACTERIUM TUMEFACIENS - STRAINS, André de Kathen and Hans-Jörg Jacobsen, Institut f. Genetik, Universität Bonn, Kirschallee 1, D- 5300 Bonn - 1, FRG

Epicotyl segments and nodus explants from etiolated pea seedlings were transformed using Agrobacterium tumefaciens-strains GV 2260 GUS int or GV 3850 HPT, carrying a neomycin- or a hygromycinphosphotransferase-gene as selectable marker. Transgenic character of kanamycin- or hygromycin-resistant tissues was confirmed by detection of NPT II- and GUS- or NOS-activities in crude protein extracts. Up to 5% of developing shoots from shoot-proliferating nodi were regenerated via organogenesis to kanamycin-resistant plantlets showing GUS- and NPT II-activity.

Transformation frequencies were found to be influenced by explant source, Agrobacterium tumefaciens-strain, the pea genotype and the duration of cocultivation. A positive effect of acetosyringone on the transformation rate could not be observed.

R 126 Comparison of various disarmed Agrobacterium tumefaciens strains and cultural conditions on transformation of Pisum sativum L., Mark C. Jordan, Monika Lulsdorf, Hans Rempel and Shaun Hobbs, Plant Biotechnology Institute, National Research Council, Saskatoon, Sakatchewan, S7N 0W9, Canada

The disarmed Agrobacterium tumefaciens strains C58C1 (pMP90), LBA4404 and EHA101, derived from C58 (nopaline), Ach5 (octopine) and A281 (succinamopine) strains repectively, containing binary vectors carrying genes for β-glucuronidase, hygromycin phosphotransferase and/or neomycin phosphotransferase II were used to inoculate regenerating and callusing explants of pea. Cultural conditions such as nurse culture during the co-cultivation stage, varying concentrations of acetosyringone, varying inoculum concentrations, duration of the cocultivation stage and the selection efficiency of hygromycin versus kanamycin were compared. Efficiency of transformation was based on callus induction from stem explants and the histochemical localization of β-glucuronidase in explants from an organogenic regeneration system.

R 127 NON-RADIOACTIVE DETECTION OF DNA-DNA HYBRIDS: APPLICATION AT SINGLE COPY LEVEL IN GENE MAPPING, Frans A. Krens, Sjefke J.H.M. Allefs and Gerard J.A.
Rouwendal, Dept. of Mol. Biol., Fndn. for Agric. Plant Breeding, P.O.Box117, 6700AC Wageningen, The Netherlands. Large-scale application of non-radioactive detection of DNA-DNA hybrids in Southern hybridization techniques has been held up so far by two major disadvantages. These were a lower sensitivity of the detection systems as compared to radioactive labeling and detection and the limited possibilities for reuse of the filters carrying the target DNA after dissociation of earlier formed hybrids. The latter problem was caused by the formation of an insoluble, blue precipitate on the filter by the BCIP-NBT detection system. New non-radioactive labeling systems and detection, which uses the conversion of chemiluminescent substrates, in combination with our results on improving blotting and target DNA fixation conditions have led to a protocol. This protocol allows the detection of 5 fg of labeled lambda DNA spotted onto nylon membranes as well as the detection of single copy gene sequences after full Southern hybridization. At present labeled probes can be stably stored at 4^0C for more than six months and can be reused at least ten times, filters can be reused at least ten times and luminogram development takes two hours or less. Data on hybridization conditions, filter utilization, optimization studies and application in our gene identification and mapping programmes will be presented.

R 128 TRANSFORMATION OF LISIANTHUS (*EUSTOMA GRANDIFLORUM*) MEDIATED BY *AGROBACTERIUM TUMEFACIENS*, Susan E. Ledger[1], Nigel K. Given[1], Steve M. Butcher[1], and Richard C. Gardner[2]. [1]Horticultural Research Centre, Ministry of Agriculture and Fisheries, Levin, New Zealand, [2]Department of Molecular and Cellular Biology, University of Auckland, Auckland, New Zealand.
Leaf pieces of the economically important cut flower, Lisianthus, were co-cultivated with *A. tumefaciens* strain A722 containing pKIWI 110. T-DNA included the nos-neomycin phosphotransferase (NPT II) gene and the 35S-β-glucuronidase (GUS) gene. Histochemical assay for GUS activity, using the substrate X-Gluc, showed a low number of positive cells two days after inoculation and positive calli 20 days after inoculation. Shoots regenerated from leaf pieces cultured on MS medium containing 0.05 ppm IBA, 1 ppm BA and 0.1 ppm GA_3. Shoots which regenerated and rooted in the presence of kanamycin and also have GUS activity are presently being screened by Southern analysis.

R 129 TRANSFORMATION AND TRANSIENT GENE EXPRESSION OF *Phaseolus vulgaris* PROTOPLASTS Patricia León[1], Federique Planckaert and Virginia Walbot[2]
Nitrogen Fixation Research Center, University of México, UNAM México[1]; Department of Biological Sciences, Stanford University, Stanford CA 94305 USA[2].
Several methods have been developed to transfer DNA into plant cell protoplasts. One of them, the electroporation, consists of applying a high electric field pulse to the naked protoplasts resulting in a reversibly change in membrane permeability which allows macromolecules can pass to the interior of the cell. Another relatively simple technique consists of incubating the protoplasts with the DNA in the presence of polyethylene-glycol. In the legume family, both electroporation and direct transfer have been used successfully to stably transform protoplasts and to study gene expression. Despite this, some members of the legume family as is the case of *Phaseolus vulgaris*, have been difficult to work with. Here we report the establishment of a suspension culture of *P. vulgaris* cv. negro jamapa and the conditions required for high level of transient gene expression. We have optimized the conditions using electroporation and PEG transformation taking into consideration both expression of a reporter gene and cell viability. The role of sequences reported to alter transient gene expression in other systems are being investigated.

R 130 ANALYSIS OF Phaseolus INTERSPECIFIC HYBRIDS BY RFLP. Mei Guo, David A. Lightfoot, Machteld C. Mok and David W.S. Mok, Department of Horticulture, Oregon State University, Corvallis, OR97331. Interspecific hybridization of P.vulgaris cv. Great Northern (GN) and P.coccineus cv. Scarlet Runner (SR) gives rise to either mature seeds or abnormal embryos depending on the direction of the cross (Shii et al 1982. Theor. Appl. Genet. 62:59-64). However beyond the initial cross normal as well as abnormal embryos occur in reciprocal progeny populations (F_2 and F_3). Moreover, a bimodal distribution of phenotypes with over-representation of the parental types has been observed. To determine whether abnormal development may be related to particular genetic combinations, RFLPs were analysed in F1 and F_2 populations using callus and surviving plants. Comparisons of complete (callus) F_2 populations with the plant (viable) subpopulation indicate the latter exhibit predominantly GN genotype with some probes. Conversely, abnormal embryos showed higher proportions of SR genotypes with the same probes. The effects of particular regions of the parental genomes in relation to embryo and plant development will be determined. In addition the accumulated data may be useful in the construction of a RFLP linkage map for Phaseolus since the frequency of RFLP between Phaseolus species was determined to be greater than 50%/probe/digest whereas within diverse P.vulgaris genotypes it was less than 1%/probe/digest.

R 131 RFLP MAPPING OF THE AUXIN-BINDING PROTEIN LOCUS (abp1) IN MAIZE

Marian Löbler, Ann M. Hirsch
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A putative auxin receptor, an auxin-binding protein, has been characterized (1,2,3) and cloned (4,5,6). We used a full length cDNA as a probe to map its locus within the maize genome. The maize families used in this experiment are described elsewhere(7). Leaf DNA was digested with Sst I, restriction fragments were separated in agarose gel electrophoresis and blotted onto genescreen membrane. The blots were probed with a radiolabelled cDNA of the ABP. RFLP data analysis showed that the locus for the ABP (abp1) maps to the long arm of chromosome 3.

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R 132 CHARACTERIZATION OF THE ACTIN GENE FAMILY AND ACTIN-BINDING PROTEINS IN *ARABIDOPSIS THALIANA*, John M. McDowell, Shurong Huang, S. Jeffery Radcliff, and Richard B. Meagher, Department of Genetics, University of Georgia, Athens, GA 30602.

Actin is involved in a number of diverse functions in various plant cell types. We have proposed that divergent plant actins could have specialized patterns of regulation and function. Actin is encoded by a multigene family in all plants examined thus far (soybean, *Petunia*, *Arabidopsis*, tomato, *Zea mays*, and rice). The soybean family contains at least three ancient classes of actin which are more diverged from each other than are muscle and cytoplasmic actin in higher animals. More detailed analysis of the *Petunia* actin gene family has shown it to contain 100-200 actin sequences in several extremely divergent classes. Data on the *Petunia* actin gene family call into question the absolute family size in these other plant species. In order to simplify our molecular genetic analysis of actin in plant cells, we have initiated several projects in *Arabidopsis thaliana*. Several actin genes have been isolated toward the complete characterization of all 10-20 members of this actin gene family. An evolutionary tree of actin-related sequences will be constructed and compared to actin genes from other species. The polymerase chain reaction is being used to assist in the isolation of tissue specific actin cDNAs. Comparative sequence analysis of actin-binding proteins from other kingdoms has revealed conserved protein sequence domains from which specific oligonucleotides are being derived. These oligonucleotides will aid in the isolation of genes encoding actin-binding proteins.

R 133 ISOLATION OF AN EFFICIENT ACTIN PROMOTER FOR USE IN RICE (*Oryza sativa*) TRANSFORMATION, David McElroy, Wanggen Zhang, Madge Rothenberg, Jun Cao, and Ray Wu, Section of Biochemistry, Molecular and Cell Biology, Biotechnology Building, Cornell University, Ithaca, NY 14853. Four unique rice actin genes, RAc1, RAc2, RAc3 and RAc7 were isolated and characterized. Pairwise comparison between the rice actin genes and a number of other plant and animal actin genes revealed that the rice actin genes are highly diverged. This suggests that the rice actin genes might display differential regulation and/or function. Gene-specific probes were used to determine the levels of the four rice actin gene transcripts in developing rice seedlings. Northern slot-blot analysis revealed that both RAc1 and RAc7 display a constitutive pattern of transcript abundance while the level of RAc2 and RAc3 transcripts decline as the rice seedling matures. Reverse-Northern blot hybridization revealed that of the four rice actin genes analyzed RAc1 has the most abundant transcript in young rice seedlings. A genomic clone containing the 5'-flanking region of RAc1 was isolated and characterized. By transient assay of rice protoplast transformed with various RAc1-β-glucuronidase (GUS) fusion constructs we found that: the region 1.3 kb upstream of the RAc1 translation initiation codon contains all of the 5'-regulatory elements necessary for efficient GUS expression; the RAc1 5'-flanking region contains a relatively efficient promoter for foreign gene expression in transformed rice protoplasts; an RAc1-GUS fusion construct lacking the RAc1 5'-intron is not expressed in transformed rice protoplasts; the intron-mediated stimulation of GUS expression is associated, in part, with an *in vivo* requirement for efficient intron splicing.The RAc1-GUS fusion constructs have been expressed in a number of different transformed rice tissues.

R 134 STABLE TRANSFORMATION OF COTTON AND SOYBEAN EMBRYOGENIC CULTURES VIA MICROPROJECTILE BOMBARDMENT. Michael D. McMullen[1,2] and John J. Finer[2], [1]USDA-ARS and [2]Dept. of Agronomy and Biotechnology Ctr., Ohio State University, OARDC, Wooster, Ohio 44691. Embryogenic suspension cultures of cotton (*Gossypium hirsutum*) and soybean (*Glycine max*) were bombarded with high density particles coated with DNA encoding hygromycin resistance. One-two weeks following bombardment, cells were placed under hygroymcin selection. Embryogenic cotton and soybean lines stably expressing hygromycin resistance were obtained. Southern hybridization analysis confirmed the presence of the introduced plasmid in the hygromycin resistant cotton and soybean cells. Addition of the scorable GUS marker gene, either on the same plasmid as the hygromycin resistance gene or on an independent plasmid (cotransformation), resulted in stable expression of GUS activity in many of the hygromycin resistant lines from both species. Plants have been regenerated from hygromycin resistant cultures of both species to determine if the introduced DNA will be stably transmitted to progeny. Microprojectile bombardment of embryogenic suspension lines appears to be an efficient method for obtaining transgenic plants of major crop species.

R 135 EVIDENCE FOR TRANSPOSITION OF MAIZE TRANSPOSON AC ELEMENT IN TRANSGENIC RICE PLANTS. Norimoto Murai, Akio Hayashimoto and Zhijian Li, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803-1720.

We are interested in establishing a method for transposon mutagenesis of transgenic rice plants to facilitate the isolation of disease-resistance loci. The Ac element sequence was placed between the promoter and coding sequences of the hygromycin resistance (HPH) gene. This would interrupt effective gene expression and render the resistance gene inactive. After transfer to rice protoplasts and subsequent Ac excision, the HPH gene function in a portion of transformants would be reactivated. The intact Ac element in pTRA137 appeared to excise from the HPH gene with high frequency in transgenic rice. The excision frequency based on the phenotypic assay was 11 to 14% in Nipponbare protoplasts and 13 to 20% in Taipei 309 protoplasts. The orientation of the Ac element in pTRA137 did not significantly influence the excision frequency. The insertion of the Tn903 fragment at the BamHI site of the Ac element in pTRA139 reduced the excision frequency to 1 to 2%. However, the insertion did not appear to inhibit the excision completely. Sixteen and 21 transgenic plants containing pTRA137 and 139, respectively, were regenerated and grown to mature fertile plants. The phenotypic variations are under vigorous scrutiny. The structure (copy number and organization) of transferred DNA was analyzed by southern blot hybridization of genomic DNA from transgenic plants. The DNA analysis indicated proper excision of the Ac element from the introduced plasmids and reintegration into the rice genome. Expression of 3.5 kb transposase RNA will be assayed. Empty donor sites in pTRA 137 and 139 will be isolated by PCR. The sequence of empty donor and integration sites will be determined to understand the mode of Ac excision in transgenic rice plants.

R 136 *cis*-ANALYSIS OF A CLASS II PATATIN PROMOTER IN TRANSIENT AND STABLE EXPRESSION ASSAYS, Jan-Peter Nap, Joke Onstenk, and Willem J. Stiekema, Molecular Plant Breeding, Research Institute ITAL, P.O. Box 48, 6700 AA Wageningen, The Netherlands. Patatin is a glycoprotein family that accounts for up to 40% of the total soluble protein in potato tubers. It is encoded by a gene family of 10 - 15 members per haploid genome, the members of which can be divided into two classes, which differ in expression pattern and promoter structure. Class II patatin mRNA constitutes up to 2% of the total patatin mRNA in tubers and is also found in roots. Understanding of the regulation of class II patatin gene expression may add to our insight in the mechanisms involved in organ specificity of gene expression in the potato plant. We have isolated and characterized a member of the patatin class II gene family. We fused the promoter sequence of this gene to GUS and obtained transgenic potato plants exhibiting the pattern of GUS activity expected for a class II promoter. To study this promoter in more detail, various 5'-deletions of this promoter were fused to GUS, either with or without the 35S CaMV enhancer sequence upstream. Transient expression of the GUS gene was assayed in leaf protoplasts after PEG-mediated DNA uptake. The same promoter deletions fused to GUS were also cloned in the binary vector BIN19 and introduced in *Solanum tuberosum* cv. Bintje using both *A. tumefaciens* and *A. rhizogenes*. The regulation of class II patatin promoter directed activity in protoplasts, transgenic plants and hairy roots will be reported.

R 137 CLONING AND GENE ORGANIZATION OF THE MAIZE BRONZE-2 LOCUS, Juli Nash and Virginia Walbot, Department of Biological Sciences, Stanford University, Stanford, CA 94025.

The maize Bronze-2 (Bz2) gene, whose product acts late in the anthocyanin biosynthetic pathway, has been cloned and its transcript mapped. The wild-type Bz2 gene was reconstructed via PCR from an existing transposon-containing clone (bz2::mu1) and an amplified product from the progenitor allele that covers the site of insertion of the transposon. Unusual features of the 850 nt transcript determined from primer extension and RNase mapping analyses include an exceptionally short 5' untranslated leader and at least one small intron (78b) in the coding region of the gene. Two strong transcription initiation sites were mapped to positions -6 nt and -17 nt upstream of the ATG. The 6b leader, resulting from initiation at the downstream transcriptional start site, is the shortest 5' untranslated leader so far reported for a higher plant gene. Additionally, there are indications that expression of Bz2 may be regulated at the level of transcript processing. The extent to which these characteristics of the Bz2 gene impact expression patterns of this locus will be discussed.

R 138 RFLP LINKAGE MAPPING IN LOBLOLLY PINE (PINUS TAEDA L.), David B. Neale and Kathleen D. Jermstad, Institute of Forest Genetics, U.S.D.A. Forest Service, Placerville, CA 95667. A genetic linkage map of loblolly pine (Pinus taeda L.), a commercially important forest species, is being developed using restriction fragment length polymorphisms (RFLPs). The genome of loblolly pine (2x=2N=24) is very large ($>10^{10}$ bp), but the estimated recombination size (2000cM) is on the order of many other plants. The generation time of loblolly pine is 8-10 years. Therefore, we have chosen an existing 3-generation outbred pedigree for mapping. This pedigree was chosen based on high isozyme and RFLP heterozygosities in the two parent trees. Loblolly pine cDNA clones are being used as probes. The detection of single-copy sequences on genomic blots is difficult due to the large genome size, but we have refined our techniques so that these hybridizations can be performed routinely. Thus far, approximately 30% of the clones have revealed polymorphisms and preliminary segregation data has been obtained.

R 139 GENETIC TRANSFORMATION OF STRAWBERRY BY *AGROBACTERIUM TUMEFACIENS* USING A LEAF DISC REGENERATION SYSTEM, Narender S. Nehra, Ravindra N. Chibbar, Kutty K. Kartha, Datla S.S. Raju, William L. Crosby and Cecil Stushnoff, Plant Biotechnology Institute, National Research Council, 110 Gymnasium Road, Saskatoon, Sask., Canada S7N 0W9

An efficient genetic transformation protocol has been developed for strawberry cv. Redcoat using *Agrobacterium tumefaciens*. The protocol relied on a high frequency (90%) shoot regeneration from leaf discs in this cultivar. For transformation, the leaf discs were co-cultured with a disarmed *Agrobacterium tumefaciens* strain carrying plasmid pBI121 which confers kanamycin resistance (NPT-II) and β-glucuronidase (GUS) activity. The co-cultivated leaf discs were precultured for 10 days on a nonselective shoot regeneration medium to facilitate efficient selection. The meristematic regions developed at the periphery of leaf discs were then selected on kanamycin (50 μg/ml) for 4-6 weeks. The light green shoots surviving on kanamycin selection medium were subsequently transferred to a rooting medium containing lower level of kanamycin (25 μg/ml). Most of the selected shoots remained green and elongated on this medium, but only few formed roots. The transgenic shoots expressed varying levels of NPT-II and GUS activity. Preliminary results of southern blotting also indicated integration of marker genes in the strawberry genome. These results and potential application of this protocol in improvement of strawberry will be discussed.

R 140 THE EXPRESSION OF AN ACTIVE *E. COLI* MNSOD IN THE CHLOROPLAST AND CYTOPLASM OF *NICOTIANA TABACUM*, Jeffery K. O'Neal, Jean C. Kridl, Andree Genez, and Jean-Michel Le Moullec[1], Calgene Inc., 1920 Fifth St. Davis, CA 95616, [1]Roussel-Uclaf, Centre de Recherches-111 Route de Noisy 93230 Romainville, France. Chimeric constructs containing *E. coli* MnSOD (eMnSOD) coding sequences driven by the cauliflower mosaic virus (CaMV) 35S promoter were transferred into *Nicotiana tabacum*. In some constructs, the transit peptide of the small subunit of soybean ribulose-1,5-bisphosphate carboxylase (SSU) was utilized to target the eMnSOD to the chloroplast. Western analysis of the resulting transgenic plants demonstrates expression of the eMnSOD in the cytoplasm of plants without the SSU transit peptide, and in the chloroplast of those plants with the transit peptide. SOD activity analysis on native polyacrylamide gels shows that the enzyme activity expressed in the cytoplasm is characteristic of eMnSOD, while the form expressed in the chloroplast has some novel characteristics.

R 141 GENE SEQUENCE, DEVELOPMENTAL EXPRESSION AND PROTEIN PHOSPHORYLATION OF RAB-17 IN MAIZE. Montserrat Pagès[1], Adela Goday[1], Miguel Angel Freire[1], Margarita Torrent[1], M.Carmen Martínez[1], Jose Maria Torné[2] andJosep Vilardell[1]. [1]Departamento de Genética Molecular. [2]Departamento de Biología Molecular y Agrobiología. Centro de Investigación y Desarrollo. C.S.I.C. Jorge Girona Salgado 18-26. 08034 Barcelona.

The ABA-induced MA12 cDNA from maize, which encodes a set of highly phosphorylated embryo proteins (Goday et al. Plant Physiol. 88 1988), was used to isolate the corresponding genomic clone. This gene, called RAB-17 (Responsive to ABA), encodes a basic, glycine-rich protein (mol. wt. 17,164) containing a cluster of 8 serine residues, seven of them contiguous. It is an homologue of the rice RAB-21 gene (Mundy J. and Chua N.H. EMBO J. 7, 2279-2286, 1988). Phosphoamino acid analysis of the isolated protein indicates that only the serine residues are phosphorylated and a putative casein-type kinase phosphorylatable sequence was identified in the protein. The pattern of expression and in vivo phosphorylation of the RAB-17 protein was studied during maize embryo germination and in calli of both meristematic or embryonic origin. ABA treatment induced the synthesis of RAB-17 mRNA and protein in calli, however, the RAB-17 proteins were found to be highly phosphorylated only in embryos.

R 142 TRANSFORMATION OF POLLEN: ANALYSIS OF GENE EXPRESSION DURING MICROSPOROGENESIS AND POLLEN FUNCTION.

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To find gene constructs applicable to fundamental research in pollen transformation, beta-glucuronidase (GUS) was histochemically analyzed in anthers and pollen of various Solanaceae. GUS activity was determined in transgenic plants containing a chimaeric GUS gene regulated by a 800 bp 35S promoter or by a pollenspecific promoter and in untransformed plants. At meiotic and postmeiotic stages of microspore and pollen development, anthers of both 35S-GUS transgenic and untransformed plants demonstrated consistently high levels of GUS activity in tapetal and sporogenic cells. In anthers of 35S-GUS transgenic plants, GUS was present in the vascular cylinder, the connectivum and the stomium at all developmental stages. This indicates that the chimaeric GUS gene product was tissue specifically localized in the anthers. It also shows that an endogenous GUS gene was expressed in a temporal- and spatial specific manner in the tapetum and pollen of untransformed plants. pH-dependency of the endogenous GUS activity provided a distinction between endogenous and transgenic GUS activity. No transgenic activity was found in pollen of plants transformed with the 35S-GUS construct. Studies are now directed towards the expression of the pollen specific promoter. GUS genes expressed in pollen will be introduced into lilium pollen by electroporation and particle gun.

R 143 AGROBACTERIUM-MEDIATED TRANSFORMATION OF RICE (OTYZA SATIVA L.) CULTURES,

Deanna M. Raineri, Paul Bottino, Milton P. Gordon, and Eugene W. Nester, Department of Microbiology SC-42, University of Washington, Seattle, WA 98195. Engineered Agrobacterium strains have been successfully used to produce transgenic plants of many dicotyledonous species. However, despite evidence that Ti-plasmid mediated T-DNA transfer into monocotyledonous cells does occur (agroinfection, Grimsley, et al., 1986), transgenic cereal plants have not been obtained. Preliminary evidence for the transformed status of callus cultures generated from mature rice embryos following Agrobacterium infection of 2 rice cultivars was suggested by phenotypic expression of T-DNA oncogenes. Further evidence was provided by Southern analysis which demonstrated the presence of T-DNA sequences in callus tissues and by GUS analysis of kanamycin-selected callus cultures when disarmed binary vectors were used. Studies to evaluate alternative selectable markers which will allow the generation of transgenic rice plants are underway. To optimize foreign gene expression, various promoters and factors which are known to enhance gene expression in other systems are also being investigated. Agroinfection data and this study, respectively, provide evidence for the transfer of T-DNA into rice plants and its integration into cultured cells. Work in progress is aimed at defining conditions which will allow its stable maintenance during plant regeneration.

R 144 GENE TRANSFER AND EXPRESSION IN CULTURED MAIZE CELLS

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Plasmids encoding selectable marker genes have been stably introduced into maize protoplasts by PEG-mediated direct DNA delivery and into suspension culture cells by microprojectile bombardment. In both cases selection for kanamycin resistance allowed for efficient recovery of callus stably transformed with varying copy numbers of the selectable gene as well as linked nonselected genes. Several characteristics of the two DNA delivery systems have been examined. i) Structural rearrangements of integrated constructs such as concatenations and deletions are frequently observed and a comparison of DNA integration patterns obtained via the two methods will be presented. ii) High frequency cotransformation (up to 80%) of two marker genes on independent plasmids has been readily achieved using both methods. iii) Fluctuation of expression levels from nonselected genes during long-term tissue culture of transformed callus has been quantified.

R 145 TRANSPOSON TAGGING STRATEGIES USING Ac AND Ds IN TOMATO

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The use of a phenotypic in situ assay based on Ac excision restoring GUS activity enabled transgenic tobacco plants to be identified in which Ac excised predominantly late in plant development. To assess the potential of late Ac excision for transposon tagging on plant cell level, a phenotypic selection assay based on Ac excision restoring hygromycin resistance was used. Protoplasts were isolated from leaves of transformants which did not display hygromycin resistance. Up to 0.8% of these protoplasts survived subjection to hygromycin. The hygromycin resistant derivatives tested were shown to represent independent transposition events. As millions of protoplasts can be screeened, it will be possible to generate in the order of 10^5 independent Ac insertions. This will enable transposon tagging of genes which can be selected for inactivation at plant cell level.

As an alternative to the autonomous transposing Ac element a two-component system comprising a trans-active Ac and a responsive Ds has been examined. A high frequency of Ds transposition in crosses between transgenic tobacco plants harbouring an active Ac and plants harbouring Ds indicates the applicability of Ds for tagging strategies. However, as Ds transposes relatively late in plant development, the F1 progeny will be chimaeric for Ds transpositions and tagging experiments should be carried out in the F2 progeny. To try and alter the timing of Ac- and Ds-excision, the regulatory sequences of Ac-transposase have been replaced by a number of tissue specific promoters. One of these is the well characterized chalcon isomerase A_2 promoter isolated from *Petunia hybrida*. A transposase production which is restricted to pollen might lead to late Ac excision and to Ds excision immediately following fertilization, which will result in non chimaeric progenies.

To set up transposon tagging in tomato, an improved Ds element was constructed which contains only those Ac sequences required for non autonomous transposition. The presence of GUS between these Ac sequences allows transposed Ds elements to be genetically followed. Initial experiments focus on determining the distances over which Ds transposes in tomato. From these results it will be decided which tomato plants containing Ds linked to the *Asc* locus on chromosome 3 will be used for transposon tagging experiments.

R 146 THE DEVELOPMENT OF POLLEN TRANSFORMATION USING ELECTROPORATION IN TOBACCO

James A. Saunders and Benjamin F. Matthews, Germplasm Quality & Enhancement Lab and Plant Molecular Biology Lab, USDA/ARS, Beltsville, MD 20705.

A technique for gene transfer in plants has been developed using high field strength electroporation procedures on germinating pollen from Nicotiana gossei. Treated pollen is used to pollinate receptive flowers and viable seed has been obtained as a result of the treatment. The pollen is germinated in buffered sucrose supplemented with borate and potassium salts prior to the electroporation treatment to initiate pollen tube growth. The pollen tube would have a drastically diminished cell wall, thus facilitating DNA uptake. We have demonstrated that DNA can be taken up by pollen treated in this manner and that DNA is expressed both in the pollen and in subsequent plants produced from the pollen. Using the GUS marker enzyme system, putative transformed plants containing the expressible GUS gene are stable for at least two generations. Transformation rates based on GUS expression is approximately 40% of the first generation of plants. Southern hybridization of the GUS DNA in transformed plants has confirmed the effectiveness of this gene delivery technique.

R 147 OPINE SYNTHESIS AND SECRETION BY TRANSGENIC PLANTS UNDER AUTOTROPHIC GROWTH CONDITIONS. M.A. Savka and S.K. Farrand, University of Illinois at Urbana-Champaign, Urbana, IL. 61801.

An *Agrobacterium*-mediated binary transformation system was constructed to introduce mannityl opine biosynthetic genes into plants. T_R right border, the three genes conferring biosynthesis of the four mannityl opines from pTi15955 along with a chimeric kanamycin resistance gene were cloned into pUCD2001. Transgenic tobacco plants expressing resistance to kanamycin were regenerated following transformation with *A. tumefaciens* carrying this construct, called pMAS4, and the Vir helper plasmid, pEHA101. Such plants exhibited resistance to kanamycin and produce the mannityl opines. While all plants synthesized mannopinic acid, mannopine and agropinic acid, not all produced agropine at detectable levels. One regenerant examined in detail showed a selfed R1 segregation pattern for kanamycin resistance of 3:1. Mannityl opine biosynthesis cosegregated with Kan^R at a frequency of 1.0 in such crosses. Analysis of R2 progeny from R1 selfings showed segregation patterns consistant with the T-DNA insert being located on a single chromosome. Opines were produced in all tissues of such plants including roots, stems, leaves, flower petals, anthers and pistils. Transgenic plants grown autotrophically in a mineral salts solution secreted the opines from their roots into the media. *Agrobacterium* strains containing genes conferring catabolism of the mannityl opines could grow in such media at the expense of the opine substrates. These results show that the three T_R genes are sufficient for the biosynthesis and secretion of the mannityl opines, that these opines are produced and secreted in plants grown under autotrophic conditions, and that the secreted opines can be utilized by plant-associated bacteria.

R 148 THE COMPETENCE OF DIFFERENT TISSUES OF **ZEA MAYS** FOR AGROINFECTION Michael Schlaeppi, Nigel Grimsley, Jose Maria Torne,and Barbara Hohn, Friedrich Miescher-Institut, Basel/Switzerland

Agroinfection, the **Agrobacterium tumefaciens** mediated transfer of cloned viral sequences into host plants, has been established for **Zea mays** (maize) using Maize Streak Virus (MSV)[1]. In young maize seedlings, only the meristematic region of the shoot apex is susceptible to agroinfection[2].

The focus of this work is to determine the earliest developmental stage of meristematic tissue competent to be agroinfected with MSV. In order to distinguish between competence of the maize tissue to be agroinfected by agrobacteria and the competence of the virus to replicate and spread in a given tissue, comparative studies of transient expression of cloned reporter genes replacing MSV are underway.

Results will be presented for germinated seedlings, mature (resting) embryos, meristematic calli, and immature embryos of different developmental stages. Preliminary results on transient expression of reporter genes will be discussed.

[1] N.H. Grimsley et al., Nature 325 (1987), 177-179
[2] N.H. Grimsley et al., Biotechnology 6, (1988), 185- 189

R 149 OCSBF-1, AN OCS-ELEMENT BINDING FACTOR FROM MAIZE. Karambir Singh[1], Elizabeth S. Dennis[1], Jeff. G. Elljs[1], Danny J. Llewellyn[1], James G. Tokuhisa[1], Jill Wahleithner[1,2] and W. James Peacock[1]. [1]C.S.I.R.O. Division of Plant Industry, GPO Box 1600, Canberra, A.C.T. 2601, Australia, [2]Department of Biochemistry and Biophysics, U.C. Davis, Davis CA 95616. The ocs-element is a 20 bp DNA sequence that is a functional component of the promoters of several plant pathogens. A maize cDNA has been isolated that encodes a protein, OCSBF-1, that binds specifically to an ocs-element sequence. The 21 kD OCSBF-1 protein is encoded by a single copy, intron-less gene that is differentially expressed in maize plants; developing leaves have a gradient of OCSBF-1 transcripts with the basal portion of the leaves containing 10-fold higher levels of OCSBF-1 transcripts than the apical portions. OCSBF-1 has a small basic amino acid region which has homology to the DNA-binding domains of the AP-1 family of transcription factors. In common with the AP-1 factors, OCSBF-1 encodes a potential leucine zipper adjacent to the DNA-binding domain. The amino-terminal 72 amino acids of OCSBF-1, encompassing the basic domain and leucine zipper motif, still binds to ocs-element sequences *in vitro*. OCSBF-1 binds to a site within each half of the ocs-element as well as to animal AP-1 and CREB sites. OCSBF-1 has similar but distinct ocs-element binding properties to the maize transcription factor OCSTF, isolated from maize nuclear extracts, which also binds to the ocs-element.

R 150 TRANSMISSION GENETICS OF NUCLEAR AND ORGANELLAR GENES UPON SOMATIC HYBRIDIZATION BY MICROFUSION OF DEFINED PROTOPLAST PAIRS, G. Spangenberg, M. Osusky, E. Freydl, J. Nagel, M. M. Oliveira* and I. Potrykus, Swiss Federal Institute of Technology, Institute of Plant Sciences, CH 8092 Zürich, Switzerland, * Faculdade De Ciencias, Lisboa, Portugal

Somatic hybrid/cybrid plants produced by one-to-one electrofusion of defined selected protoplast+protoplast and protoplast+cytoplast pairs (**microfusion**) of male fertile N. tabacum and different cms tobacco alloplasmic lines and microculture of the fusion products were analyzed for their organellar and nuclear composition. The fate of chloroplasts was assessed by streptomycin resistance/sensitivity assay using somatic leaf tissue (Ro generation) and R1 seedlings. For the analysis of mitochondrial (mt) DNA, species specific patterns were generated by Southern hybridization of restriction endonuclease digests of mtDNA and total DNA with DNA probes of N. sylvestris mitochondrial origin. Nuclear fate was characterized by Southern hybridization analysis as well as expression assays for the functionality of selectable marker genes: npt II (Km^r) and hph (Hm^r) previously introduced by direct gene transfer into the parental nuclear genomes. In addition, flower morphology from independent regenerants was analyzed by developmental histology and SEM studies on floral bud formation. In this way, transmission genetic analysis concerning organellar and nuclear fate, as well as experimental evidence for the predictable transfer under controlled conditions of agronomical traits (eg. cytoplasmic male sterility) in somatic hybrids/cybrids obtained by microfusion of defined preselected protoplasts and subprotoplasts is presented for the first time.

R 151 TRANSIENT EXPRESSION FROM MICROPROJECTILE-MEDIATED DNA TRANSFER IN PINUS TAEDA L.
Anne-Marie Stomp* Arthur Weissinger and Ronald R. Sederoff* Departments of Forestry* and Crop Science, North Carolina State University, Raleigh, NC 27695
Transfer of plasmid DNA to Pinus taeda L. (loblolly pine) cotyledonary cells by microprojectile bombardment has been demonstrated using beta-glucuronidase (GUS) as an unselected marker. Histochemical staining for GUS indicated the presence of active enzyme in localized centers (blue spots) 24 hours after bombardment. GUS expression declined as a function of time in culture, but could be detected 62 days post-bombardment. The number of GUS-positive blue spots was a function of the developmental stage induced in culture. The highest frequencies of GUS-positive blue spots correlated with the developmental switch from cotyledon to meristem, as determined by changes in the patterns of cell division. Serial sections revealed that localized expression centers resulted from predominantly single-cell events. The staining pattern of GUS-positive blue spots was complex, with indigo found in both the central target cell and in adjacent cells. Indigo appeared compartmentalized within cells. GUS-positive cells containing one, multiple, or no microprojectiles have been identified. Cellular damage sustained by GUS-positive cells ranged from virtually non-detectable to sufficiently extensive to presumably cause cell death. Microprojectile bombardment provides a useful method with which to assay transient gene expression in loblolly pine and has potential for the production of transgenic plants in this valuable crop plant.

R 152 DIFFERENTIAL GENE EXPRESSION DURING CULTIVAR/RACE-SPECIFIC INTERACTIONS BETWEEN POTATOES AND PHYTOPHTHORA INFESTANS, Guenter Strittmatter, Norbert Martini and Klaus Hahlbrock, Dept. of Biochemistry, MPI fuer Zuechtungsforschung, D-5000 Koeln 30, FRG.
Infection of Solanum tuberosum with Phytophthora infestans results either in a compatible (host susceptible/fungus virulent) or an incompatible (host resistant/fungus avirulent) interaction. Our interest focuses on the role of transcriptional regulation during the early decisive stages of cultivar/race-specific interactions. So far, three clones of host genes with a differential mRNA accumulation in compatible and incompatible interactions 1 hour postinoculation were found. Two of them were isolated by differential screening of a genomic library from potato cultivar Datura; the corresponding mRNAs are predominantly accumulated in the incompatible interaction. The third clone was identified by the use of a heterologous probe for an elicitor-inducible gene from parsley cell cultures. In potato cultivar Datura, the homologue to this gene is preferentially expressed in a compatible interaction 1 hour postinoculation. The accumulation of mRNA for PR1, a PR protein from potatoes with striking similarity to a soybean heat shock protein, starts at a later time point and with no significant difference between the two types of interaction. These genes are used as test genes to identify cis elements and the corresponding trans factors responsible for differential mRNA accumulation during early time points of infection. In parallel, clones for putative transcription factors were isolated by screening a genomic library from potato cultivar Datura with heterologous probes for conserved domains in known transcription factors. The goal with these clones is to find members in the respective transcription factor groups involved in the transcriptional regulation during cultivar/race-specific interactions.

R 153 AN RFLP MAP OF THE SOYBEAN GENOME, Scott Tingey and Antoni Rafalski, Biotechnology Research, Agricultural Products Department, E.I. du Pont de Nemours and Co (Inc.) P.O.Box 80402 Experimental Station, Wilmington, DE 19880-0402, Tel. (302) 695-7252.
The genetics of soybean, one of the major U.S. and world crops, is relatively underdeveloped in comparison with other crop plants like corn, tomato or wheat. To partly remedy this we created a genetic map of the soybean genome using RFLP markers. Over three thousand low copy number soybean genomic clones were used to identify polymorphisms between Bonus, a cultivar of *Glycine max* and PI 81762, an accession of *Glycine soja*, using five restriction enzymes. Only approximately 15% of the probes revealed polymorphisms, which were subsequently mapped in a population segregating from a cross of Bonus X PI 81762. Over four hundred RFLP markers, including several cloned genes were mapped to date in the soybean genome. These markers define 23 linkage groups, and a total of 2700 cM of DNA. The average distance between markers is 7.2 cM, with only three gaps larger then 37 cM. Our estimate places 95% of the genome within 3.5 cM of a RFLP marker. The detailed genetic map of soybean will be useful in an effort to map agronomically important genes.

R 154 PLANT CELL TRANSFORMATION USING HIGH VELOCITY MICROPARTICLES, Rubén H. Vallejos, Mónica López, Martin I. Reggiardo, Lelia M. Orsaria, Hugo R. Permingeat, Marcelo A. Spitteler and José Luis Arana, Centro de Estudios Fotosintéticos y Bioquímicos (CONICET, F.M.Lillo, U.N.R.), Suipacha 531, 2000 Rosario, Argentina.

Cell penetration employing high velocity microparticles coated with DNA is one of the more recently developed methods for plant cell transformation. The present work shows the results obtained with this technique in the transient transformation of maize, tobacco, sunflower and wheat cells. Coleoptiles isolated from maize kernels were incubated in N6 medium supplemented with 10 μM 2,4-D. Basal leaf segments obtained from the two inner leaves of 25 days old maize plantlets were cultured in SH medium in the presence of 30 μM Dicamba. Tobacco leaf discs were placed abaxial side up on MS medium. After 48 h at 26 °C the materials were bombarded in a partial vacuum with tungsten microparticles coated with the plasmid pBI 221 or PAI1 GUSN that contains the GUS coding region. Transiently transformed cells were detected by histochemical assay. Coleoptiles showed an average of 20 transformed cells per cm^2. In tobacco leaf discs and in sunflower hypocotyl segments, transformed stomata occlusive cells were frequently observed. The expression of a foreign gene in basal leaf segments of maize and in wheat embryos was also observed. The results are in agreement with those obtained in other laboratories and suggest that this technique should be useful in obtaining transgenic plants from species which, like gramineae, are difficult to transform by other methods.

R 155 APPLICATION OF THE POLYMERASE CHAIN REACTION IN BACKCROSS BREEDING Edward A. Weck, Douglas F. Mead, Christine L. Bredenkamp, Michael M. Kiefer, Diana Beckman and *Douglas Roberts, Northrup King Research Center, Stanton, MN 55081 and *Sandoz Crop Protection, Palo Alto, CA 94304-1104 Traditional backcross breeding theory estimates a 99% recurrent parent recovery after 6 generations of backcrossing. Restriction Fragment Length Polymorphism (RFLP) analysis of near isogenic corn lines created with the single genes Rp1d and Ht1 indicates that actual levels of recurrent parent recovery are 89-91% after 6 backcross generations. The utilization of RFLP analysis should allow plant breeders to more closely monitor specific genomic segments in backcross breeding programs. In addition, a more rapid conversion to the recurrent parent genotype should be feasible. The analysis of a backcross 2 population with RFLP markers will be presented. The applicability of PCR in this backcross 2 population will be presented by comparison with with RFLP probe data.

R 156 COMPARISON OF GENETIC MAPS OF TWO *LACTUCA* SPECIES. Lore Westphal, Richard V. Kesseli and Richard W. Michelmore, Department of Vegetable Crops, University of California, Davis, CA 95616

Genetic maps of *Lactuca sativa* and *Lactuca saligna* were compared in order to study their evolutionary relationship. Genetic maps of two intraspecific crosses were developed by analyzing linkage between resistance genes, morphological markers, isozyme, and RFLP loci. The detailed map of *L. sativa* includes over 170 loci, most of them are distributed into 12 linkage groups. The preliminary map of *L. saligna* contains more than 50 loci and is divided into at least 9 linkage groups. Corresponding linkage groups of both species differ with regard to their genetic distances and, in some cases, to the localization of particular loci, indicating genomic rearrangements during the evolution of *L. sativa* and *L. saligna*.

R 157 SEPARATION OF MEGABASE-SIZED PLANT DNA BY PULSED-FIELD GEL ELECTROPHORESIS, Fang-sheng Wu and Bruce Cahoon, Department of Biology, Virginia Commonwealth University, Richmond, VA. 23284-2012. Pulsed-field gel electrophoresis(PFGE) was used to separate megabase-sized DNAs from several different plant species including Brassica oleracea, B. napus, Zea mays, petunia, potato, and rice. Protoplasts were isolated and purified from either mesophyll of plant or hypocotyls of etiolated seedlings and were embedded in agarose as agarose plugs. These plugs were then treated with proteinase K and detergents to lyse the protoplasts and release the DNA before they were subjected to PFGE using a contour clamped homogeneous electrophoresis(CHEF) system. A number of parameters including sample treatment, pulse length, pulse length ramping, and electrophoretic voltages were tested to optimize the separation of DNAs with different sizes. Using intact chromosomal DNA from Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Plasmodium falciparum as standards, the sizes of plant DNAs that were separated by PFGE ranged from 200 to over 4,000 kilobase pairs. The chromosomal DNA of cyanobacteria can also be separated by the CHEF system. The agarose plugs containing intact chromosomal DNA can also be digested with octanucleotide-recognizing restriction enzymes to produce large DNA fragments which can then be separated by PFGE, transferred to nitrocellulose membranes, and hybridized to specific DNA probes.

R 158 RFLP'S IN POLYPLOIDS : ESTABLISHING LINKAGES AND DISTINGUISHING AUTO FROM ALLO-POLYPLOIDS. Wu K.K. , W.L. Burnquist, M.F. Sorrells, T.L.Tew, P.H. Moore, and D.J Heinz. Hawaiian Sugar Planters' Association, HI., Copersucar SP, Brazil, and Dept of Plant Breeding, Cornell University, NY.14853. Construction of RFLP linkage maps in diploid species can now be considered a straightforward procedure. RFLP likage maps in polyploid species , however have not been developed with exception to those established on diploid relatives. Complex segregation patterns and the inability to identify RFLP genotypes, due to the fact that individual bands may actually represent single, double or multi-dose bands have impeded the progress of polyploid mapping. To overcome these problems, individual bands from RFLP phenotypes need to be analysed. Bands can be identified as single, double or multi-dose by the observed segregation of the band in progeny evaluations.
In this study, a hypothetical octoploid species is used to illustrate the procedures for identifying single-dose bands, detecting linkages, and distinguishing allo- from auto-octoploidy, without prior knowledge of the allelic relationships between the RFLP bands. Population size required, probability of linkage, and attributes of different mapping populations are discussed. Population size required for detecting single-dose bands with 99% level of confidence is 75. A population size of 75 also is adequate for distinguishing linkages with cross over rate of 0.25 from cross over rate of 0.5 at the 99% level of confidence. If the number of linkage pairs determined to be in coupling phase is about equal to that in repulsion , the species can be considered an allo-polyploid species; and if none of the linkage pairs detected are in repulsion phase, it can be considered an auto-octoploid species. For the mapping population, one parent should have high heterozygosity to assure a high frequency of single-dose bands and the second parent should have low level of heterozygosity to increase the chance for detecting unique bands in the first parent. The procedures should be applicable to different levels of ploidy with minor modifications.

R 159 RAPID PROLIFERATION OF *ACTIVATOR* (*Ac*) IN TRANSGENIC TOMATO John I.Yoder, Department of Vegetable Crops, University of California-Davis, Davis, CA 95616. The maize transposable element *Ac* is somatically active in transgenic tomato plants. I have examined the segregation behavior of transposed *Ac* elements in successive generations of transformants containing single copies of *Ac* and found that *Ac* can rapidly proliferate in the tomato genome. In one transgenic line, the number of Ac elements increased from one to over twenty in only two generations. Within a plant, each copy was at a unique chromosomal location, suggesting copy number increases are associated with transposition. Amplified copies do not appear by Southern analysis to be structurally defective. Lines with large numbers of *Ac* elements have reduced fertility and vigor while isogenic siblings with few or no elements are normal. These lines will be useful for studying the evolution of transposable element families and for examining mechanisms by which plant genomes adapt to invasion by foreign transposable elements.

R 160 A PROTEIN SERINE/THREONINE KINASE FROM MAIZE IS RELATED TO THE TYROSINE KINASE GROWTH FACTOR RECEPTORS, Ren Zhang and John C. Walker, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.
The protein kinase family of enzymes mediates the response of eukaryotic cells to both inter- and intracelluar signals. In many organisms a great deal is known about both the structure and function of the two classes of protein kinases, the serine/threonine-specific and the tyrosine-specific. However, in higher plants our knowledge of these important enzymes lags behind. We have undertaken a molecular biological approach to identify serine/threonine-specific protein kinases in maize and characterize the primary sequences of these enzymes to obtain insights into their structure and possible involvement in signal transduction. We have used oligonucleotides directed against conserved sequences in the catalytic domains of the serine/threonine-specific protein kinases as PCR primers to clone members of this family from maize. One of the cDNA clones that we have isolated is homologous to a 2800 nucleotide mRNA that is expressed in both root and shoot tissues. The deduced amino acid sequence derived from the sequence of this cDNA clone has 25% identity to the EGF receptor and 53% homology when conservative amino acid substitutions are taken into account. In addition, this putative protein kinase consists of three structural domains that are reminiscent of the tyrosine kinase growth hormone receptors: a cysteine-rich sequence that may function as a extracellular ligand-binding domain, a single hydrophobic membrane spanning segment and an intracellular domain that includes the sequences specifying a serine/threonine-specific protein kinase. These structural features suggest that this maize protein kinase is involved in signal transduction processes.

Crop Improvement and Environmental Stress

R 200 EXPRESSION OF SUPEROXIDE DISMUTASE GENES IN PLANTS SUBJECTED TO ENVIRONMENTAL STRESS, Randy D. Allen, Luis Wong-Vega, Sibel H. Isin and Phat M. Dang. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409
Superoxide dismutase (SOD) is a key enzyme in the reactive oxygen detoxifying system of aoerbic organisms. Superoxide radicals (O^-), which are normally formed in plants during cellular metabolism, increase dramatically under stressful conditions. These radicals react directly or indirectly with bio-molecules to damage essentially all cellular components. Superoxide dismutase catalyzes the dismutation of O^- to hydrogen peroxide and molecular oxygen and thus forms the first line of defense against this toxic species. Increased levels of superoxide dismutase have been found to have protective function in plants exposed to environmental insults such as paraquat, photo-oxidation, pollutants and temperature extremes. We have investigated the expression of specific SOD genes in response to environmental stress using SOD cDNAs. These results are being used to help us understand the role of various SOD isoforms in the development of stress tolerance in plants. This knowledge can then be used in attempts to optimize the stress adaptation mechanisms of crop species.

R 201 TRANSGENIC TOBACCO RESISTANT TO A BACTERIAL DISEASE BY THE DETOXIFICATION OF PATHOGENIC TOXIN, Hiroyuki Anzai, Katsuyoshi Yoneyama* and Isamu Yamaguchi*, Pharmaceutcal Research Center, Meiji Seika Kaisha,Ltd. Morooka-cho,Yokohama,222,Japan, * The Institure of Chemical and Physical Research(RIKEN),2-1 Hirosawa,Wako,351-01,Japan
Some plant pathogens produce toxins which cause disease in infected plants. One of the pathogenic toxins, tabtoxin, is produced by *Pseudomonas syringae* pv. *tabaci*, which causes wildfire of tobacco. A tabtoxin resistance gene (*ttr*) coding for an acetyltransferase isolated from *P.syringae* pv. *tabaci* was fused to the 35S promoter of the cauliflower mosaic virus(CaMV) to construct a chimeric gene for introduction into tobacco cells by *Agrobacterium*-mediated transformation. The transgenic tobacco plants showed a high specific-expression of the *ttr* gene and no chlorotic symptoms caused by tabtoxin treatment or with infection by *P.syringae* pv. *tabaci*. These results demonstrate a successful approach to obtain disease-resistant plants by detoxification of the pathogenic toxins which play an important role in pathogenesis.

R 202 AMPLIFICATION OF A MUTANT ACETOHYDROXYACID SYNTHASE GENE IN A SULFONYLUREA RESISTANT TOBACCO CELL LINE, Susan L. Armour, Joe J. DiMaio, Raymond D. Shillito, Christian T. Harms, and George C. Jen, CIBA-GEIGY Corp. Agricultural Biotechnology Research Unit , P.O. Box 12257, Research Triangle Park, NC 27709-2257.

Mutagenized tobacco cell lines resistant to a sulfonylurea (SU) herbicide CGA131'036 were isolated and screened for strong cross-resistance to a second structurally dissimilar SU herbicide CGA136'872. The SU27 cell line was identified to have broad resistance to both these and several other SU herbicides. Southern analysis showed the tobacco SuRB AHAS gene was amplified approximately 20 fold in SU27 cells. A lambda library of the SU27 DNA was constructed, and multiple independent clones of the SuRA and SuRB AHAS genes were isolated. DNA sequencing showed all SuRB AHAS gene clones to contain an identical missense mutation in the AHAS coding sequence. No mutations were detected in the SuRA AHAS gene clones. The SU-resistant states of the AHAS gene clones were assayed by tobacco cell transformations. These studies showed that all of the SuRB AHAS gene clones and none of the SuRA AHAS gene clones gave rise to SU-resistant transformants.

R 203 RELATIONSHIP OF A HEAT-INDUCIBLE PEPTIDE TO THE WHEAT STORAGE PROTEIN GLIADIN, *+Caron S. Blumenthal,+Ian L. Batey,+Frank Bekes,+Colin W. Wrigley and #*Snow Barlow, *Department of Biological Sciences, Macquarie University, North Ryde 2109, Australia; +CSIRO Division of Plant Industry, Wheat Research Unit, North Ryde 2113, Australia; #Faculty of Horticulture, University of Western Sydney, Richmond 2753, Australia. Australian wheat varieties produce a 14 amino acid peptide (Mr 1550) in addition to the normal group of heat shock proteins in response to temperatures in excess of 40°C. This peptide is produced in response to heat-shock, ethanol and sodium arsenite in wheat coleoptiles, leaves and roots. Under heat shock conditions, the production of the peptide was directly related to the thermotolerance exhibited by a particular cultivar and also to an individual cultivar's reputation for heat tolerance in the field. The sequence of this peptide is homologous to the N-terminal of the wheat storage protein alpha/beta gliadin. Quantitation of the peptide production under heat shock conditions in a range of Chinese Spring aneuploids revealed that the peptide gene was located on the same chromosome arm as one of the gliadin genes. Examination of the published nucleotide sequences for the alpha/beta gliadin gene revealed the presence of 5 heat-shock promoter elements upstream of the TATA box. Evidence for the heat-inducibility of the gliadin gene was obtained from head culture experiments where preferential incorporation of ^{14}C-amino acids into gliadin occurred under heat shock conditions. Further evidence was obtained from glasshouse and field trials where a high temperature stress during grain filling increased the gliadin content of the flour produced from the wheat grain. These changes were also cultivar specific. These results indicate that wheat quality as well as yield must be considered, when evaluating the possible role of heat shock proteins in protecting wheat under high temperature conditions.

R 204 STRATEGIES FOR PRODUCING NON-TOXIC VARIANTS OF CERATO-ULMIN, A DUTCH ELM DISEASE TOXIN, USING SYNTHETIC DNA, Mark G. Bolyard and Mariam B. Sticklen, Pesticide Research Center, Michigan State University, East Lansing, MI 48824-1311.
Dutch elm disease (DED) is responsible for the destruction of millions of susceptible elms throughout the world. The fungus Ceratocystis ulmi, which is responsible for DED, produces a pathogenic toxin known as Cerato-ulmin (CU). Our approach to further characterizing this toxin is to determine relationships between structure and toxicity through biochemical means, then to exploit these findings by producing mutated peptides using recombinant DNA technology. Preliminary assays were performed, using native CU (provided by S. Takai), to determine the extent of denaturation and reduction, followed by dialysis against water, which would return the toxin to its active form. Native CU is extremely insoluble and reports indicate that soluble material is not toxic [Stevenson et al., Phytochemistry 18:235-238, 1979]. Treatment with 8 M urea or 10 mM 2-mercaptoethanol produced insoluble material following dialysis, but the two treatments together produced a soluble peptide. As disulfide bonds are believed to be produced co-translationally, the removal of cysteines which form disulfide bonds may yield a non-toxic molecule. We have assembled oligonucleotides, based on the amino acid sequence of the native peptide (Yaguchi et al., manuscript in preparation; provided by Dr. S. Takai), which encode recombinant CU. We plan to insert the synthetic DNA into pGEX-2T (Pharmacia) to express CU as a fusion with glutathione-S-transferase (GST). This is done to maintain stability and to facilitate purification of the fusion from the remaining bacterial proteins using a glutathione-Sepharose column. GST can be separated from CU by treatment with thrombin, then cyanogen bromide can be used to remove residual amino terminal amino acids. In addition, we have synthesized oligos to replace the cysteines with serines to examine the effect of the loss of disulfide bonding on the toxicity of the peptide. Toxicity studies are being performed in collaboration with Drs. S. Takai and W. Richards.

R 205 THE ROLE OF SUPEROXIDE DISMUTASE IN PROVIDING STRESS TOLERANCE IN PLANTS, Chris Bowler, W. Van Camp, E. Tsang, M. Van Montagu and D. Inzé, Laboratorium voor Genetica, Rijksuniversiteit Gent, B-9000 Gent (Belgium)

Superoxide dismutases (SOD) are metalloproteins which catalyze the formation of hydrogen peroxide from the superoxide radical ($O_2^{\cdot-}$). Since many stress conditions have been shown to generate highly reactive, reduced oxygen species such as superoxide radicals, our ultimate aim is to determine whether a plant which has higher levels of SOD is better protected against the stresses imposed on it by the environment.

As a first step in this study we have characterized the SOD enzymes present in *N. plumbaginifolia*. In summary, we have found a manganese SOD (MnSOD) within the mitochondria, copper/zinc SOD (Cu/ZnSOD) located in the cytosol and an iron SOD (FeSOD) present in the chloroplasts. cDNAs encoding all of these have now been isolated and characterized. We have found that each *SOD* gene is highly responsive to stress conditions; however, they are all differentially regulated, reflecting the fact that each enzyme is protecting a different subcellular compartment against superoxide radical-mediated damage.

We have constructed expression vectors designed to target these SODs to mitochondria, cytosol, and chloroplasts. Transgenic plants have now been obtained which overexpress SOD in one or more compartments of the cell. As a model system to study oxidative stress we have been using methyl viologen, a compound known to generate superoxide radicals in vivo. Our data shows that overexpression of SOD can protect the photosynthetic pigments and the membranes against methyl viologen-induced damage. We are currently assessing the behaviour of these plants to other more natural stress conditions.

Bacillus thuringiensis (BT) subsp. *aizawai* strain EG6346, a novel grain dust isolate, has been analyzed by Southern blot hybridization for its toxin gene profile. Strain EG6346 lacks typical *cryIA* toxin genes, yet does possess novel related toxin gene sequences. A recombinant genomic plasmid library has been constructed for strain EG6346 in *Escherichia coli*. One recombinant plasmid, pEG640, which was isolated from the library contains an apparently novel toxin gene, designated *cryIE*, related to, but distinct from, the published sequences for other *cryI* genes. A second truncated novel toxin gene sequence is also located on pEG640, approximately 500 bases downstream from *cryIE*. Introduction of *cryIE* into a *Cry-* BT recipient strain via electroporation has enabled production of purified protein for bioassay analyses. Results of the sequence analyses coupled with insect bioassay data confirm *cryIE* as a unique P1-type BT toxin gene with possible utility for the construction of insect resistant transgenic plants.

R 207 STRUCTURE AND BIOCHEMISTRY OF A LOW MOLECULAR WEIGHT CHLOROPLAST HEAT SHOCK PROTEIN, Qiang Chen and Elizabeth Vierling, Department of Biochemistry, University of Arizona, Tucson, AZ 85721. In plants, low molecular weight (LMW) chloroplast-localized HSPs have been found in many species. In our laboratory, cDNA clones for chloroplast LMW HSPs from pea, petunia and *Arabidopsis* have been sequenced. Comparison of LMW chloroplast HSPs from five divergent plant species reveals that the proteins show 60% identity and 80% similarity. Two major conserved domains have been identified in these chloroplast HSPs: A carboxyl-terminal domain which is homologous to cytoplasmic HSPs of plants and other eukaryotes; and an amino-terminal domain which forms an amphipathic α-helical "methionine bristle". To study HSP21 structure and expression, antibodies against pea HSP21 have been produced. HSP21 cannot be detected at non-stress temperatures, but accumulates in both leaves and roots during heat stress. We estimate that HSP21 accumulates at least 200-fold during stress at 38°C, and that the protein has a half-life of 52 hours. The majority of HSP21 is found in the soluble protein fraction of the chloroplast in a 200 kD particle. Similar HSP21 particles have also been identified in pea roots. Import of *in vitro* translated HSP21 into chloroplasts revealed that HSP21 could assemble into the 200 kD complex in isolated organelles. The HSP21-containing particle is being purified to determine if HSP21 is associated with other chloroplast proteins. We suggest that the 200 kD complex is the functional form of HSP21 and that HSP21 functions in a way similar to LMW cytoplasmic HSPs which also form high molecular weight particles. These data also suggest that HSP21 functions in both photosynthetic and non-photosynthetic plastids during stress and recovery from stress.

R 208 COLD-INDUCED CHANGES IN GENE EXPRESSION IN SUGAR BEET.

Sue K. Crosthwaite & Gareth I. Jenkins. Departments of Biochemistry and Botany,University of Glasgow, Glasgow G12 8QQ, Scotland, UK.

We are interested in changes in gene expression involved in cold acclimation and vernalization in sugar beet. To monitor changes in gene expression we have used two-dimensional gel electrophoresis to examine accumulated leaf proteins and *in vitro* translation products of RNA. We have compared changes in gene expression in the biennial sugar beet, which requires vernalization in order to flower, with the annual which does not.

Transfer of plants from normal temperatures into the cold consistently induces the accumulation of two polypeptides of 21-22 kDa, pI approx. 3.5. Both proteins are in the medium range of abundance. These proteins can be detected after two days of low temperature treatment and persist for at least two months. They are present in leaves of all ages. We have found that the proteins are induced by cold in both the annual and biennial and it is therefore unlikely that they are involved in the vernalization response. The proteins also appear following wounding, but not heat shock, and may therefore be representatives of a class of general stress proteins. Two-dimensional gels of *in vitro* translation products of RNA extracted from young leaves of biennial plants show several changes in response to cold; some translation products are induced by the cold treatment while others decrease. These proteins are being investigated by molecular cloning.

R 209 INDUCTION OF CAM BY SALT STRESS: REGULATION OF THE PEPCase GENE FAMILY IN *MESEMBRYANTHEMUM CRYSTALLINUM*.

John C. Cushman, Christine B. Michalowski, Michael S. Meiners and Hans J. Bohnert. Department of Biochemisty. University of Arizona, Tucson, AZ 85721 USA.

Salt stress induces a shift from C3 photosynthesis to Crassulacean Acid Metabolism (CAM) in the facultative halophyte, *Mesembryanthemum crystallinum*. One of the central enzymes of CAM, phosphoenolpyruvate carboxylase (PEPCase; E.C. 4.1.1.31), catalyzes the fixation of CO_2 (HCO_3^-) into oxaloacetate (OAA). OAA is subsequently converted into malate and stored in the vacuole. We have characterized two members of the PEPCase gene family in *M. crystallinum*. Only one member of this small gene family (designated *Ppc1*) responds to salt stress via transcriptional activation. In order to understand the basis of this differential transcriptional activation, we have begun to examine the interaction of DNA-binding proteins to the 5' upstream region of *Ppc1*. In addition, we are studying the expression of this promoter region in transgenic glycophytic species. In *N. tabacum*, the *Ppc1* promoter yields expression of a GUS reporter gene at levels comparable to that obtained with the 35S CaMV promoter. Understanding the mechanisms of gene activation due to salt stress will help gain insight into how certain plants cope with environmental stresses such as salinity and drought. Supported in part by grants from the NSF, USDA, and the Arizona Agricultural Experiment Station.

R 210 TRANSCRIPTION OF PROLINE BIOSYSNTHESIS GENES IN CYANOBACTERIA GROWN UNDER DIFFERENT SALT CONCENTRATIONS. **Henry Daniell, Mehdi M. Naderi, and Leneon Wang, Department of Biological Sciences, University of Idaho, Moscow, ID 83843.** **Many species of cyanobacteria accumulate organic compounds under salt stress. Different organisms, bacteria, plants, and some marine animals increase the cytoplasmic proline concentration in response to environmental osmotic stress. In enteric bacteria, production of proline from glutamate is sequentially mediated by the gene products of proB (γ-glutamyl kinase), proA (glutamate semialdehyde dehydrogenase), and proC (1-pyrroline 5-carboxylate reductase). In this study, Southern analysis has revealed that proB is located in the genomes of Anacystis nidulans and Synechosystis 6803. Therefore, bacterial proB gene has been used as a probe to analyze the rate of transcription of proB gene in Anacystis nidulans and Synechosystis 6803 grown under different salt concentrations. We have developed a new method for improved isolation of intact cyanobacterial RNA. Size fractionation of total RNA has been accomplished by rate zonal centrifugation in linear sucrose gradients to enrich proB mRNA. Northern analyses are in progress to quantify mRNA levels of proB in cyanobacterial cells grown under different concentrations of NaCl.** **This study is supported by NSF-EPSCoR Grant RII-8902065 to H. Daniell.**

R 211 CLONING AND CHARACTERIZATION OF A MAIZE METALLOTHIONEIN GENE, Anic de Framond and Michael Koziel, Department of Plant Molecular Biology, CIBA-GEIGY Biotechnology Research, P.O Box 12257, Research Triangle Park, NC 27709-2257

A maize gene, expressed differentially throughout the plant has been cloned and sequenced. The 8 kD protein encoded by this gene was identified as a metallothionein-like protein after comparison with the putative pea metallothionein (MT) sequence recently described by Evans et al. (1) The corn MT gene is very strongly transcribed in roots without exposure to high levels of metals that are known to regulate mammalian MT genes. Moderate levels of transcription are found in green leaves , and low levels in pith and anther. Genomic Southern analysis indicates that there is a related gene in the corn genome, which cross-hybridizes weakly with the MT gene. The activity of the MT gene promoter , fused to the GUS reporter gene, was analyzed in transgenic tobacco. The pattern of expression of this chimeric gene in tobacco is being compared with that of the native gene in maize.

(1) Marta Evans, Laurence N. Gatehouse, John A. Gatehouse, Nigel J. Robinson, Ronald R.D. Croy , 1989 submitted

R 212 HSP70 EXPRESSION DURING SEED DEVELOPMENT. Amy DeRocher[1], Lisa Lauzon[2], Elizabeth Vierling[1,2], Departments of [2]Biochemistry and of [1]Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721. In eukaryotes, HSP70 (Heat Shock Protein, 70 kDa) is encoded by a multigene family with stress inducible and constitutively expressed members. HSP70s are thought to assist in the proper folding of nascent or misfolded proteins in both stressed and unstressed tissue. Since seed development presents unusual physiological conditions; desiccation, and the accumulation of seed storage components, we wanted to know what role these HSPs might play in development. We have cloned and sequenced cDNAs encoding two HSP70 homologues of pea (Pisum sativum): a 70 kDa protein present in unstressed, vegetative tissues (HSC70), and a 72 kDa protein which accumulates in vegetative tissues only after heat stress (HSP72). In order to study the expression of these proteins during development, peas were grown to maturity in a growth chamber on a 16hr/8hr, 18C/14C day/night cycle. HSP72 and HSC70 mRNAs are present in developing seeds and during early phases of imbibition, as are proteins which co-migrate with HSP72 and HSC70 on western blots. HSP72 first appears during the storage protein accumulation phase and peaks in the mature seed. It is expressed in both the axis and the cotyledon of the developing seed, but not in the seed coat or pod. HSP72 persists in the imbibing seed through radical emergence. HSC70 is expressed in both the embryonic and maternal tissues throughout development, but the amount of HSC70 in the axis and cotyledon appears to decrease as HSP72 increases.

R 213 FIELD EVALUATION OF GLUFOSINATE TOLERANT CROPS BEARING A MODIFIED PPT-ACETYLTRANSFERASEGENE FROM STREPTOMYCES VIRIDOCHROMOGENES

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L-glufosinate (L-Phosphinothricin = PPT), the active ingredient of the herbicide Basta® can be inactivated by N-acetylation. Highly specific PPT-acetyltransferases are known from the aminoacid sequence of the Streptomyces viridochromogenes PPT-Acetyltransferase (PAT), a gene has been synthesized with a codon usage optimized for expression in plants. This synthetic gene was fused between the 35S promotor and terminator of Ca MV and transferred to crop plants via Agrobacterium tumefaciens. Field tests with transgenic tobacco and alfalfa plants were performed. The tobacco plants were completely protected against high rates of glufosinate. Even though the transgenic alfalfa plants expressed the PAT gene at lower levels, they were not visibly damaged by a Basta application at a rate which is equivalent to 2 kg glufosinate/ha. Data on the molecular characterization and field performance of the two species will be presented.

R 214 SEQUENCE COMPARISON OF THE COAT PROTEIN GENE OF CASSAVA COMMON MOSAIC VIRUS WITH OTHER POTEXVIRUSES SHOWS COMMON FEATURES AND SUGGESTS IMPORTANT SEQUENCES FOR THE COAT PROTEIN GENE EXPRESSION IN TRANSGENIC PLANTS, Claude Fauquet, Lee Calvert, Didier Bogusz, Claudine Franche, Christian Schopke and Roger N. Beachy, Department of Biology, CB 1137, Washington University, St Louis, Mo 63130, USA.

Cassava common mosaic virus (CCMV), a potexvirus infecting cassava in several South American countries, is one of the viral targets of the International Cassava-Trans Program. The goal of the research is to produce transgenic cassava plants resistant to viruses using the coat protein mediated protection strategy. About 50% of the 3' end of the CCMV genome has been cloned and the sequence of the last 1200 nucleotides has been completed. This sequence revealed only one major open reading frame corresponding to a protein of 229 amino-acids with predicted molecular weight of 25 K. This sequence includes the sequence of two peptides resulting from trypsin treatment of the purified viral coat protein. Comparison with sequences of coat proteins of five other potexviruses shows a relatively low percentage of homology, varying from 41% to 65%, but also shows core regions that are highly conserved; reaching 75% homology. Upstream of the initiation codon (14 nucleotides), there is a sequence of 17 nucleotides that is present in all potexviruses upstream of the CP gene as well as of the 26K gene. No function has been assigned to this sequence but its involvement into the production of subgenomic messenger RNAs has been suggested. In order to apply the coat protein mediated protection strategy against CCMV to cassava plants, different chimaeric gene constructions with or without that sequence have been made and are under evaluation in transgenic plants.

R 215 CHARACTERISATION OF THE GENES ENCODING THE RUBBER ELONGATION FACTOR (REF) OF HEVEA BRASILIENSIS. Elisabeth Goyvaerts and Nam-Hai Chua*. Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511. *The Rockefeller University, New York, New York 10021-6399. In Hevea brasiliensis, the rubber particle, RP, is the site of rubber (cis 1-4 polyisoprene) biosynthesis. The most abundant RP protein is the 14 kDa REF that renders cis specificity to the polymerisation reaction (Light et al., Dennis and Light, JBC 264). In vitro expression of 710-800 bp REF cDNAs produce a 14 kDa protein, that is precipitated with anti-REF antibodies. Comparison of the amino acid sequence deduced from the cDNAs with the protein sequence data (Dennis et al. JBC 264) reveals that REF is not made as a precursor protein, indicating a cytoplasmic assembly of the RP. The cDNAs only differ at the site of polyA tail addition. Southern blot analysis of Hevea DNA shows 2 REF genes, both of which have been isolated and sequenced. They encode REF in 3 exons and their nucleotide sequences are 95% identical. The 3' untranslated region of gene1 contains a 17 bp AT rich insertion absent from gene2 and from the cDNA clones. Gene1 diverges from gene2 downstream of the first polyA addition site, 170 bp after the translation stop codon. The 5' untranslated leader is 130 bp long and a putative TATA box is located at -30. Northern blot analyses show that REF is expressed higher in younger than older tissue. The expression level decreases from stem to petiole to leave to root. REF expression is specific to the laticiferous cells within the phloem tissue. REF promoter-GUS fusions might be highly expressed in the phloem of transgenic plants. Extensive studies of REF expression in commercial rubber trees under different exploitation systems may enable us to use the pattern of expression as an early determinant of high rubber yield.

R 216 Alcohol dehydrogenase gene expression in Petunia

Robert Gregerson and Judith Strommer Department of Genetics, University of Georgia, Athens Georgia

Alcohol Dehydrogenase (Adh) genes have been isolated and used as probes for gene expression in a number of plant species. Every plant studied has at least one Adh gene whose expression is inducible by anaerobic treatment, and those plants with more than a single Adh gene show differential expression of Adh genes in various tissues. Petunia has two Adh genes, one of which is expressed predominantly in the pollen, anther and seed of the plant, and is also somewhat anaerobically inducible, and a second Adh gene which is highly induced by anaerobiosis in the root, stem and leaf of the plant. Several Adh-GUS fusions have been constructed to define the sequences involved in this regulation.

R 217 CHARACTERIZATION OF A PEA GENE WHOSE TRANSCRIPTION IS RAPIDLY INDUCED BY WILTING, Felix D. Guerrero, Jennifer Jones, John Mullet and Lyle Crossland,CIBA-GEIGY Biotechnology Research, Box 12257, Research Triangle Park, North Carolina, 27709-2257. Reduction of turgor in pea shoots caused the induction of several poly(A) RNAs. cDNA clones to three of the induced poly(A) RNAs were isolated and the expression of their corresponding genes studied. By 4 hours after wilting, the concentration of the three poly(A)s had increased 4-6 fold. Run-on transcription experiments using pea nuclei from wilted and non-wilted control shoots showed transcription of these RNAs was induced within 30 minutes following reduction of shoot turgor. The cDNA and genomic clone DNA sequence has been determined for one of the turgor responsive RNAs. The transcriptional start site has been mapped and promoter analysis initiated using gel retardation and DNA footprinting analysis.The genomic clone's 5' region has been subcloned upstream of the GUS coding region of the plant transformation vector pBI101 and expression of the GUS reporter gene studied in wilted transgenic tobacco plants.

R 218 PROTEINS WHICH INTERACT WITH THE 5' REGION OF A WHEAT ABA INDUCIBLE LEA CLASS GENE Mark J. Guiltinan and Ralph S. Quatrano, Department of Biology, University of North Carolina, Chapel Hill, NC 27599
The wheat Em gene is expressed in maturing embryos and in drought or osmotically stressed seedlings in response to elevated levels of the phytohormone abscisic acid (ABA). DNA sequences from the 5' region of this gene contain the signals necessary to confer ABA inducibility on a foreign gene.[1] Several AT rich sequences have been identified in the 5' region which are responsible for high levels of expression of the Em gene. In addition, a GC rich sequence has been shown to contain the signals necessary for ABA induction. Both of these sequences are bound by wheat embryo nuclear extracts in gel shift and footprinting experiments. Several cDNA clones have been isolated from a lambda gt11 expression library made from wheat embryo RNA which encode putative DNA binding proteins. One recognizes the AT rich sequences and two the GC rich ABA response element. Progress on the analysis of these clones will be discussed.

1) Marcotte et al., The Plant Cell 1:969-976, 1989. Supported by a USDA grant (89-37262-4456) to R.S.Q.

R 219 CLONING AND CHARACTERIZATION OF SALT-STRESS INDUCED GENES IN THE ROOTS OF LOPHOPYRUM ELONGATUM REVEALS THE EARLY RESPONSE, Patrick Gulick and Jan Dvorak, Department of Agronomy and Range Science, University of California at Davis, CA 95616. We have isolated eleven independent cDNA clones of genes which are induced in the roots of Lophopyrum elongatum within 6 hours after the initiation of salt stress. L. elongatum is a highly salt tolerant relative of cultivated wheat that is extremely interesting as a source of genes for the improvement of wheat because its salt tolerance has been shown to be expressed in the genetic background of wheat when its chromosomes are introduced into wheat by cytogenetic methods.

cDNA clones of salt stressed induced genes were isolated by +/- screening of an enriched cDNA library that was constructed by a novel application of formamide-phenol emulsion reassociation to a subtractive hybridization enrichment. The eleven genes show a similar regulation, typically showing enhanced expression within two hours and peak expression within six to twelve hours and a return to background level by 24 hours after the initiation of stress. The functional relationship of these genes to salt tolerance are unknown. Experiments to characterize their expression in sensitive and tolerant genotypes are in progress.

R 220 HERBICIDE - RESISTANT TRANSGENIC PLANTS CARRYING MUTATED ACETOLACTATE SYNTHASE GENES, Mary E. Hartnett, Chok-Fun Chui, C. Jeffry Mauvais, Raymond E. McDevitt, Susan Knowlton, Julie K. Smith, S. Carl Falco, and Barbara J. Mazur, E. I. du Pont de Nemours and Co., Inc., Agricultural Products Department, Experimental Station, Wilmington, Delaware 19880-0402

Acetolactate synthase (ALS) is the target enzyme for three unrelated classes of herbicides, the sulfonylureas, the imidazolinones, and the triazolopyrimidines. We have cloned the genes which specify acetolactate synthase from a variety of wild type plants, as well as from plants which are resistant to these herbicides. The molecular basis of herbicide resistance in these plants has been deduced by comparing the nucleotide sequences of the cloned sensitive and resistant ALS genes. By further comparing these sequences to ALS sequences obtained from herbicide-resistant yeast mutants, two patterns have become clear. First, the ALS sequences that can be mutated to cause resistance are in domains that are conserved between plants, yeast and bacteria. Second, identical molecular substitutions in ALS can confer herbicide resistance in both yeast and plants. These findings have been extended by oligonucleotide directed *in vitro* mutagenesis of plant ALS genes, followed by introduction of the mutated genes into sensitive plants. The herbicide-resistant transgenic plants so produced provide additional evidence for the commonality of mutations which specify herbicide resistance in ALS genes.

R 221 MOLECULAR STUDIES ON CLIMATE-INDUCED DORMANCY IN BARLEY

Freek Heidekamp, Rob Schuurink, Joke van Beckum and André Schram, Center for Phytotechnology RUL/TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

Some of the good malting barley varieties which are currently in use show high levels of post-harvest dormancy. Impaired germination caused by dormant barley seeds can cause severe problems in the malting process. It would therefore be very attractive if barley varieties became available which only show limited post-harvest dormancy. In order to identify such barley varieties a detailed insight into the physiological and molecular mechanisms of dormancy is needed.

The current knowledge on how dormancy in barley seed occurs is limited. It is however generally assumed that dormancy is generated during grain development. In this process the plant hormone ABA is thought to play an important role. As a first step towards a better understanding of the molecular mechanisms behind dormancy we investigated whether the degree of seed dormancy in a given barley variety can be reproducibly determined by the growing conditions. Malting barley varieties which showed different degrees of dormancy in practice were grown in phytotrons under climatic conditions which lead to high and low degrees of dormancy in the mature seed. In order to investigate whether this impaired germination is correlated with differences in gene expression, the expression pattern of proteins in developing seeds of plants which were grown under dormant or non-dormant conditions was studied. Furthermore, the expression patterns of genes responsive to ABA were studied in developing seeds of these plants. Results of these studies will be presented.

R 222 EXPRESSION OF LOW MOLECULAR WEIGHT HEAT SHOCK PROTEINS DURING SEED DEVELOPMENT, Kenneth W. Helm, Amy DeRocher, Lisa Lauzon and Elizabeth Vierling, Department of Biochemistry, University of Arizona, Tucson, Az. 85721.

We previously demonstrated that mRNAs encoding certain heat shock proteins (hsps) are present in quiescent pea (*Pisum sativum, L.*) embryos. We are studying the developmental expression of hsps to learn more about the function(s) of hsps, as well as about embryogenesis. We have isolated and sequenced three cDNA clones encoding low molecular weight (lmw) hsps of pea: hsp18, hsp17.7, and hsp22. Each cDNA is representative of a distinct, multigene family. These families of lmw hsps are found in all angiosperms surveyed. Multiple sequence comparisons of hsps within two gene families (represented by hsp18 and hsp 17.7) indicate that between species each family is 60-70% identical at the amino acid level. In contrast, within a species the amino acid sequence homology of the different gene families is not more than 40%. The derived amino acid sequence of hsp22 indicates that it possesses a signal sequence, and may be localized to a discrete subcellular compartment. These sequence data suggest each lmw hsp gene family is functionally unique. The mRNAs encoding hsp18 and hsp17.7 are present in mature pea embryos. Rabbit antibodies have been raised against a hsp18/TrpE fusion protein made in *E. coli*. A similar approach is being used to raise antisera against hsp17.7 and hsp22. Western analysis of developing pea embryos grown in a controlled environment demonstrates that hsp18 begins to accumulate by 18 days after flowering. The relative concentration of the protein continues to increase throughout embryogenesis and is maximal in the mature seed.

R 223 **VIRUS RESISTANCE IN POTATO CULTIVARS**, Marianne J. Huisman, Erik Jongedijk, Dinie Posthumus, André Hoekema, Lucy Molendijk, Peter J.M. van den Elzen, Ben J.C. Cornelissen. MOGEN International nv, Einsteinweg 97, 2333 CB Leiden The Netherlands. The commercial potato (*Solanum tuberosum*) cultivars Bintje, Desiree, and Escort were transformed efficiently after optimizing the conditions for regeneration from potato tuber discs. For transformation discs were cocultivated with *Agrobacterium tumefaciens* using a disarmed binary vector system. This system allowed the introduction of a chimaeric gene encoding the coat protein (CP) of potato virus X (PVX) into two cultivars most susceptible to this virus, Bintje and Escort. Expression levels of the PVX CP were determined. Those transgenic plants revealing the highest PVX CP expression levels were tested for PVX resistance in laboratory tests. Extensive field trials with PVX containing transgenic Escort and Bintje plants showed that 41% of the transgenic Bintje plants and 82% of the transgenic Escort plants are true to type. In the field trials, plants with low, medium and high expression levels for PVX CP were analyzed for 50 morphological characteristics as described in the UPOV guidelines for the conduct of tests for distinctiveness, homogeneity and stability. These characteristics comprise of 21 plant, 10 flower, 7 tuber and 12 light sprout characteristics. Field trials have been performed with tissue-culture grown plants the first year and with tuber grown plants the second year. Results obtained this far indicate that: -potato varieties can be genetically engineered with preservation of their intrinsic properties (including yield); -the frequency of deviant transgenic clones may differ between varieties; -deviant light sprout characteristics are invariably associated with a deviant plant phenotype. Transgenic potato clones may thus be prescreened on the basis of light sprout characteristics.

R 224 Toxin Gene Expression and Regulation in *Bacillus thuringiensis* subsp. *kurstaki*. AMY JELEN, JUDITH A. CHAMBERS, R. GENE GROAT, CHRISTINE JANY, TIMOTHY B. JOHNSON and CYNTHIA GAWRON-BURKE. Ecogen Inc., Langhorne, PA 19047-1810.

We have chosen *Bacillus thuringiensis* (BT) subsp. *kurstaki* strain EG2059, which has significant *Spodoptera exigua* insecticidal activity and contains multiple toxin genes, as the prototype strain in which to investigate BT toxin gene expression. A genomic library was constructed in a high copy plasmid vector using total DNA prepared from EG2059. The *cryIA(a)*, *cryIA(c)* and *cryIIA* genes were isolated from the library, characterized by restriction mapping, and subsequently introduced into either a *Cry*- BT recipient strain or EG2059. We present data, from SDS/PAGE and Western blot analyses with delta-endotoxin specific antibody probes, on the relative toxin gene expression in these recombinant strains as compared to the native host (EG2059). These data coupled with bioassay analyses, further our understanding of BT toxin gene expression and its contribution to insecticidal activity. Furthermore, the CRYI(a), CRYI(c), and CRYIIA antibody probes may be useful in analyses of delta-endotoxin expression in genetically engineered plants containing multiple BT genes.

R 225 CLONING AND CHARACTERIZATION OF MAIZE ACETOHYDROXYACID SYNTHASE GENES, George C. Jen, Susan L. Armour, and Sandra L. Volrath, CIBA-GEIGY Corp. Agricultural Biotechnology Research Unit, P.O. Box 12257, Research Triangle Park, NC 27709-2257.

Acetohydroxyacid synthase (AHAS), an enzyme in the biosynthetic pathway of branched-chain amino acids, is the target of several classes of herbicides. We have cloned the maize genes encoding AHAS from a lambda library using an *Arabidopsis* AHAS gene fragment. Two different AHAS genes were isolated. Genomic Southern analysis showed maize to contain two AHAS genes and that they matched in restriction digest patterns to the two lambda clones we isolated. Both of the maize AHAS genes have been sequenced and shown to lack introns. The two AHAS genes have 96% nucleotide homology and encode mature proteins which are approximately 98% homologous. The transcription initiation sites as well as the tissue-specific expression patterns of the two AHAS genes have been determined. The results of these studies will be presented.

R 226 THE ROLE OF TRANSIENT GENOMIC MODIFICATION IN THE DEVELOPMENT OF FREEZING TOLERANCE IN Brassica napus cv. Jet Neuf. A. M. Johnson-Flanagan, W. Kuhnle, A. Laroche, and J. Singh, Department of Plant Science, University of Alberta, Edmonton, Alberta, T6G 2P5 (WK and AMJ-F), Lethbridge Research Station, Agriculture Canada, Lethbridge, T1J 4B1 (AL) and Plant Research Centre, Agriculture Canada, Ottawa, K1A 0C6 (JS). Young expanding and mature leaves developed different degrees of hardening during growth at 2 C. After 3 weeks of acclimation, LT_{50}s of -13.5 C and -9.6 C were recorded for young and mature leaves, respectively. Results also showed that incorporation of ^{3}H-thymidine during acclimation was two times higher in young expanding leaves when compared to mature leaves. Together, these results suggest that changes associated with cell division are important for hardening. Thus, research focused on transient genomic modification. Decreased cytosine methylation revealed by HPLC analyses and doubling in rDNA copy numbers were observed in leaf DNA isolated from cold hardened plants. Interestingly, the relative amount of 5-methyl cytosine was significantly lower in the young expanding leaves compared with the mature leaves. Furthermore, differences in the restriction pattern of rDNA genes attributed to differential methylation were observed. Conversely, low temperature growth of the spring cultivar Topas was not associated with increases in rDNA copy number, nor were differences in the restriction pattern of rDNA genes observed. While these results suggest rDNA changes are involved in the acclimation process, they may equally reflect vernalization responses. This possibility was explored by determining variations in base composition of total DNA and copy numbers and restriction patterns of rDNA genes in a cell suspension culture of Brassica napus, cv Jet Neuf, hardened at room temperature with ABA.

R 227 RECOGNITION PROCESSES IN THE INTERACTION OF *RHYNCHOSPORIUM SECALIS* AND BARLEY. Wolfgang Knogge, Matthias Hahn and Lutz Wevelsiep. Dept. Biochemistry, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Federal Republic of Germany.

R.secalis is a perthotrophic pathogen of barley. Fungal race US238.1 is virulent on cultivar Atlas (resistance locus *Rrs2*) where it forms a dense subcuticular stroma in the leaves; it destroys the underlying plant cells and finally sporulates. However, it is avirulent on the near-isogenic cultivar Atlas 46 (*Rrs1* and *Rrs2*) where fungal development is prevented very soon after hyphal penetration of the cuticle. This pathosystem is being used as an experimental model to characterize the role of effector molecules (*e.g.* toxins, elicitors, suppressors, activators) in plant-pathogen communication. The final goal is an understandingof the function of resistance locus *Rrs1*. Small necrosis-inducing proteins (M_r < 10 kDa) were detected in fungal culture filtrates and purified to homogeneity. These toxins are cultivar non-specific and appear to be essential tools of the fungus to open the plant's nutrient supply. Two of them function as activators of the plant plasma membrane ATPase. Results from affinity chromatography and photoaffinity labeling experiments using one of these toxins as ligand suggest specific binding to plant plasma membrane proteins which are not identical with the catalytic subunit of the ATPase. As another class of fungus-derived effector molecules elicitors of plant defense reactions such as a rapid and strong peroxidase induction are analyzed. Finally, in a reciprocal approach fungal toxin cDNAs are being isolated to screen for molecules of plant origin which affect toxin expression.

R 228 THE REDUCTION OF THE FREEZING POINT OF TOBACCO PLANTS TRANSFORMED WITH THE GENE ENCODING FOR THE ANTIFREEZE PROTEIN FROM WINTER FLOUNDER, JungSook Lee*[1], M. Selim Cetiner[2], William J. Blackmon[2], and Jesse M. Jaynes[1], Departments of Biochemistry[1] and Horticulture[2], Louisiana State University, Baton Rouge, LA 70803.

The winter flounder, *Pseudopleuronectes americanus*, can survive in seawater at temperatures below freezing by producing antifreeze proteins which depress the freezing point of their cellular fluids. The antifreeze gene encodes a 91 amino acid protein which is composed of a mature protein of 53 amino acids and a preproprotein of 38 amino acids. The gene encoding the mature antifreeze protein, including a start methionine, was placed under the control of the cauliflower mosaic virus 35S promoter residing on the binary vector pBI 121. In order to obtain enhanced expression, the gene was also introduced into a plasmid which would allow expression from the double 35S CaMV promoter and the construct subcloned into the intermediate vector pMON200 and the binary vector pBI 121. After triparental mating and infection of tobacco leaf-disks with *Agrobacterium tumefaciens* containing pBI 121-AF and pBI 121-fAF, transgenic plantlets were obtained which were kanamycin resistant and GUS positive. Southern analysis confirms the presence of single copy gene integration. Several individual plants were selected and tested for the reduction of the freezing point of leaf tissue by differential calorimetry. The data conclusively demonstrates the freezing point depression in transgenic plants of about 3-5°C compared to transformed controls. These results have confirmed the ability of this fish protein to confer increased frost-tolerance to plants.

R 229 Gene Expression Associated with Abscisic Acid Induced Freezing Tolerance in Bromegrass (*Bromus inermis*) Cell Cultures. Stephen P. Lee and Tony H.H. Chen, Dept. of Horticulture, Oregon State University, Corvallis, OR 97331

Abscisic acid (ABA) has been shown to increase frost tolerance of bromegrass (*Bromus inermis* Leyss) cell suspension cultures at room temperature (Plant Physiol. 73:71-75, 1983). Bromegrass cultures treated with 75uM ABA increased cold hardiness from an LT_{50} of -7C to below -30C in 5 days. Our objective was to study gene expression associated with ABA induced frost tolerance. The products of *in vitro* translation of poly(A^+) RNA isolated from 5 day old bromegrass culture with or without 75uM ABA treatment were separated by 2-D SDS-PAGE and visualized by fluorography. The appearance of 15 new peptide spots, MW ranging from 53kD to 18.4kD and pI from 7.5 to 4.5 and the disappearance of a 29kD (pI=6.4) spot was observed in the ABA treated cells. A time course study (0-7days) showed that most of those ABA induced mRNAs were present after 1 day of ABA treatment. In fact at least 4 ABA induced translatable mRNAs were present within 1 hr and others appeared by 8-24 hrs of ABA treatment. mRNA encoding a polypeptide of 29kD(pI=6.4) disappeared by 8 hrs of treatment. Cold treatment (+4C) also induced some of the ABA induced mRNAs but there were fewer changes in gene expression than with ABA treatment. Induction of gene expression associated with ABA induced freezing tolerance thus seems to occur very rapidly (within 1 hr). As ABA induced freezing tolerance depends on ABA concentration, various levels of ABA (0-10^{-4}M) were also tested to determine threshold levels triggering the expression of the novel mRNAs. The majority of ABA induced mRNAs appeared at 10^{-5}M or higher. The results suggest that ABA induced expression of specific genes contribute to increased freezing tolerance. Further characterization of these ABA induced mRNAs by cDNA cloning are underway.

R 230 SELECTION OF STABLE TRANSFORMANTS FROM MAIZE SUSPENSION CULTURES USING THE HERBICIDE BIALAPHOS

P.G. Lemaux, T.M. Spencer, M.L. Mangano, T.R. Adams, R.J. Daines, J.V. O'Brien, W.G. Start, W.R. Adams, S.A. Chambers, N.G. Willetts, R.W. Krueger, A.P. Kausch C.J. Mackey, and W.J. Gordon-Kamm. Plant Genetics Research, DeKalb-Pfizer Genetics, Groton, CT 06340

Microprojectile bombardment of nonembryogenic and embryogenic maize suspension cultures was used to generate stable genetic transformants. Suspension culture cells were bombarded with two plasmids: one encoded the nonselected enzyme, β-glucuronidase; the other contained the *bar* gene from *Streptomyces hygroscopicus*, which is responsible for resistance to the herbicide bialaphos. Bombarded cells were selected on medium containing bialaphos, which is cleaved in plant cells to yield phosphinothricin, an inhibitor of glutamine synthetase. Bialaphos-resistant colonies were recovered from a nonembryogenic culture and four embryogenic suspension cultures. Such colonies contained integrated copies of the *bar* gene and expressed the herbicide-inactivating enzyme encoded by *bar*. Coexpression and cotransformation of the nonselected gene was confirmed in a subpopulation of the bialaphos-resistant colonies from these experiments.

R 231 CHARACTERIZATION AND CLONING OF THE POKEWEED ANTIVIRAL PROTEIN, Jennifer K. Lodge, Wojciech Kaniewski and Nilgun Tumer. Monsanto Company, St. Louis, MO, 63198. Pokeweed antiviral protein, a powerful inhibitor of translation, is found in the cell walls of leaves and seeds of pokeweed (*Phytolacca americana*). Three forms of this protein have been identified -- PAP from spring leaves, PAP-II from summer leaves and PAP-S from seeds. Others have shown that PAP, when applied exogenously to leaves of local lesion hosts, protects against virus infection of TMV, CMV and SBMV (all RNA viruses). Recently, we have demonstrated that exogenously applied PAP-S is very effective against five different viruses from different groups, including DNA viruses.

We have isolated a cDNA clone for the spring leaf form of the protein by using degenerate oligonucleotides made to the previously published 30 N-terminal amino acids. The predicted amino acid sequence of our clone matches the 30 N-terminal amino acids of PAP perfectly and is very similar to internal portions of PAP-S for which we have partial protein sequence. The mature protein encoded by our cDNA is 291 aa long which agrees well with the molecular weight of 30Kd of the protein. We will present the characterization of this clone.

R 232 USE OF PATHOGEN-INDUCIBLE PLANT PROMOTERS TO EXPRESS PRESUMABLE PATHOGEN-RESISTANCE GENES IN TRANSGENIC PLANTS.

Jürgen Logemann, Barbara Siebertz, Susanne Reinold, Isolde Häuser, Lothar Willmitzer, Jeff Schell. Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne Weg, 5000 Köln 30, FRG.

A wound-inducible gene *wun1* has been isolated from potato. Northern blot data as well as in situ hybridisation experiments demonstrate that a fungal plant pathogen (*Phytopthora infestans*) as well as viral plant pathogens (potato leafroll virus, Potato virus Y) are able to induce *wun1* in potato plants. *wun1* promoter analysis revealed that a 1,2 Kb large 5`upstream region is sufficient for wound-inducible expression of GUS in transgenic tobacco. This expression is specific for the epidermis cells of leaves and stems. As a result of a double promoter fusion (fusion of the wound-inducible Tr-promoter in front of the *wun1*-promoter) wound-inducible GUS expression could be specifically targeted to the vascular system. Both promoters will be useful for the expression of resistance genes depending on whether a pathogen attacks a plant through the epidermis or through the vacsular system. Several genes with proposed pathogen inhibitory functions have been fused to the *wun1*-promoter and are under investigation in transgenic plants.

R 233 GENETIC ENGINEERING OF TOBACCO AND COTTON PLANTS FOR RESISTANCE TO PHENOXY HERBICIDES, Bruce R. Lyon, Colin L.D. Jenkins, Yvonne L. Cousins, John L. Huppatz, Danny J. Llewellyn, Elizabeth S. Dennis and W. James Peacock, CSIRO Division of Plant Industry, GPO Box 1600, Canberra City, ACT 2601, Australia. Phenoxy herbicides such as 2,4-D are still widely used in Australia for the control of broadleaf weeds in grain crops and fallow. For sensitive crops such as cotton, protection from accidental spray drift and, ideally, resistance to direct herbicide application, would be a valuable agronomic trait. As an experimental system, we have introduced a bacterial gene encoding a 2,4-D degradation enzyme (2,4-D monooxygenase) into tobacco plants by Agrobacterium transformation. Transgenic plants possessing this gene are at least ten-fold more tolerant to 2,4-D than controls and can withstand spraying with several times the normal herbicide field application rate. In order to increase resistance to the higher level we believe will be necessary for cotton, we have investigated the effect of using different promoter constructs and of increasing the gene copy number. The 2,4-D resistance gene has now been introduced into tissue from Coker 315 cotton and Australian commercial cotton cultivars, and the regeneration of transgenic cotton plants is proceeding. Our current research is focused on the analysis of the biochemical and molecular nature of the 2,4-D monooxygenase with the aim of broadening the specificity of the enzyme towards a range of useful phenoxy herbicides.

R 234 CLONING GENES FOR THE SHIKIMATE PATHWAY ENZYMES OF PISUM SATIVUM.

F. MacLeod, S.P. Granger, K. Taylor, & J.R. Coggins. Department of Biochemistry, University of Glasgow, GLASGOW G12 8QQ, Scotland.

The shikimate pathway enzymes have been extensively characterised in *Pisum sativum*, and to further these enzyme studies clones for the individual enzyme genes were required. Initial attempts to use fragments of the *ARO 1* gene of *Saccharomyces cerevisiae* as heterologous probes for the corresponding *P.sativum* genes were not successful. In contrast, heterologous probing of a pea cDNA library with a cDNA for *Petunia hybrida* 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase yielded cDNA clones for EPSP synthase ; other higher plant derived probes for the shikimate pathway enzymes were not available. However we have been able to purify small quantities of several of the *P.sativum* enzymes and our progress using the alternative route for gene cloning, involving microprotein sequencing followed by synthetic oligonucleotide probe design, will be described.

R 235 EXPRESSION OF MOUSE METALLOTHIONEIN GENES IN TOBACCO, Indu B. Maiti, Ricky Yeargan, George J. Wagner, and Arthur G. Hunt, Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY40546-0091

We have expressed a mouse metallothionein (MT) gene in tobacco under control of the cauliflower mosaic virus (CaMV) 35S promoter and a pea ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS*) gene promoter. Seedlings in which MT gene expression is driven by the 35S promoter are resistant to toxic levels of cadmium. Mature plants carrying the 35S-MT gene accumulate less Cd in their leaves when exposed to low levels of Cd in laboratory growth conditions. Plants with the *rbcS*-MT construction express this gene in a light-regulated and tissue-specific manner, as expected. Moreover, the MT levels in leaves of these plants are about 20% of those seen in 35S-MT plants. These plants are currently being tested for Cd resistance. In addition, a small field evaluation of 35S-MT lines for Cd levels is being evaluated. These experiments will address the possibility of using MTs to alter Cd levels in crop species.

R 236 WOUND-INDUCIBLE EXPRESSION OF A GENE FROM TOMATO, Belinda Martineau and Catherine M. Houck, Calgene, Inc., 1920 Fifth Street, Davis, CA 95616.

We have isolated a cDNA clone, designated pZ8, from *L. esculentum* cv. UC82B that represents an mRNA highly abundant in severely wounded leaf tissue. Accumulation of pZ8 RNA in non-wounded tomato leaves appears to vary (perhaps a consequence of the handling of the plants) but can be approximately 100 times less than in wounded leaves. Based on comparisons with known amounts of pZ8 RNA prepared using a "riboprobe" system (Promega Biotec), pZ8 RNA makes up 0.01-0.02% of the total cellular RNA in wounded leaves of tomato cultivars UC82B and Bonnie Best. Assuming polyadenylated RNA makes up approximately 1% of total cellular RNA, pZ8 RNA accumulates in wounded leaves to levels two and 10 times those of two other tomato wound-inducible genes, Inhibitor I and Inhibitor II, respectively [1].

From DNA blot hybridization experiments we have estimated that two to five pZ8 genes are present in the tomato genome. pZ8 gene regulatory regions may be useful for expressing target genes designed to promote plant tolerance to insect attack.

1. J.S. Graham, G. Hall, G. Pearce and C.A. Ryan (1986) *Planta* **169**:399-405.

R 237 TWO YEARS OF TRANSGENIC FLAX FIELD TESTS: WHAT DO THEY TELL US? Alan McHughen, Mark Jordan and Sandra McSheffrey, Crop Development Centre, University of Saskatchewan, Saskatoon, Sask. S7N 0W0 CANADA

In 1988 and 1989, a total of four transgenic field tests were conducted with 14 lines of commercial flax (Linum usitatissimum) transformed with disarmed Agrobacterium tumefaciens carrying one of several modified Ti plasmids. Flax is grown on approx. 2 million acres in Canada and northern US as an oilseed. Two of the tests were of lines containing T-DNA carrying a chimeric NPT-II gene (conferring kanamycin resistance) as a marker to study outcrossing frequency. These data are necessary to develop safe isolation distances for use with more environmentally risky constructs. Four transgenic lines contained modified EPSP Synthase constructs to confer glyphosate (Roundup) resistance (this test was conducted in collaboration with Monsanto co.), and eight lines contained a mutant Arabidopsis ALS gene conferring resistance to sulfonylurea herbicides. The results from these tests show that environmental risks can be minimized by careful planning, that T-DNA insertions do not routinely interfere with the normal agronomic functioning of transgenic crop plants in the field, and that crop improvement (at least herbicide resistance) is a viable goal of transformation technology.

R 238 EXPRESSION OF THE POTATO LEAFROLL VIRUS COAT PROTEIN GENE IN TRANSGENIC POTATOES.
Joan McPherson, Larry Kawchuk and Bob Martin. Department of Plant Science, University of British Columbia, Vancouver, V6T 2A2, Canada and Agriculture Canada Research Station, Vancouver, British Columbia, Canada.

The Potato Leafroll Virus (PLRV) Coat protein gene has been isolated and inserted into the genome of potato cultivars Desiree and Russet Burbank. Chimaeric genes consisting of the Cauliflower Mosaic Virus 35S duplicated enhancer ligated to the coding region of the virus coat protein were introduced into explants using the modified *Agrobacterium tumefaciens* vector system. Western blot analysis using specific antibodies has demonstrated up to 0.1% of total protein in the leaves of transgenic plants. Immuno electron microscopy did not detect assembled protein structures. These potato plants are presently being inoculated with PLRV and their level of infection will be monitored with ELISA.

R 239 LOCALIZATION OF PROLINE BIOSYNTHESIS GENES IN PISUM SATIVUM, Mehdi M. Naderi and Henry Daniell Department of Biological Sciences, University of Idaho, Moscow, ID 83843. Plants subjected to environmental osmotic stress are known to accumulate proline. Proline is also known to be localized within chloroplasts. In enteric bacteria, production of proline from glutamate is sequentially mediated by the gene products of proB (-glutamyl kinase), proA (glutamate semialdehyde dehydrogenase) and proC (1-pyrroline 5-carboxylate reductase). In this study, Southern analysis of pea nuclear and chloroplast DNA, using bacterial proB gene as a probe, has revealed that proB gene is located in the chloroplast genome but not in the nuclear genome. Isolated pea chloroplast DNA is free of nuclear DNA as tested by probing with ribulose bisphosphate carboxylase small subunit (rbcS) fragment from pea. Chloroplast DNA filters that hybridized with bacterial proB also show hybridizing fragment with ribulose bisphosphate carboxylase large subunit (rbcL) gene which is known to be chloroplast encoded. Presently, using our pea chloroplast DNA library we have localized hybridizing clones using appropriate probes. Northern analyses are in progress to localize the site of transcription of proB gene using poly A and non-poly A RNA fractions isolated from pea. This study is supported by a grant from SBOE 88-022.

R 240 THE SENSITIVITY OF CEREALS TO HERBICIDES DEPENDS ON THE DNA STATEMENT IN GROUND MERISTEMS. Irina A.Oginova. Department of Biophysics, Research Institute of Biology, State University, Dniepropetrovsk 320625, Ukraine, USSR. In the ground meristem of Zea mays and Sorghum bicolor, on the base of the data obtained in laboratory and field tests for many years, it is established that the number of cell in the G_1 interphase period increases whereas the relative quantity of metabolically active DNA decreases depending on the acting herbicide substance when using high doses. The firm statistic connection between these parameters and the plant height, between leaf area, chlorophyll content and the state of reproductive organs is demonstrated (correlation coefficient is 0.71-0.93). That allows to use those for the diagnostics of the plantation state.

R 241 EXPRESSION AND GENETIC ORIGIN OF THE MEMBERS OF THE ACETO HYDROXYACID SYNTHASE GENE FAMILY IN *BRASSICA NAPUS*. Thérèse Ouellet, Robert G. Rutledge and Brian L. Miki, Plant Research Centre, Agriculture Canada, C.E.F., Ottawa, Ont., Canada K1A 0C6.

The acetohydroxyacid synthase (AHAS) catalyzes the first common step in the biosynthesis of branched-chain amino acids and is the target for several classes of herbicides. Southern blot analyses have revealed the presence of four distinct AHAS genes in the *Brassica napus* rapeseed, three of which have been isolated and sequenced. Two of these genes (AHAS1 and AHAS3) code for proteins that are highly homologous to the *Arabidopsis* AHAS protein, while the third one (AHAS2) codes for a distinctive AHAS isozyme. The genetic origin of each of the four AHAS genes was determined by Southern blot analysis of genomic DNA from the two parental species of *B. napus* (*B. campestris* and *B. oleracea*). Expression analyses using RNase protection assays have established that AHAS1 and AHAS3 are constitutively expressed at a low level in all major tissues. This is in contrast to AHAS2, which was found to be expressed only in flowers and in developping fruits.

R 242 INDUCTION OF SYNTHESIS OF PROTEINS BY ALUMINUM IN WHEAT (TRITICUM AESTIVUM L.) ROOT TIPS. Magaly Rincon and Robert Gonzales, The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahom 73402.

It is well known phenomenon that aluminum (Al^{3+}) reduces meristematic activity in wheat (Triticum aestivum L.) root tips of sensitive cultivars. However, root growth in some cultivars is less affected or required higher Al^{3+} concentrations to cause inhibition of root growth. Aniol (Plant Physiol., 76: 551-555, 1984) suggested that induction of synthesis of a Al^{3+} binding protein(s) might be the mechanism involved in protecting the cells against the deleterious effects of Al^{3+}. To test this hypothesis, wheat seedlings of a sensitive and tolerant cultivars (Tam 105 and Bounty 203-A, respectively) were treated with Al^{3+} and the proteins were labeled with [^{35}S] methionine. One-dimensional SDS-PAGE analysis of the newly synthesized proteins showed that Al^{3+} (50 µM), within 24h, induced the synthesis of new proteins in both cultivars. The appearance of 37, 32 and 18.5 KD proteins was reproducible. Al^{3+} (50 µM) inhibited root growth of TAM 105 but not that of Bounty 203-A. Two-dimensional PAGE analysis of in vivo labeled proteins as affected by Al^{3+} is in progress. Mechanisms of Al^{3+} toxicity and tolerance will be discussed.

R 243 EFFECTS OF (R,S) - ABSCISIC ACID, (S) - ABSCISIC ACID, PHASEIC ACID AND 2', 3' - DIHYDROABSCISIC ACID ON FREEZING TOLERANCE AND PROTEIN CHANGES IN BROMUS INERMIS CELL SUSPENSION CULTURES, Albert J. Robertson, Masaya Ishikawa, M.J.T. Reaney, S.R. Abrams and L.V. Gusta. Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada (S7N OWO).

Exogenous applications of 75 uM (R,S)-ABA to bromegrass cell suspensions cultures at 25° for 7 days increased freezing tolerance to -40°C compared to -3°C for the control. Also, ABA treated cells had increased resistance to heat, salt and osmotic stresses. Electrophoretic analyses identified three major protein bands (25, 30 and 200 kD) which increased in intensity during both ABA and low temperature induced hardening. Pulse labelling at 24 hour time periods showed increased incorporation into 25, 28 and 30 kD proteins in cultures hardened at 8°C and at 25°C in the presence of 75 uM ABA. After six days of dehardening at 25°C, synthesis of proteins in the 25-30 kD area decreased. Comparative 2-D SDS-PAGE of total cell protein isolated from control cultures and those treated with 75 uM (R,S)-ABA showed major increases in 16, 20, 22 to 28, 30 to 31, 36 to 38, 41, 43, 47, 82 and 100 kD proteins. Cells treated with 75 uM (S)-ABA developed freezing tolerance levels similar to but less than (R,S) - ABA. Two dimensional protein maps showed (S)-ABA increased the same proteins sensitive to (R,S)-ABA, but to a lesser degree. Phaseic acid, a metabolite of ABA, at 75 and 100 uM had no effect on freezing tolerance. Two -D protein maps of total cell protein isolated from phaseic acid treated cells were similar to control protein maps except for a quantitative increase in two (16, 20 kD) proteins which were also increased by (R,S) and (S)-ABA. An analog of ABA, 2', 3'-dihydro abscisic acid increased freezing tolerance to -25°C at 75 uM concentrations. Protein analysis indicated that this ABA analog increased the same set of proteins as (RS)-ABA.

R 244 ISOLATION AND CHARATERIZATION OF A MUTANT ACETOLACTATE SYNTHASE GENE FROM IMIDAZOLINONE-RESISTANT ARABIDOPSIS THALIANA VAR. COLUMBIA, Kanagasabapathi Sathasivan[1], George W. Haughn[2], and Norimoto Murai[1].1) Department of Plant Patholgy and Crop Physiology, Louisiana State University, Baton Rouge LA 70803. 2) Department of Biology, University of Saskatchewan, Saskatoon, Canada S7N 0W0. Acetolactate synthase (ALS), the first enzyme in the biosynthetic pathway of leucine, isoleucine, and valine is inhibited by three groups of herbicides: imidazolinones, sulfonylureas, and triazolo pyrimidines. Mutant forms of ALS enzymes resistant to the sulfonylureas have been extensively studied and a single amino acid change was known to confer sulfonylurea resistance in bacteria and plants. In order to understand the molecular basis of imidazolinone herbicide resistance , a homozygous mutant GH-90 of *Arabidopsis thaliana* , isolated and characterized by Drs. George Haughn and Chris Somerville (In press) was analysed. The mutant plant and its ALS are atleast 100 times more resistant to the imidazolinone herbicide imazapyr than the wild type. A southern hybridization analysis of the genomic DNA from the mutant and the wild type plants indicated that there is no gross structural change or amplification in the ALS gene in the mutant to confer herbicide resistance. A genomic DNA library was constructed from the GH-90 DNA in the lambda vector EMBL3 and screened by an ALS gene probe. Among the three positively hybridizing clones identified, one was selected and purified. The ALS gene was subcloned in a 5.8 kb Xba I fragment into pUC 18 and pBluscript KS+/- . Nested deletions were created using ExonucleaseIII and Mung bean nuclease treatment. The DNA sequence was determined by both double and single stranded sequencing methods. Identified a G to A mutation in the ALS gene at the 1958th nucleotide.This would result in serine to asparagine substituition at the 653rd amino acid in the matured protein. Restriction analysis indicated that the mutant ALS gene also has altered flanking sequence, as indicated by the new Cla I restriction site at 1.2 Kb 5' ustream . The importance of the latter mutation is not clear. Chimeric vector was constructed to express the mutant gene in tobacco and the expression studies are in progress.

R 245 ANALYSIS OF BARLEY NITRATE REDUCTASE GENES AND THEIR AZIDE INDUCED MUTANT, Kirk M. Schnorr and Andris Kleinhofs, Department ofAgronomy and Soils, Washington State University, Pullman, WA 99164-6420
Nitrate reductase(NR) is responsible for the conversion of nitrate to nitrite in the nitrate assimilation pathway. Nitrate reductase is a homodimer with a subunit size of about 110 kilodaltons. Our lab has identified several barley NR deficient mutants that are unable to transfer electrons from the primary electron donor NADH to the terminal electron acceptor nitrate. Nitrate reductase cDNAs from both a wild type barley cv. Steptoe and an azide induced mutant Az32(narlh, Steptoe background) have been cloned and sequenced. Sequence comparison of the 1.1kb 3' end of the NR genes revealed 23 base pair changes in the coding region. Three of these changes result in amino acid substitutions. A 630bp fragment with significant homology to NADH cytochrome b_5 reductase and possibly including the mutagenic lesion resulting in the Az32 phenotype was identified for further study. This region also contains 15 of the 23 base pair changes and all three of the amino acid changes. The polymerase chain reaction(PCR) was employed to amplify this 630 base pair region from genomic DNA isolated from wild type and mutant genomic DNA. The resulting PCR products were cloned and sequenced. Comparison of the sequence data suggests that more than one gene fragment of equal size is present in the genomic DNA. Analysis of PCR products from both mutant and wild type genomic DNA and cDNA is in progress in order to identify and characterize these fragments.

R 246 IN VITRO SELECTION OF HEAT STRESS RESISTANT MUTANT CELL LINES OF WHEAT AND MOLECULAR STRATEGY FOR BREEDING HEAT STRESS RESISTANT VARIETIES,
X.M. Shang, M. Yucel, W.C. Wang and H.T. Nguyen, Department of Agronomy, Horticulture and Entomology, Texas Tech University, Lubbock, TX 79409
Heat stress resistant mutant cell lines of wheat (Triticum aestivum var. Mustang) have been selected under high temperature conditions which were 37°C for 280h, 40 °C for 105h, and 48°C for 40h. Higher percentage viable cells for these mutants were recovered than control lines after a lethal stress temperature of 50°C for 1.50h, indicating that the selected mutants were more heat resistant. The unique aspects of the heat shock protein (HSP) profiles from these mutants revealed by isoelectric focusing polyacrylamide gel electrophoresis of in vivo radiolabeled proteins are: **a.** There are several significant differences in HSPs 16, 17, 33, and 70 kD range with pI from 7.2 to 9.8, comparing the selected mutants with the non-selected cell lines. **b.** All of the mutants can maintain most of the normal protein synthesis under the 40°C, 4h heat shock condition, as opposed to suppressed levels of normal protein synthsis observed in non-selected cells and tissues. Gene amplification and rearranged genomes of these mutants were characterized by the occurrence of the double minute chromosomes, high frequency of micronuclei, and enlarged eu- and hetero-chromatin segments of wheat genomes detected by the HCl-KOH-Giemsa banding technique. Strategically, we utilize the selected cell lines as the novel resources to study further the genetic mechanisms of the thermoresistance of the mutants, and to construct cDNA and genomic libraries to identify, isolate and transform the desired heat stress resistant gene(s) into susceptible varieties.

R 247 GENES INVOLVED IN SYNTHESIS AND MODIFICATION OF PHYTOCHELATINS IN SCHIZOSACCHAROMYCES POMBE. David M. Speiser, Lisa Kreppel, Gretchen Scheel, Daniel F. Ortiz, Susan L. Abrahamson, Agnes M. Soriano, and David W. Ow. Plant Gene Expression Center, USDA/ARS/U.C. Berkeley. 800 Buchanan St., Albany, CA 94710.

Pollution by heavy metals threatens agriculture and the environment in many portions of the world. Animals respond to heavy metal contamination by inducing the synthesis of metallothionein, a metal-chelating protein, which in mammalian cells is encoded by a multigene family. In contrast, plants utilize small metal-binding peptides which are products of glutathione metabolism. Commonly called phytochelatins, they are also known as cadystins, γ-glutamyl peptides, and Cd-binding peptides. Some fungi including the fission yeast *Schizosaccharomyces pombe* share this same response to heavy metals and so are ideal model systems to study this phenomenon. In *S. pombe*, PCs have been shown to occur in two forms, one of which contains acid-labile sulfide as part of the chelation complex.

We isolated and characterized Cd-sensitive mutants of *S. pombe* and have shown that several mutants are deficient in synthesis of sulfide-containing PC but which produce normal amounts of sulfide. Two different DNA clones were isolated from *S. pombe* genomic DNA which restore Cd-resistance and synthesis of the sulfide-containing Cd-complex. Gene disruption using the isolated clones demonstrated that these loci are involved in PC-Cd complex assembly. The two cloned genetic loci were assigned to different *S. pombe* chromosomes by Southern hybridization of chromosomes separated by pulsed-field gel electrophoresis. Deletion analysis of the original clones identified the portions responsible for encoding the complementing activity. The size of the message corresponding to each clone, the direction of transcription, and the pattern of Cd-induction was determined by Northern analysis of RNA isolated from Cd-induced and -uninduced yeast.

R 248 THE SEARCH FOR AN ATTENUATING SATELLITE FOR THE CONTROL OF GRAPEVINE FANLEAF VIRUS (GFLV), Doreen F. Stabinsky, Boni K. Gregory and George E. Bruening, Department of Plant Pathology, University of California, Davis, CA 95616

Many satellite RNAs supported by economically significant plant viruses have been shown to mitigate viral symptoms and offer at least partial protection to the plant against the virus. The nepovirus, family has multiple examples of such satellite RNAs, although grapevine fanleaf virus (GFLV), an important grape pathogen, has no such detected parasitic RNA. We have some evidence to support the hypothesis that satellite sequences are present in plants; this presents a possible origin of satellite RNAs. We present results of experiments here designed to 1) generate, through serial passage of GFLV through plants with satellite-like genomic DNA sequences, a satellite unique to the virus; and 2) to adapt the satellite RNA of tobacco ringspot virus to be effectively replicated by GFLV. Further uses of these RNAs are also discussed.

R 249 HERBICIDE RESISTANT MUTANTS OF SUGAR BEET (BETA VULGARIS).

Peter Stougaard, Kirsten Bojsen, and Tove Christensen.

DANISCO A/S, Biotechnology Research Division, DK-1001 Copenhagen K, Denmark.

Sugar beet suspension culture cells resistant to both imidazolinone and sulfonyl urea herbicides were selected. The mechanism of resistance was found to be caused by a changed acetohydroxy acid synthase.

Two different acetohydroxy acid synthase genes were isolated from these tetraploid sugar beet cell cultures. The only difference between the predicted amino acid sequences of the two genes, AHAS I and AHAS II, was at amino acid pos. 37 where AHAS I coded for Leu and AHAS II for Pro.

In the cell culture mutant 1 the predicted amino acid sequence of the AHAS I gene contained two amino acid substitutions. Mutant 2 had a wild type AHAS I gene but contained an AHAS II gene coding for an enzyme with three amino acid substitutions one of them being the same as in the resistant form of AHAS I, at pos. 569.

In order to determine which of the mutations that would mediate herbicide resistance we separated the mutations and cloned the various gene constructs into an E.coli expression vector. AHAS enzyme synthesized from such recombinant E.coli cells was found to be herbicide resistant only if it contained amino acid substitution at pos. 569 (Trp --> Leu).

Plant transformation vectors containing different AHAS genes have been constructed and used for transformation of tobacco and sugar beet.

R 250 CELL WALL PROTEINS OF EXPANDING AND FULLY EXPANDED LEAVES OF COTTON AFTER STRESS,
Norma Trolinder*[1], Tze-Chen Hsieh[2], Alesia Reinisch[1], and Wen Chung Wang[2], [1]USDA-ARS, Route 3, Box 215, Lubbock, TX 79401. U.S.A. [2]Department of Agronomy, Horticulture and Entomology, Texas Tech University, Lubbock, TX 79409. U.S.A.

Two wild cotton lines respond to environmental stress differently. T25 leaves remain very turgid even under extreme environmental stress, whereas T169 leaves wilt dramatically (1). The objective of our research was to determine if this response was related to changes in cell wall protein patterns during stress.

The two lines were grown in stress and non-stressed environments. The first unfolded leaf and the third and fifth leaves distal to the shoot meristem were harvested and cell wall proteins were extracted by infiltration with sucrose or $CaCl_2$. Gel electrophoresis, followed by silver staining, revealed changes in cell wall protein patterns between stages of leaf development and between cotton lines. There were major shifts in several bands. We believe that one of the major limitations to growth under conditions of environmental stress may be cell wall extensibility which could be reflected by changes in cell wall proteins (2).

References.
1. Quisenberry J, Jordan W, Roarke B, & Fryrear D (1981) Crop Science 21:889-895.
2. Bozarth C, Mullet J, & Boyer J (1987) Plant Physiol 85:261-267.

R 251 **EXPRESSION OF HEAT SHOCK PROTEIN GENES IN RESPONSE TO 2,4-DINITROPHENOL,** Kailash C. Upadhyaya and Ram Kishore, School of Life Sciences, Jawaharlal Nehru University, New Delhi-110067, India.
Seedlings of pigeon pea (Cajanus cajan), a tropical legume plant, has been shown to synthesize at least 35 polypeptides ranging in molecular weight from 15-91 kD at heat shock temperature (40°C) as resolved by two-dimensional nonequilibrium pH gel electrophoresis. The effect of a known uncoupler of oxidative phosphorylation, 2,4-dinitrophenol which generates endogenous heat signals during the oxidation of $NADH^+$ has been investigated in activating the heat shock (hsp) genes. Approximately 84 polypeptides, 12 over 60 kD, 41 between 30-60 kD and 31 with a molecular weight below 30 kD, are synthesized in the presence of DNP at normal growth temperature (30°C). The synthesis of 14 hsps is not initiated by DNP whereas an equal number of polypeptides appear to be specific for the DNP treatment and they are not synthesized following heat shock. However, 17-19 hsps appear to be synthesized in the presence of DNP. Thus, the DNP treatment induces the synthesis of two different sets of polypeptides, (i) unique to the DNP treatment and (ii) whose synthesis could either be initiated by heat shock or by the DNP treatment.

R 252 MOLECULAR EVIDENCE OF GENETIC DIVERSITY OF HEAT-SHOCK GENE EXPRESSION AND ITS CORRELATION WITH PHOTOSYNTHESIS GENE EXPRESSION BETWEEN HEAT-TOLERANT AND -SUSCEPTIBLE WHEAT LINES, Jian Weng, Richard A. Vierling, and Henry T. Nguyen, Department of Agronomy, Horticulture and Entomology, Texas Tech University, Lubbock, TX 79409
Our objective was to determine the molecular basis of natural genetic diversity of different HS responses and correlation between HSP synthesis and thermal tolerance in wheat. Two wheat lines "Mustang"(heat-tolerant) and "Sturdy"(heat-susceptible) were selected due to their genotypic differences in thermal tolerance. Several sets of RNA from leaf tissues exposed to different HS temperatures, HS durations and diurnal stress periods were isolated and hybridized with HSP cDNA probes, photosynthesis-related nuclear encoded gene probes, and plastid encoded gene probes by Northern dot-blots. HSP profiles from *in vitro* translation products separated on 2-D gels were also compared. The Northern dot-blot data from a direct HS treatment and time course experiment obviously show that the heat-tolerant line "Mustang" synthesizes low molecular weight HSP mRNA faster at the optimum HS temperature, reaching higher levels sooner than the heat-susceptible line "Sturdy", especially the chloroplast localized HSP. The data from hybridization of photosynthetic gene probes also show that in "Mustang" the mRNA level recovers much quicker than in "Sturdy". It seems there is a good correlation between increased chloroplast-localized HSP synthesis and increased heat-protection in photosynthesis system. We also constructed several cDNA libraries from control and HS mRNA isolated from heat-tolerant and -susceptible genotypes using expression vector Lambda ZAP-II. More than 30 positive clones were picked up by screening with HSP cDNA probes. Characterising individual clone using *in vitro* hybrid selection or immunoscreening methods and sequencing positive clones are under way. In general, results of molecular analysis of HS gene expression in wheat lines differing in thermal tolerance will be presented. The role of HSP synthesis in relation to genetic diversity in thermal tolerance and a strategy for genetic engineering of thermal tolerance in plants will be discussed.

R 253 EXPRESSION OF MODIFIED POTATO PROTEINASE INHIBITOR II GENES IN TRANSGENIC PLANTS,
Derek W.R.White*, Michael T. McManus*, Paul M. Ealing* and Peter G.McGregor+,
*Grasslands Division and +Plant Protection Division, Department of Scientific and Industrial Research, Palmerston North, New Zealand.
Potato proteinase inhibitor II (PPi II) is an inhibitor of the serine endopeptidases, trypsin and chymotrypsin, and accumulates systemically in response to mechanical wounding of leaves. To determine whether the introduction of PPi II would confer resistance to insect damage we have examined the expression of modified forms of the gene in transgenic plants. The coding region of a PPi II gene was fused to the following 5' regulatory regions; the intact CaMV 35S promoter, the root-specific domain of the CaMV 35S promoter, and the TR 1'-2', wound-inducible promoter from Agrobacterium. These constructs were introduced into tobacco and PPi II-specific transcription analysed in transgenic plants by Northern blotting. A polyclonal antibody, raised against purified PPi II protein, was used in ELISA and Western blotting analysis to detect PPi II protein accumulation in these plants. PPi II protein, extracted from transgenic plants, is active as a proteinase inhibitor in vitro. These experiments, and results from insect feeding trials on transgenic and control plants will be presented.

R 254 SALT TOLERANCE IN ALFALFA: GENE ACTIVATION IN SALT TOLERANT CALLUS AND PLANTS REGENERATED FROM SALT TOLERANT CALLUS. Ilga Winicov, Departments of Biochemistry and Microbiology, University of Nevada, Reno; Reno, NV 89557. In order to identify genes involved in cellular salt tolerance of alfalfa (**Medicago sativa**) we have selected cell lines that are tolerant to 1% NaCl in the growth medium. This phenotype is maintained in absence of selective pressure, indicating a stable change in gene expression leading to tolerance. Photosynthesis related genes of both nuclear and chloroplast origin are activated in the salt tolerant lines in culture as shown by mRNA accumulation. This activation appears to be correlated with the tolerance phenotype as shown by light requirement and sensitivity to inhibitors of PSII. We have also isolated three different cDNA clones from salt inducible mRNAs from the tolerant callus. These mRNAs do not appear under conditions of salt stress in the salt sensitive sister cultures. A number of plants have been regenerated from several salt tolerant lines and they show increased tolerance to growth in the presence of NaCl under greenhouse conditions. These plants are self fertile. Experiments are in progress to identify the tissue specific expression of genes activated in salt tolerant cell culture at the whole plant level.

R 255 HEAT SHOCK GENE REGULATION DURING SOMATIC EMBRYOGENESIS OF CARROT, J. Lynn Zimmerman, Nestor Apuya and Cindi O'Carroll, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, MD 21228.
We have determined that carrot somatic embryos exhibit an unusual program of heat shock gene regulation. Specifically, we have observed that globular embryos both *synthesize* and *accumulate* significantly less heat shock mRNA than embryos of any other stage or than callus suspension cells. In fact, there appears to be no transcriptional induction of heat shock genes in response to thermal stress during this time period. However, in spite of the absence of transcriptional induction of heat shock genes, all embryos synthesize the full complement of heat shock proteins (hsps) compared to hsps of callus cells. They appear to accomplish this by both stabilizing the existing heat shock mRNAs and more efficiently translating those heat shock mRNAs which are available. This translational control is currently under investigation. Superimposed on this unusual pattern of regulation, we have observed that mid-globular embryos can be irreversibly arrested in their development if they are exposed to a brief and carefully timed heat shock exposure. No other stage of somatic embryo development is similarly arrested by heat shock. In addition to representing an interesting system for studying the molecular details of heat shock gene regulation, these observations may have important implications for seed-crop productivity if a similar phenomenon occurs during zygotic embryogenesis.

R 256 THE USDA ARS PLANT GENOME MAPPING PROGRAM, Jerome P. Miksche, Director, Office of Plant Genome Mapping, Building 005, Beltsville, MD 20705. The nation is faced with major agricultural challenges of water quality, climate changes, sustainable agriculture, need for new crops, new uses for crop and forest products, food quality and safety, germplasm exploration and enhancement, and biocontrol. To meet these challenges and find answers to the complex issues involved, research is needed to locate related plant genes. Presently, however, little is known about protein products of genes controlling structure, physiological functions, and disease resistance in plants. Without this knowledge we cannot utilize the new biotechnology tools necessary to solve these major agricultural problems.

Biological Stress and Oil Seed Improvement

R 300 MOLECULAR EVENTS IN PLANT CELLS TRIGGERED BY FUNGAL COMPONENTS. A.J. Anderson, J. Cardon, C. Tepper, K. Blee. Biology, Utah State University, Logan, UT 84322-5305. Extracellular products from the fungal pathogen Colletotrichum lindemuthianum enhance the production of active oxygen species in suspension cultured bean cells. The increased production, measured by chemiluminescence, occurs rapidly within 30 minutes. Sensitivity to catalase and cyanide indicates that plant peroxidase activity is involved in the response. Direct activation of plant peroxidases by the fungal components was not observed. The intensity of the signal was higher in interactions between fungal components from races incompatible on the test cultivar than with compatible cultivars. Signals from responding cells were rapidly transmitted through plant cell layers as determined by in situ hybridization of mRNA corresponding to altered expression of defense-related genes.

R 301 MOLECULAR GENETIC ANALYSIS OF *ARABIDOPSIS THALIANA* RESISTANCE TO THE BACTERIAL PATHOGEN *PSEUDOMONAS SYRINGAE* PV. *TOMATO*. Andrew F. Bent, Roger W. Innes, and Brian J. Staskawicz, Dept. of Plant Pathology, University of California, Berkeley, CA, 94720.

We are using *A. thaliana* to study the molecular nature of gene-for-gene disease resistance. Initial work has included a screen of geographically diverse *P.s.* pv. *tomato* strains and *A. thaliana* ecotypes (>30 of each) for differential disease symptoms. Most *A. thaliana* ecotypes are resistant to leaf infection by most *P.s.* pv. *tomato* strains, showing no visual symptoms upon leaf infiltration with a 10^6 cfu/ml bacterial suspension. However, four of the *P.s.* pv. *tomato* strains surveyed cause extensive cell death and chlorosis in *A. thaliana* ecotype Col-0 upon inoculation at 10^6 cfu/ml. We have identified other ecotypes that are resistant to one or more of these strains. In addition, genomic libraries from *P.s.* pv. *tomato* 1065 and T-1 (strains that do not cause disease on Col-0) have been mobilized into the pathogenic strain DC3000, and five independent putative avirulence loci have been obtained. Characterization of these loci (subcloning, sequencing, gene fusions) is underway. For three of the five clones, *A. thaliana* ecotypes that do not recognize DC3000 carrying the avirulence loci have been identified. Genetic tests are in progress to determine if the plant resistance determinants segregate as single dominant loci. Progeny will be analyzed using RFLP probes to genetically map the resistance genes in *A. thaliana* Col-0 corresponding to the *P.s.* pv. *tomato* avirulence genes.

R 302 GENETIC ANALYSIS OF AGGLUTINABILITY OF BENEFICIAL ROOT COLONIZING PSEUDOMONADS. C.R. Buell and A.J. Anderson. Department of Biology, Utah State University, Logan, Utah 84322-5305.
Binding of certain beneficial fluorescent pseudomonads to the root surface involves an interaction between a plant glycoprotein, an agglutinin, and bacterial cell surface structures. Analysis of an agglutinable parent strain and Agg$^-$ mutants indicates that the agglutination phenotype is not correlated with major outer membrane proteins, LPS structure or negatively charged polymers. Agglutination is heat labile and sensitive to proteases. Agglutinability is regulated by growth medium and growth phase; root components induce the phenotype. Genetic analysis of Tn5 and EMS Agg$^-$ mutants reveal multiple loci are involved. A 2.7KB EcoRI-Hind III parental fragment restored agglutinability to one mutant but did not complement other mutants or a non-agglutinable isolate of *P. fluorescens*.

R 303 CLONING OF cDNA AND CHROMOSOMAL LOCATION OF GENES ENCODING THE THREE TYPES OF SUBUNITS OF THE WHEAT TETRAMERIC INHIBITOR OF INSECT α-AMYLASE. Pilar Carbonero, Federico García-Maroto, Carmen Maraña and Montaña Mena, Bioquímica y Biología Molecular, E.T.S. Ingenieros Agrónomos-UPM, E-28040 Madrid (Spain).
Wheat endosperms cDNA clones, corresponding to proteins CM1, CM3 and CM16, which represent the three types of subunits of the wheat tetrameric inhibitor of insect α-amylases, have been characterized. The deduced amino acid sequences of the mature polypeptides are homologous to those of the dimeric and monomeric α-amylase inhibitors and of the trypsin inhibitors. The mature polypeptides are preceded by typical signal peptides. Southern-blot analysis of appropriate aneuploids, using the cloned cDNAs as probes, has led to the location of genes for subunits of the CM3 and of the CM16 type within a few kb of each other in chromosomes 4A, 4B and 4D, and those for the CM1 type of subunit in chromosomes 7A, 7B and 7D. Protein subunits of the tetrameric inhibitor corresponding to genes from the B and D genomes have been previously characterized. No proteins of this class have been found to be encoded by the A genome in hexaploid wheat (genomes AA,BB,DD) or in diploid wheats (AA) and no anti α-amylase activity has been detected in the latter, so that the A-genome genes must be either silent (pseudogenes) or expressed at a much lower level.

R 304 MOLECULAR GENETIC ANALYSIS OF *PSEUDOMONAS SYRINGAE* PV *TOMATO LYCOPERSICON ESCULENTUM* INTERACTIONS. Francine Carland, Pamela Ronald, John Salmeron, Brian Kearney, and Brian Staskawicz. Dept. of Plant Pathology, University of California, Berkeley, CA 94720.
Pseudomonas syringae pv *tomato* is the causal agent of bacterial speck disease of tomato. Resistance to the pathogen occurs in cultivars containing the dominant resistant gene *Pto* that specifically corresponds to the dominant avirulence gene *avrPto* in the pathogen in a classic gene for gene fashion. We are interested in studying this particular host pathogen interaction because it allows us to determine the basic mechanisms responsible for host pathogen specificity and the molecular basis of disease resistance. Analysis of both *Pto* and *avrPto* is a requirement for a thorough understanding of the interaction. *avrPto* has been cloned and is currently being characterized at the nucleotide level. We have also been mapping *Pto* and have confirmed earlier reports that the gene resides on chromosome 5 and is linked to the recessive mutation anthocyanin free (af). However, the map position obtained from our initial experiments is not consistent with the previously assigned map position. Presently, we are analyzing segregating populations and mapping the *Pto* gene in relation to both morphological markers and RFLP markers. Furthermore, we have introduced the maize transposable element Activator (Ac) into a cultivar of tomato containing the *Pto* gene and are in the process of mapping the chromosome location of the T-DNA inserts. Once we have found linkage of the T-DNA to the *Pto* gene, we will try to identify and clone the *Pto* gene by transposon tagging.

R 305 TRANSFER OF NON-HOST DISEASE RESISTANCE RESPONSE GENE FROM PEAS TO HETEROLOGOUS HOSTS, Chin C. Chiang, Ming Mei Chang and L. A. Hadwiger, Molecular Biology of Disease Resistance Laboratory, Washington State University, Pullman, WA 99164-6430.
The inevitable reductions due for chemical pesticides underline the need for strategies for plant defense without chemicals. The single Mendelian traits for disease resistance within a species which have traditionally been available to breeders, are limited and as yet are not cloned for engineering purposes. Non-host resistance is an unlimited source of defense genes, e.g. most plants except potatoes can resist most potato pathogens. We have cloned several disease resistance response genes (DRRG) of peas which appear to function in the pea's non-host resistance to non-pea pathogens. The DRRG49-homologous product(s) constitutes a major portion (up to 1/4) of the legume protein following fungal challenge. In order to characterize the DRRG49 gene products, the antiserum has been developed using DRRG49-β-galactosidase fusion protein expressed in E. coli. The DRRG49 structural gene under 35S promoter from cauliflower mosaic virus has been constructed and transferred into tobacco and potato via agrobacterium mediated gene transfer. The mRNA and protein levels of DRRG49 will be monitored. The effect of pea gene 49 on the transformed tissue's resistance response will be presented.

R 306 RFLP MAPPING IN FLAX WITH PARTICULAR REFERENCE TO THE ISOLATION OF FLAX RUST RESISTANCE GENES. Christopher A. Cullis, Mark. B. Gorman*, James Bader, Norman Alldridge and K. Jane Alldridge. Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106. *Biology Department, Baldwin-Wallace College, Berea, Ohio 44107.
A genetic map of flax is being constructed using morphological markers, protein polymorphisms and RFLPs. The source of the RFLPs are PstI clones isolated from total genomic DNA. More than 90% of these clones are low copy number sequences, and the majority have proved to be useful markers in the test material. The primary objective of the mapping effort is to use the linked polymorphisms to isolate the rust resistance genes from flax. There are 30 known rust resistance genes in flax. These 5 loci, are termed K,L, M, N, and P. There are 2, 13, 7, 3, and 5 gene specificities at these loci respectively. Three of these loci, K, N, and P, are on the same chromosome while the other two are linked to any of the other loci. We have mapped the M locus to a specific chromosome. A series of near isogenic lines will be used to approach, and ultimately clone, the locus.

R 307 MOLECULAR CLONING AND DEFENSE-RELATED EXPRESSION OF THE ALFALFA CHALCONE SYNTHASE GENE FAMILY. Karen Dalkin and Richard A. Dixon. The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402.
When challenged with phytopathogens, plants activate a number of defense mechanisms, such as modification of the cell wall, induction of lytic enzymes and synthesis of phytoalexins. Alfalfa cell suspension cultures accumulate high concentrations of the pterocarpan phytoalexin medicarpin following exposure to an elicitor preparation from the cell walls of the fungus Colletotrichum lindemuthianum. Chalcone synthase (CHS) catalyses the first and key regulatory step in the branch pathway of phenylpropanoid biosynthesis specific for synthesis of flavonoid/isoflavonoid compounds. We have shown that CHS is encoded by a multi-gene family in alfalfa. Treatment of alfalfa cells with Colletotrichum elicitor results in the accumulation of a number of distinct CHS transcripts encoding a set of CHS isopolypeptides of 43-44 kDa, with differing pI's in the range pH 6-7. Several full length CHS cDNA clones have been isolated from an elicitor-induced alfalfa cDNA library, based on cross hybridization with a bean CHS cDNA. Sequence analysis and restriction mapping have revealed considerable polymorphism between individual CHS cDNA clones. The isolation of genomic clones for alfalfa CHS and studies on the differential regulation of CHS genes in response to various stimuli are in progress.

R 308 THE INTRODUCTION INTO PLANTS OF GENES WHICH ENCODE SOME OF THE NATURAL COMPONENTS OF THE HUMORAL IMMUNE RESPONSE OF *HYALOPHORA CECROPIA*, Luis J. C. Destéfano-Beltrán*[1], M. Selim Cetiner[2], Timothy P. Denny[3], Christopher A. Clark[4], John H. Dodds[5], and Jesse M. Jaynes[1], Departments of Biochemistry[1], Horticulture[2], and Plant Pathology[4], Louisiana State University, Baton Rouge, LA 70803, Department of Plant Pathology[5], University of Georgia, Athens, GA 30602, and the International Potato Center[5].

A number of plant pathogenic bacteria and fungi have been shown to be highly sensitive to SB-37, a derivative of cecropin B (one of the three cecropins from the giant silk moth, *Hyalophora cecropia*). The lytic activity exhibited by SB-37 has been shown to increase up to 20-fold in the presence of chicken lysozyme. The cDNA genes encoding SB-37, attacin E (another component of the humoral response of *H. cecropia*), and chicken lysozyme have been introduced into the intermediate vector pMON316. Following the standard leaf-disk infection protocol, these genes have been integrated into the genome of tobacco. The transformation of at least ten independent lines from each gene has been confirmed by Southern blot analysis. In addition, these genes have been placed under the control of the double 35S promoter and the proteinase inhibitor II promoter from potato and introduced into pBI 121. *Agrobacterium tumefaciens*, strain LBA 4404, was subsequently transformed with these constructs and tobacco and potato tissue were infected. Gus-positive and kanamycin resistant plants have been obtained and Southern blot analysis has shown the effective introduction of these genes. Selfing of all plants has yielded material which is currently undergoing challenge experiments by *Pseudomonas solanacearum*. Gus-positive potato plants, regenerated from hairy roots, produced by infection with *A. rhizogenes* R1000 containing the same constructs have also been obtained. The introduction of this suite of genes into plants may significantly augment the level of resistance to bacterial and fungal disease.

R 309 CLONING OF 1,3-β-D-GLUCANASE cDNA AND INDUCTION OF GLUCANASE AND CHITINASE mRNA IN BEAN CELL SUSPENSION CULTURES. Brent Edington and Richard A. Dixon, The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma, 73402.

Hydrolases such as 1,3-β-D-glucanase and chitinase are induced in plants in response to pathogen attack, ethylene, or treatment with elicitors. This may be a key factor in defense against pathogenic fungi, as these enzymes have the potential either to hydrolyse the walls of hyphal tips, thereby causing cell lysis, or to release fungal cell wall oligomers which can potentiate the host resistance response. Utlilizing degenerate oligos as primers, which were derived by comparison of conserved regions of 1,3-1,4-β-D-glucanase from barley and 1,3-β-D-glucanase from tobacco, bean (Phaseolus vulgaris) 1,3-β-D-glucanase sequences were amplified from a cDNA library using the polymerase chain reaction. Amplified sequences of the appropriate size were cloned into pGEM7z+ and sequenced using the dideoxy chain termination procedure. The 120 base pair region between the primers was 65% homologous at the nucleic acic level with the same region of the 1,3-β-D-glucanase from tobacco. This PCR-produced clone was used to isolate a longer (approx. 1 Kb) cDNA clone which exhibited 67% homology to the tobacco 1,3-β-D-glucanase. Southern hybridization using the PCR-derived probe showed that 1,3-β-D-glucanase genes comprise a small gene family of possibly 2 or 3 members. Elicitor-mediated induction of glucanase and chitinase in suspension cultures was analyzed at the level of enzyme activity and mRNA accumulation. These hydrolases appear to have the same pattern of induction in response to elicitor.

R 310 TERMINAL ENZYMES OF ISOFLAVONOID PHYTOALEXIN BIOSYNTHESIS IN ALFALFA, Robert Edwards, Ganesan Gowri, Nancy Paiva and Richard A. Dixon, The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402.

Legumes, such as alfalfa, respond to fungal pathogens by synthesizing antifungal isoflavonoid phytoalexins from a specific branch of the phenyl propanoid pathway. The terminal enzymes of isoflavonoid biosynthesis are of interest because they determine the antifungal characteristics of the phytoalexin by modifying functional groups and by producing active isomers. In addition, expression of the genes encoding these enzymes may be under more selective environmental and developmental control than is the case for the more ubiquitous enzymes of general phenylpropanoid biosynthesis. Using cell cultures of alfalfa (Medicago sativa) as a model system, we are studying the enzymes isoflavone O-methyl transferase (IOMT), isoflavone reductase (IFR) and pterocarpan synthase (PTS) which are specifically involved in the biosynthesis of the phytoalexin medicarpin. Recent studies have shown that the specific activities of IOMT, IFR, and PTS increase in alfalfa cells in response to treatment with fungal elicitor and that this is accompanied by the accumulation of medicarpin. In cell cultures this enzyme system specifically synthesizes the minus (-) isomer of the phytoalexin. Current work on the characterization of these enzymes and the strategies for the cloning of their genes, will be presented, and the potential for manipulating the terminal steps of isoflavonoid biosynthesis discussed.

R 311 CHARACTERIZATION OF THE SOYBEAN MOSAIC VIRUS-N NIa-LIKE PROTEIN, Alan L. Eggenberger and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130. Soybean mosaic virus-N (SMV-N) is a member of the potyvirus group of plant viruses. Potyviruses have a positive-sense RNA genome approximately 10 kb in length. The genomic RNA encodes a single polyprotein which is processed by one or more viral proteases. One of these proteases, the NIa protein, has been shown to be responsible for most of the polyprotein cleavages. Based on cDNA sequence data, the consensus recognition site of the SMV-N NIa-like protein is predicted to be (V or L) (S or T) (L or V) Q / (S, G, or A). Work is in progress to better characterize the SMV-N NIa-like protein and its recognition sites.

R 312 **MOLECULAR GENETIC CHARACTERIZATION OF DISEASE RESISTANCE LOCI OF LACTUCA SATIVA USING YEAST ARTIFICIAL CHROMOSOMES**
Marc G. FORTIN and Richard W. MICHELMORE. Dept. Vegetable Crops, Univ. Calif at Davis, Davis CA. 95616
We are interested in chromosome walking over large distances from RFLP markers to clone disease resistance genes in Lactuca sativa. We will also characterize the frequency of recombination in areas surrounding these resistance loci. We have isolated large molecular weight DNA from L. sativa cv Kordaat (>7 Mbp) that we have cloned as yeast artificial chromosome (YAC) clones. YACs of 100 to 250 Kbp were obtained. We have developed a YAC vector (for EcoRI partial digest cloning) that facilitates chromosome walking and mapping using RNA promoters. Rare restriction enzyme sites were added either side of the cloning site to facilitate identification of terminal sequences in the cloned DNA. The frequency of recombination over defined physical distances in different areas of the genome, specially surrounding resistance loci, will be determined using RFLP segregation analysis of the terminal sequences from YAC clones.

R 313 IMMUNOCYTOLOCALIZATION OF HYDROXYPROLINE RICH GLYCOPROTEIN PC-1 DURING MAIZE DEVELOPMENT, Sue E. Fritz, Elizabeth E. Hood, and Kendall R. Hood, Department of Biology, Utah State University, Logan, UT 84322-5305. Hydroxyproline rich glycoproteins (HRGPs) are a main component of the primary cell wall. PC-1, the major HRGP isolated from maize pericarp, has been partially characterized and quantified (see E.E. Hood et al. related poster). Poly- and monoclonal antibodies to PC-1 protein and have been used as probes in an indirect alkaline phosphatase antibody conjugate reaction against three maize varieties. Tissue from stems, leaves, roots, kernels and cobs were printed on nitrocellulose; while kernels at six developmental stages were embedded in glycol methacrylate and sectioned. The objective of these studies was to localize PC-1 in different tissues and stages of kernel development. India ink staining of total soluble protein and polyclonal antibody staining of tissue prints give comparable results. Kernel morphology can be easily seen. Golden x Bantam (GxB), a sweet corn, stains nearly uniformly black and purple, respectively, throughout development. At 10 and 20 days after pollination (DAP) the staining is most intense in the pericarp and less intense in the developing endosperm and central portions of the glume. Popcorns, South American Yellow (SAY) and Japanese Hullness (JHL), show less soluble protein primarily at late stages of development (30 and 40 DAP) when endosperm has hardened. Embryos stain darkly. Pericarp tissue from all three varieties reacts strongly with monoclonal antibody beginning at early stages of development, becoming most intense around 10 and 20 DAP. Internal areas of kernels stain more lightly indicating either lesser quantities of HRGPs or diffusion from adjacent tissues during printing. Embryonic tissues of the later stages of SAY and JHL stain more strongly than pericarp. Immunocytolocalization with monoclonal antibodies on embedded kernel tissue show intense pericarp staining with little or no background staining. The staining intensity varies among corn varieties, areas of kernel pericarp, and increases with kernel maturity.

R 314 ORGAN-SPECIFIC AND DEVELOPMENTAL EXPRESSION OF A BARLEY THIONIN GENE IN TOBACCO.
F. García-Olmedo, J.A. Fernández, M.J. Carmona, J.J. López-Fando. Cátedra de Bioquímica y Biología Molecular, E.T.S.Ingenieros Agrónomos-UPM, E-28040 Madrid, Spain.
Thionins are sulfur-rich polypeptides of about 5 kDa, which are active against plant pathogenic microbes (1,2). They are synthesized as precursors that undergo at least two processing steps: the co-translational excission of a signal peptide and the post-translational elimination of a C-terminal acidic peptide. Both cDNA and genomic DNA encoding a barley endosperm thionin have been cloned. Two introns interrupt the sequence corresponding to the C-terminal peptide. Constructions involving various lengths of sequence upstream of the thionin gene fused to the glucuronidase reporter gene, as well as others involving the 35S promotor fused to the coding sequences of the cDNA and the genomic DNA, have been used to transform tobacco plants. It is concluded that the barley thionin promoter directs seed-specific, temporally-regulated expression in tobacco and that the introns from the monocot gene are splied when the gene is expressed in a dicot.
1) Fernandez de Caleya et al. (1972) Applied Microbiology 23: 998-1000.
2) García-Olmedo et al. (1987) Oxford Surveys of Plant Molecular and Cell Biology (B. Miflin ed.) Vol. 4: 275-334. Oxford University Press.

R 315 MOLECULAR ANALYSIS OF L-PHENYLALANINE AMMONIA-LYASE (PAL) FROM ALFALFA (MEDICAGO SATIVA L.). Ganesan Gowri, Karen Dalkin and Richard A. Dixon. The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402.
L-Phenylalanine ammonia-lyase (PAL) catalyses the first step in the biosynthesis of phenylpropanoid plant secondary metabolites among which are the isoflavonoid phytoalexins of the leguminosae. PAL has been the subject of much interest in view of its induction by a number of environmental factors including elicitation, stress, light and growth regulators. In bean and alfalfa there are several isoforms of PAL that differ in their relative abundance and kinetic behaviour. Previous studies have shown that application of fungal cell wall preparation (elicitor) to bean cell suspension cultures causes a rapid increase in PAL activity and mRNA transcription. In alfalfa cell cultures, increased activity of PAL upon exposure to elicitor has recently been reported. In order to investigate molecular mechanisms of defense against fungal infection in alfalfa, we have isolated three putative PAL cDNA clones from an elicitor induced alfalfa cDNA library using anti-(alfalfa PAL) antibody. One of these cDNA clones (PAL-3), which appears to be near full-length, hybridized strongly to a partial PAL cDNA clone from bean. Genomic Southern analysis using PAL-3 revealed the presence of at least six different genes for PAL in alfalfa. Nucleotide sequence analysis of this cDNA clone as well as kinetics of induction of PAL mRNA by the fungal elicitor in alfalfa suspension cultures will be presented. The possible involvement of the transcripts encoded by the other two cDNA clones in fungal elicitation in alfalfa is also being studied.

R 316 IDENTIFICATION OF RFLPs MARKERS LINKED TO THE Ht1 GENE BY THE USE OF ISOGENIC LINES, C.Guitton, S. Bentolila , N.Bouvet, G.Freyssinet, Rhône-Poulenc Agrochimie, BP 9163, Lyon Cédex 09, France.
We have identified several RFLP markers linked to the Ht1 gene of maize, which confers resistance to the fungal pathogen: Helminthosporium turcicum race 1.
This was accomplished by the use of four pairs of near isogenic lines (NILS) namely:A619, B73, W153R, CM105, each of them differing by the presence or the absence of the Ht1 gene.
A total of 64 probes, with some of them in groups of three pooled together, were labelled and probed against southern blots of these NILS digested with three restriction enzymes: EcoRI, BamHI, HindIII.
Tightly linked markers with Ht1 gene are distinguished by the fact they must exhibit polymorphism between all of the NILS studied. two probes, UMC125 and UMC150, from D.HOISINGTON'S genomic bank, localized on the chromosome 2L, fulfil this requirement. The distance between them is approximatively 30 cM, suggesting either a big drag effect during the breeding program of introgression (big wild segment retained in the introgressed lines) or a co-introgression.
The estimation of map distances between Ht1 gene and these RFLPs markers is being carried out by linkage analysis on a F2 population.

R 317 STRATEGIES FOR THE MOLECULAR CLONING OF A DNA-BINDING PROTEIN. Maria J. Harrison[1], Mike A. Lawton[2], Christopher J. Lamb[2], and Richard A. Dixon[1]. [1]The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402. [2]The Salk Institute, Plant Biology Laboratory, P. O. Box 85800, San Diego, California, 92138.

Chalcone synthase (CHS) catalyses the first biosynthetic step in the flavonoid/isoflavonoid branch of the phenylpropanoid pathway. In Phaseolus vulgaris, CHS is coordinately induced along with other enzymes in the pathway in response to environmental stress and pathogen attack. The end products of the pathway are thought to play an important role in the resistance of the plant to pathogen attack (1). The promoter region from an elicitor inducible chalcone synthase gene has been analyzed by gel retardation and footprinting using nuclear extracts from bean and alfalfa suspension cultured cells. Three protein binding sites have been identified in the distal half of the promoter mapping to a region which appears to be involved in the quantitative regulation of the promoter. Attempts to purify the binding protein/protein complex will be described.

1. Dixon, R.A., et al. Phil. Trans. R. Soc. London B. 314, 411-426, 1986.

R 318 **RFLP-ASSISTED MOLECULAR CHARACTERIZATION OF POWDERY MILDEW RESISTANCE GENES FROM BARLEY**, **Karin M. Hinze, Paul Schulze-Lefert, Richard D. Thompson and Francesco Salamini, Department of Pflanzenzüchtung und Ertragsphysiologie, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30.**

Erysiphe graminis f. sp. hordei **is the causal agent of powdery mildew infections of barley, one of the severest diseases in Europe. Although classical genetical analysis has identified several barley encoded resistance loci clustered on chromosomes 4 and 5 so far nothing is known molecularly about the corresponding genes. We have chosen two of these loci for a detailed molecular characterization: the *ml-o* locus, conferring recessive, horizontal resistance and the *Ml-g* locus, mediating dominant, race-specific resistance. As a first step towards cloning the corresponding genes we are using a locus targeted RFLP-identification procedure based on recurrent parent backcross lines (1). Analyzing near-isogenic lines of all known *ml-o* mutants and *Ml-g* alleles, we have isolated so far tightly linked RFLP-markers derived from a barley genomic PstI-library. Marker identification is facilitated through a screening procedure employing simultanous hybridization of 4-6 genomic PstI fragments to Southern blots. Their chromosomal origin is verified by wheat/barley chromosome addition lines. The final goal is a collection of locus-linked RFLP-markers with a density that allows physical linkage of markers on pulsed-field electrophoresis gels.**

(1) N.D. Young, D. Zamir, M.W. Ganal and S.D. Tanksley (1988), Genetics 120, p. 579-585.

R 319 COMPARISON OF HYDROXYPROLINE-RICH GLYCOPROTEINS IN SWEET CORN AND POPCORN PERICARPS, Elizabeth E. Hood, Kendall R. Hood, and Sue E. Fritz, Dept. of Biology, Utah State Univ., Logan, UT 84322-5305.

Hydroxyproline-rich glycoproteins (HRGPs) are an integral structural part of many plant cell walls and are accumulated during normal development. They are primarily associated with tissues that support or protect the plant. PC-1, an HRGP from maize pericarp has been previously isolated and partially characterized. Here we demonstrate the accumulation of salt soluble and insoluble PC-1 and total hydroxyproline (hyp) in maize pericarp during seed development. Pericarp samples were taken at several times post pollination from three greenhouse grown maize varieties. Pericarp cell wall (CW) dry weight (DW) increased to a higher level in one popcorn than in the sweet corn; the other popcorn accumulated CW DW to levels similar to the sweet corn even though the kernels are much smaller. Hydroxyproline accumulated to a higher level in both popcorns with their tough pericarps (up to 6 μg/mg DW), than in the hybrid sweet corn with soft pericarp (up to 2 μg/mg DW). However, the most striking difference between the popcorns and sweet corn was in the amount of hyp per pericarp--up to 25.1 μg per pericarp in popcorn versus 9.8 μg per pericarp in the sweet corn. Wall proteins from these 3 maize varieties are similar in size, charge, and antigenicity as shown by gel electrophoresis and western blot analyses. In a related poster (see S.E. Fritz & E.E. Hood) pictorial data are presented showing tissue and cell types containing PC-1 and related antigens in developing maize seed cell walls. Potential roles of these proteins in structure and in defense against pathogens and wounding will be discussed.

R 320 STRATEGY FOR CLONING AVIRULENCE GENES FROM BREMIA LACTUCAE (LETTUCE DOWNY MILDEW), Howard Judelson, Erik Legg, and Richard Michelmore, Dept. of Vegetable Crops, University of California, Davis, 95616.
Our goal is the identification of the molecular basis of specificity in the interaction between the Oomycete pathogen, Bremia lactucae, and lettuce. Incompatible interactions are determined by avirulence (Avr) genes in the fungus and matching resistance (Dm) genes in the host. A genetic map of B. lactucae is being constructed using RFLP markers, and to date contains over 80 loci. RFLP loci linked to 6 Avr alleles have been identified. A probe tightly linked to Avr6 is being used to start a chromosome walk to this Avr locus. Cosmid clones linked to Avr loci will be introduced into virulent isolates of the pathogen to test for functional Avr genes. Procedures for the DNA-mediated transformation of B. lactucae are therefore being developed. Initially, vectors for transformation of Oomycete fungi were constructed. Promoter and polyadenylation elements from highly expressed genes from B. lactucae were fused (transcriptionally and translationally) to bacterial drug resistance genes (NPTII, HPT). The vectors incorporated B. lactucae sequences from either heat-shock gene hsp70 or gene ham34. Both genes are expressed constitutively throughout the life cycle. The function of ham34 is unknown but the gene is transcribed at a high level (6% of mRNA in germinating spores). B. lactucae is a obligate biotroph therefore traditional methods for transformation are not feasible. DNA is being introduced by high-velocity microparticle bombardment. Transformants will be selected by growing the fungus on drug-imbibed lettuce cotyledons.

R 321 MOLECULAR CLONING OF A PROTEIN ASSOCIATED WITH SOYBEAN SEED OIL BODIES WHICH IS HOMOLOGOUS TO THIOL PROTEASES OF THE PAPAIN -CATHEPSIN FAMILY, Andrzej Kalinski, Jane M. Weisemann, Benjamin F. Matthews and Eliot M. Herman, Plant Molecular Biology Laboratory, USDA/ARS, Beltsville, MD 20705
A 34 kDa (P34) polypeptide is one of the four major proteins observed in SDS/PAGE of isolated soybean seed oil bodies. A soybean lambda ZAP II cDNA library of mRNA of midmaturation seeds was screened with two different monoclonal antibodies elicited against P34. A putative full length cDNA clone of 1358 bp was isolated. The cDNA clone encodes a 780 bp open reading frame with a deduced polypeptide sequence of 28939 Da which aligns with the first 23 of 25 amino terminal residues of P34. A comparison of the deduced sequence of P34 with those deposited with GenBank shows that P34 is homologous to the thiol proteases of the papain/cathepsin family. Northern analysis of RNAs from soybean tissues shows the highest levels of P34 mRNA in midmaturation seeds and trace amounts in mature leaves and germinating seeds. Hybridization pattern on a Southern blot of Eco RI digested genomic DNA probed with the P34 cDNA indicates that there is more than one copy of the P34 gene in the soybean genome. P34 genes were analyzed on Southern blots of restricted chromosome-sized DNA molecules (50-1300 kb) resolved by pulsed field gel electrophoresis and probed with P34 cDNA. Supported in part by USDA Competitive grant CRCR-86-1-2021 to EMH.

R 322 THE ROLE OF CYTOCHROME b_5 IN Δ-12 DESATURATION BY MICROSOMES OF CARTHAMUS TINCTORIUS, Ellen V. Kearns, Suzanne H. Thomashow, Christopher R. Somerville, MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48823.
Cytochrome b_5 has been demonstrated to be the electron donor for desaturase enzymes in mammalian liver endoplasmic reticulum. In plants, electron donors for the membrane-bound desaturases have not been identified. The cytoplasmic domain of cytochrome b_5 was purified from Brassica oleracea, and polyclonal antibodies were made in mouse. Dilutions of immune serum and ascites fluid blocked virtually all electron flow through cytochrome b_5 as shown by the inhibition of NADH-dependent cytochrome c reduction in Carthamus tinctorius microsomes. Preimmune serum dilutions did not block electron flow. Results indicating the effect of these antibodies on Δ-12 desaturation will be presented.

R 323 GENOMIC DISTRIBUTION, VARIABILITY AND ORIGIN OF DISEASE RESISTANCE GENES IN LETTUCE

Rick Kesseli, Oswaldo Ochoa and Richard Michelmore
Department of Vegetable Crops, University of California, Davis, CA. 95616.

Thirteen resistance genes to lettuce downy mildew (Dm) have been located in four clusters on our detailed RFLP generated genetic maps of lettuce. Three other resistance genes to pathogens have now been mapped Anthracnose (Ant), Corky root (cor) and Lettuce mosaic virus (mo). With RFLP's as genetic markers we have surveyed 68 lines including cultivars and wild relatives. These data have defined higher levels of allelic variation in the genomic regions of the resistance genes. The history of genetic change in two regions containing clusters of resistance genes is traced by analysing pedigrees leading to modern cultivars. For example, two distinct sources of Dm5/8 are founded in the cultivars surveyed and these were introduced from Russian and western European accessions.

R 324 ACYL CARRIER PROTEIN IS MODIFIED IN A CoA DEPENDENT REACTION UPON IMPORT INTO THE CHLOROPLAST, Gayle Lamppa and Michael Fernandez, Department of Molecular Genetics and Cell Biology, University of Chicago, 920 E. 58th St., Chicago, IL 60637

A cDNA coding for spinach ACP was used to investigate import and proteolytic processing of the ACP precursor (preACP) by isolated spinach chloroplasts, and to examine if the addition of the phosphopantetheine prosthetic group is essential for these events. Import of preACP resulted in two products of ~14 and 18 kD. The slower migrating form is a derivative of the 14 kD peptide, as demonstrated in a time course experiment. Serine 38 was converted to alanine, eliminating the phosphopantheine attachment site. Upon import of the mutant ACP precursor (preACPΔSer) only the 14 kD peptide was found, showing that the production of the 18 kD peptide depends on the presence of serine 38. Furthermore, these results demonstrate that the prosthetic group is not necessary for ACP translocation and processing. The modified peptide exhibits enhanced mobility relative to the 14 kD peptide when analyzed on non-denaturing polyacrylamide gels. This enhanced mobility of the modified form, which requires serine 38, suggested that the modification is the addition of the phosphopantetheine group. Cleavage of the precursor was reconstituted in an organelle-free reaction. Using preACP, the major product was the 14 kD peptide with a low level of the 18 kD peptide, whereas preACPΔSer gave only the 14 kD peptide. CoA normally donates the phosphopantetheine to ACP in a reaction catalyzed by holo-ACP synthase. The addition of CoA to the organelle-free reactions gave complete conversion of the 14 kD peptide to the modified form at 10 uM. Mg^{++} also stimulates the modification reaction, whereas it is inhibited by EDTA at concentrations that do not affect the removal of the transit peptide. Our results support the conclusion that there is a holo-ACP synthase activity in the soluble compartment of spinach chloroplasts.

R 325 *ARABIDOPSIS THALIANA* HOST DIFFERENTIAL RESPONSES TO THE PHYTOPATHOGEN *XANTHOMONAS CAMPESTRIS* PATHOVAR *ARMORACIAE*, Gerard R. Lazo and Robert A. Ludwig, Department of Biology, Sinsheimer Laboratories, University of California, Santa Cruz, CA 95064.

We have tested *Arabidopsis thaliana* for susceptibility to phytopathogenic bacteria. Fifteen genetically diverse strains of *Xanthomonas campestris* pathovars *campestris* and *armoraciae* were inoculated on five different *A. thaliana* ecotypes: Columbia, Hannovrisch, Landsberg erecta, Niederzenz, and Wassilewskija. When challenged with *X. campestris* pv. *campestris*, all five ecotypes exhibited chlorotic leaf symptoms that started at leaf margins. In contrast, with strains of *X. campestris* pv. *armoraciae* (a leaf spot pathogen of crucifers) host differential responses were detected; leaf spot symptoms appeared on susceptible *A. thaliana* ecotypes. Five other *X. campestris* pathovars (of different host plants) failed to yield disease symptoms on all five *A. thaliana* ecotypes tested. Plant infection responses were assayed on one-month-old vegetatively grown plants spray-inoculated with bacterial suspensions and maintained under high humidity. Disease symptoms were usually visible one week after inoculation. As a second screening assay, plants grown in Petri dishes yielded similar, differential host responses. To map *A. thaliana* resistance loci, we are now determining segregation patterns from progeny of crossed *A. thaliana* ecotypes. We are using a two step protocol to identify and clone plant resistance genes. Genomic DNA libraries from resistant ecotypes are first being constructed in *Agrobacterium tumefaciens* pTi-derived plasmids. Using transformation protocols designed to screen genomic libraries, susceptible ecotypes will then be tested for phenotypic rescue of disease resistance.

R 326 EXPRESSION OF ANTI-SENSE RNA IN TRANSGENIC TOBACCO PLANTS CONFERS RESISTANCE TO GEMINIVIRUS INFECTION, **Conrad Lichtenstein, Anthony G. Day & Eduardo Rodriguez-Bejarano, Centre for Biotechnology, Imperial College, London SW7 2AZ, England.**

We chose Tomato Golden Mosaic Virus (TGMV), a gemini virus, to use as a model system for producing virus-resistant plants by expression of anti-viral RNAs. Using the *Agrobacterium tumefaciens* transformation system, transgenic tobacco plants expressing antisense RNA, targeted against TGMV genes, were constructed to challenge with TGMV to ask whether these plants have acquired resistance to this virus. Our genetic cassettes also contained, on the same transcription unit, a gene encoding antibiotic resistance allowing selection for concomitant expression of the antisense gene.

Following infection with TGMV the frequency of symptom development was very significantly reduced in a number of transgenic lines and could be correlated with a marked reduction in viral DNA replication as seen in a leaf disc inoculation assay.

R 327 STRUCTURE OF TOBACCO BASIC β-1,3-GLUCANASE GENES, W. Donald MacRae, Sylvia Soder, Linda Dhani, Graham Rayner and Roger J. Kemble, Allelix Crop Technologies, 6850 Goreway Drive, Mississauga, Ontario, Canada L4V 1P1

β-1,3-glucanases of higher plants are important modulators of plant-microorganism interaction as well as having yet poorly understood roles in normal plant development. Their accumulation is regulated by the infection process, chemical elicitors and hormones and by normal development in floral tissue. Acidic and basic β-1,3-glucanases have been distinguished in various isoforms. The basic β-1,3-glucanases are known to be encoded by a multigene family in tobacco. We have isolated three genomic clones from a tobacco library in the vector λEMBL3 on the basis of their hybridization with a cDNA clone for a basic β-1,3-glucanase. The clones differ in their restriction maps and appear to represent different members of the multigene family. We will report the results of restriction enzyme mapping and nucleotide sequencing of these β-1,3-glucanase genes.

R 328 CHITINASE AND GLUCANASE FROM FUNGALLY-INFECTED ALFALFA. Eileen A. Maher and Richard A. Dixon, The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402.

Chitinase and 1,3-β-D-glucanase may participate in defense reactions against fungal pathogens by degrading fungal cell walls and/or releasing fragments that could elicit additional plant responses to infection. To determine whether chitinase and glucanase could enhance disease resistance, we plan to evaluate resistance in transgenic alfalfa (Medicago sativa) with increased expression of these two hydrolases. In preliminary experiments, chitinase and glucanase from fungally-infected alfalfa were characterized and potential probes for chitinase and glucanase were evaluated. Multiple chitinase and at least two glucanase isozymes were identified by chromatofocusing of intercellular washing fluids from alfalfa following inoculation with Colletotrichum trifolii, an alfalfa pathogen. Four protein bands from fractions with chitinase activity reacted with an anti-(bean chitinase) antibody when evaluated by Western blotting, and activity associated with these specific proteins was confirmed in glycol chitin polyacrylamide gels. High levels of chitinase and glucanase were induced in both compatible and incompatible interactions and varied with cultivar. Isolation of chitinase and glucanase clones from alfalfa cDNA libraries is in progress.

R 329 BIOCHEMISTRY AND MOLECULAR BIOLOGY OF OIL-BODY PROTEINS IN ARABIDOPSIS AND OILSEED CROPS, Denis J. Murphy, Ceri Batchelder, Ian Cummins, Eira-Wyn Edwards, Charles H. Shaw, Helen V. Whitfield, Oilseeds Research Group, Department of Biological Sciences, University of Durham, Durham DH1 3LE, UK.

The major proteins, termed oleosins, that are associated exclusively with the storage lipid bodies found in all oil-storing plant tissues, have been characterised from a wide variety of crop species. In many cases, the major oleosins were highly hydrophobic proteins of 15-25kDa. Antibodies raised against the major oleosins of a particular plant family readily recognised oleosins of a similar size within each family, e.g. 19-20kDa for Cruciferae, 20kDa for Compositae, 24kDa for Leguminosae. In addition to these intra-family similarities, extensive cross-reactivity was found between different families. These immunological studies combined with peptide mapping experiments indicate that oleosins from many diverse plant species may share common structural attributes. This was confirmed by direct sequencing of the major oleosins from rapeseed and radish and by sequence data deduced from cDNA sequences. Comparison of these sequences with the sequence derived for the major maize oleosin (Vance & Huang, 1987) shows many striking similarities. A physical structure for oleosins will be proposed and their role and potential biotechnological usefulness will be discussed.

R 330 THE INTRODUCTION OF A SYNTHETIC GENE, ENCODING A NOVEL LYTIC PEPTIDE, INTO PLANTS WHICH THEN EXHIBIT INCREASED BACTERIAL DISEASE RESISTANCE, Pablito G. Nagpala*[1], M. Selim Cetiner[2], Timothy P. Denny[3], Christopher A. Clark[4], William J. Blackmon[2], and Jesse M. Jaynes[1], Departments of Biochemistry[1], Horticulture[2], and Plant Pathology[4], Louisiana State University, Baton Rouge, LA 70803 and Department of Plant Pathology[3], University of Georgia, Athens, GA 30602.

A 38 amino acid long peptide, Shiva-1, was designed and chemically synthesized in our laboratory. Shiva-1 has only 46% amino acid homology with the naturally occurring lytic peptide from *Hyalophora cecropia* known as cecropin B. However, hydrophobic properties and charge density of the natural molecule were conserved at 100% in the synthetic peptide. This novel lytic peptide was shown to be capable of killing a number of different species of plant pathogenic bacteria at nanomolar concentrations. Comparative studies indicate that Shiva-1 is more effective in this bacteriolytic activity than cecropin B. The gene for Shiva-1 has been chemically synthesized and cloned into the binary LBA 4404/pBI 121 under the control of a wound inducible plant promoter and in A281/pMON530 under the cauliflower mosaic virus promoter 35S. Tobacco leaf disks were transformed using these binary systems containing the gene encoding Shiva-1 and transgenic plants have been obtained. These plants were shown to be kanamycin resistant and those infected with LBA 4404/pBI 121 have GUS activity indicating effective plant transformation. Southern blots have confirmed the presence of single copies of the Shiva-1 gene integrated into the genomes of individual transgenic plants. Northern analysis verifies that the expression of the gene is triggered by mechanical and pathogen induced wounding. F1 plants demonstrate a 3:1 segregation pattern for kanamycin resistance. Preliminary results indicate that these plants exhibit delayed symptoms, reduced disease severity and mortality after infection by *Pseudomonas solanacearum* when compared to transgenic control plants.

R 331 SPECIFIC BINDING OF SUPPRESSOR OF PHYTOPHTHORA INFESTANS TO MEMBRANE SITE OF POTATO CELLS, Naotaka Furuichi and Joichi Suzuki

Developmental and Reproductive Biology Center, Yamagata 990, Japan. Treatment of potato tissues with suppressors of Phytophthora infestans results in the inhibition of hypersensitive cell death, a typical resistance reactions against microbial infection. To identify putative suppressor targets in potato tuber cells, immunochemical methods were used. The fluorescent labeled anti-suppressor monoclonal antibodies disclosed suppressor binding site(s) in membrane surface of the host protoplasts. The glucan binding proteins of potato cells were isolated after passage through the potato tuber homogenate to affinity gels, to which glucan suppressors from compatible or incompatible race were coupled. After the binding proteins of the suppressors being fractionated by SDS-PAGE, the proteins of the R1- and r-gene cultivars revealed 66 kD protein, and 41.5 kD and the lower mol. wt. proteins, respectively. These results suggested that the binding between suppressors and the binding proteins regulate the switching on or off with regard to hypersensitive reaction of potato cells.

R 332 PROTEINS ASSOCIATED WITH THE MAIZE LESION MUTANT lls1, Ron J. Okagaki and Oliver E. Nelson, Laboratory of Genetics, University of Wisconsin, Madison, WI 53706 There are over 20 lesion genes in maize that produce necrotic lesions in the plant. Lethal leafspot-1 (lls1) is one such gene, and its expression gives necrotic lesions that resemble lesions made by the fungal pathogen Helminthosporium carbonum race 1. Expression of several other lesion genes also give lesions resembling infections by pathogens. This has suggested to some workers that the lesion genes may be a useful model system for studying disease resistance. However, others believe that the resemblance is coincidental. We are looking at the proteins that appear during lesion formation in lls1 plants. One dimensional SDS-PAGE analysis comparing proteins from lls1 and their non-mutant sibs reveal several new bands appearing in the lls1 plants. Of interest here are two low molecular weight, acid extractable proteins that may be pathogenesis-related proteins. Pathogenesis-related proteins are induced after pathogen infection and may play a role in disease resistance, however other stresses are known to induce these proteins. We are hoping to learn more about the relationship between the accumulation of these and other proteins and the formation of necrotic lesions in lls1 plants.

R 333 MARKER SATURATION OF GENOMIC REGIONS OF *Lactuca sativa* CONTAINING DISEASE RESISTANCE GENES. Ilan Paran, Richard V. Kesseli, Kiotaka Nakahara and Richard W. Michelmore. Department of Vegetable crops, University of California Davis, CA 95616. Genes that code for resistance to downy mildew in lettuce are clustered in four linkage groups. The major linkage group consists of seven mapped resistance (*Dm*) genes that span over 19 cM. We are analysing linkage between these genes and RFLP markers in three different crosses. Enrichment for linked RFLPs to *Dm* genes is being done using near-isogenic lines that differ for a particular *Dm* gene. Currently, four markers have been found linked. The closest marker is a member of a multi gene family. Two members of this family are linked to two *Dm* genes in two different linkage groups. The cDNA clone was sequenced and found to be homologous to the glycolytic enzyme Triose Phosphate Isomerase. Additional markers up to 200 Kb from existing ones will be obtained by screening a general jumping library that is under construction in this laboratory. These markers will be used as starting points for chromosomal walking to clone the *Dm* gene.

R 334 WOUND AND LIGHT REGULATED EXPRESSION OF TWO TOMATO TRANSCRIPTS, Barbara L. Parsons and Autar K. Mattoo, Plant Molecular Biology Laboratory, USDA/ARS, Beltsville Agricultural Research Center-West, Beltsville, MD 20705 Differential blot hybridization was used to isolate cDNA clones corresponding to tomato fruit RNAs whose expression is altered upon wounding. Two of these cDNA clones, designated pT52 and pT58, hybridize to RNAs of approximately 450 and 820 nucleotides in length, respectively. Both the transcripts accumulate with time after wounding of tomato pericarp tissue. This time-dependent induction is characterized by cyclic increases and decreases in steady-state levels. Northern blot analysis revealed that both the transcripts accumulate to the highest steady-state levels in early-red fruit. Analysis of different tomato plant tissues demonstrated that more pT52 RNA is found in the stem than in the leaf or root, whereas pT58 RNA is present in equally high levels in both the stem and root. In addition, the steady-state level of the pT52 transcript is regulated by light. We are using these and other cDNA clones as tools to study the molecular mechanisms underlying the wound response.

R 335 IN VIVO ANALYSIS OF SPINACH ACYL-ACYL CARRIER PROTEINS IN DEVELOPING SEED AND LEAF, Martha A. Post-Beittenmiller[1], Jan G. Jaworski[2], and John B. Ohlrogge[1],
1. Department of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48824-1312;
2. Department of Chemistry, Miami University, Oxford, OH 45056.

Although plant oils are a major commodity, worth nearly $20 billion annually, relatively little is known regarding the regulation of their biosynthesis. In order to determine what step(s) might be involved in limiting production of de novo fatty acids, we have begun an analysis of the in vivo pools of the acylated and nonacylated forms of spinach acyl carrier protein (ACP). Liquid nitrogen frozen tissue was homogenized in the presence of N-ethylmaleimide at pH 6.0 and immediately run on polyacrylamide urea gels. Acyl-ACPs with fatty acid chain lengths from C_3 (malonyl) to $C_{18:1}$ could be resolved by varying the urea concentration in this gel system. In spinach leaf the level of total acyl-ACPs was approximately 20% of the level of free, nonacylated ACP. Both ACP-I and II were similar in their pattern of distribution of acyl-ACPs and the relative abundances of acyl-ACP-I and acyl-ACP-II indicated that both isoforms had similar activity in vivo. In developing spinach seed there is a single isoform, ACP-II. The proportion of acyl-ACP to free ACP-SH was higher in seed than leaf such that approximately 40% of ACP was acylated. Only very low levels of malonyl-ACPs were detected in either leaf or seed, suggesting that malonyl-ACP or the steps leading up to malonyl-ACP may be limiting rates of fatty acid synthesis in plants.

R 336 *ARABIDOPSIS THALIANA* AS A HOST FOR *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*, Robert B. Simpson and Lori Johnson, Molecular Biology Group, Plant Cell Research Institute, Inc., 6560 Trinity Court, Dublin, CA 94568

One of the most challenging areas in plant pathology is the isolation of genes which control a plant's interaction with pathogens. The difficulty in isolation of these genes stems from the complex nature of most higher plants. We suggest that the cruciferous weed *Arabidopsis thaliana* may serve as a model organism in plant pathology since its biology, genetics and genome structure are particularly amenable to the isolation of plant genes. We have shown that *Arabidopsis* is a host for the phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot in cruciferous crops. Furthermore, two ecotypes of *Arabidopsis* show differential responses to *X.c.* pv. *campestris*, suggesting that disease resistance genes are present in the *Arabidopsis* gene pool and may be isolated in a straightforward manner. We are in the process of screening 91 ecotypes of *Arabidopsis* for their responses to five strains of *X.c.* pv. *campestris* and will report the results.

R 337 ALTERED ACETYL-COENZYME A CARBOXYLASE CONFERS HERBICIDE TOLERANCE IN MAIZE, D.A. Somers, W.B. Parker, L.C. Marshall, P. Dotray, D.L. Wyse, J.W. Gronwald, and B.G. Gengenbach, Department of Agronomy and Plant Genetics and U.S. Dep. of Agriculture-Agricultural Research Service, University of Minnesota, St. Paul, MN 55108.

Acetyl-coenzyme A carboxylase (ACCase) from most grasses including maize is inhibited by cyclohexanedione and aryloxyphenoxypropionate herbicides. Inhibition of ACCase blocks the ATP dependent carboxylation of acetyl-CoA leading to depletion of malonyl-CoA, which is the primary precursor for fatty acid synthesis. Dicot ACCases are not inhibited by these herbicides. Five mutants tolerant to sethoxydim (cyclohexanedione) and/or haloxyfop (aryloxyphenoxypropionate) were selected from maize tissue cultures. Herbicide tolerance was expressed in plants regenerated from the tolerant tissue cultures. Four mutants were tolerant to both herbicides whereas one mutant exhibited tolerance to haloxyfop only. In all mutants, herbicide-tolerance was inherited as single, partially dominant, nuclear genes. Homozygous progeny of the most characterized mutant, S2, treated with up to 0.8 kg/ha sethoxydim (4-fold the normal field rate) did not exhibit herbicide injury symptoms and had the same grain yield as untreated susceptible wildtype plants. The ACCase activities of wildtype and homozygous mutant S2 seedlings were inhibited 50% by 10 and >1000 μM sethoxydim and 3.5 and 56 μM haloxyfop, respectively. Analyses of ACCase activities from homozygous mutant seedlings derived from the four other mutant tissue cultures also indicated that the mutant ACCases were less sensitive to herbicide inhibition. These results indicate that herbicide tolerance was controlled by partially dominant nuclear mutations in the ACCase structural gene that conferred herbicide-insensitivity to this plastid-localized enzyme. The relationship of these genes or alleles in the different mutants is currently under investigation. Furthermore, sufficient herbicide tolerance was exhibited in homozygous mutant maize to warrant development and evaluation of herbicide tolerant maize varieties.

R 338 USE OF RFLP MARKERS FOR LOCATING GENES CONTROLLING MORPHOLOGICAL TRAITS IN *BRASSICA RAPA*, Keming Song and Thomas C. Osborn, Dept. of Agronomy, University of Wisconsin, Madison, WI 53706. One hundred thirty four mapped RFLP markers were used to study genes controlling 21 morphological traits in *Brassica rapa* (syn.*campestris*). Ninety five F2 individuals from a cross of Chinese cabbage by Spring broccoli were analyzed for segregation of RFLP loci and for variation in leaf, stem, and flowering characteristics. We were able to locate genes controlling all of the traits measured and to dissect the genetic control of complex traits. For example, we found that eight out of 21 traits seemed to be controlled by single major genes plus minor genes, whereas other traits were controlled by multiple genes with similar effects. Also, we found a group of genes controlling days to flowering were located on one linkage group covering 118 map units, and these genes also controlled days to budding. Two unlinked markers representing duplicated RFLP loci were found to be associated with different genes controlling petiole morphology, suggesting expression of duplicated genes.

R 339 ASSAY OF COMPOUNDS THAT ACTIVATE PLANT DEFENSE USING CHS PROMOTER-REPORTER GENE FUSIONS IN TRANSGENIC TOBACCO. Bruce A. Stermer[1], Jurg Schmid[2], Christopher J. Lamb[2] and Richard A. Dixon[1]. [1]The Samuel Roberts Noble Foundation, Plant Biology Division, P. O. Box 2180, Ardmore, Oklahoma 73402. [2]The Salk Institute, Plant Biology Laboratory, P. O. Box 85800, San Diego, California, 92138.
Transgenic plants containing infection- or stress-activated promoters coupled to reporter genes may be useful in the identification of elicitors, inter-cellular secondary messengers or agrichemicals able to induce plant defense. To examine this possibility, transgenic tobacco plants containing 5'-flanking sequences from two bean chalcone synthase (CHS) genes, λ15 and λ8, fused upstream of the coding region of a β-glucuronidase (GUS) reporter gene, were tested for promoter activation by abiotic elicitors or an incompatible isolate of Pseudomonas syringae. Shortwave UV light or 1 mM $HgCl_2$ induced the expression of both promoter-GUS constructs in transgenic tobacco. Glutathione, an elicitor of bean cells, was unable to activate the fusion genes in tobacco. P. syringae or 100 mM oxalate, both able to induce systemic disease resistance in cucumbers, induced expression of the λ8-GUS but not the λ15-GUS construct. Both P. syringae and oxalate induced GUS activity in leaf tissues several cm away from the treatment site, implicating the involvement of a secondary messenger. In contrast, wounding or UV light induced GUS activity only in the immediate treatment area. These results indicate that defense gene promoters fused with a reporter gene and expressed in transgenic plants may be a useful tool to screen or assay defense-inducing molecules.

R 340 STEREOISOMERISM IN PLANT DISEASE RESISTANCE: PROPERTIES AND REGULATION OF THE STEREOISOMERIC PRINCIPLE IN PISUM SEEDLINGS, Yuejin Sun, Hans D. Van Etten[1] and Geza Hrazdina, Institute of Food Science, Cornell University, Geneva, NY 14456 and [1]Department of Plant Pathology, The University of Arizona, Tucson, AZ 85721
Numerous examples in the plant pathological literature illustrate the phenomenon of induced resistance in plants. One of the common responses associated with the elicitation of the resistant state is the accumulation of phytoalexins. Although there is evidence that phytoalexins are not an important determinant of resistance in all of the interactions, studies from our laboratories with the *Nectria haematococca*/pea interaction argue that phytoalexins may play an important role in some cases.
Pea seedlings normally synthesize only the (+) isomer of pisatin. This is quite unusual, since most legumes produce only the (-) isomers of the isoflavonoid phytoalexins. The synthesis of the unusual optical isomer of a phytoalexin in peas suggests novel strategies for engineering disease resistant plant varieties. Here we report some of the characteristics of 2',7-dihydroxy-4',5'-methylenedioxyisoflavone reductase, the regulatory principle responsible for the production of (+) pisatin, the isoflavonoid phytoalexin of pea seedlings.

INDUCED TRANSCRIPTS ARE MEMBERS OF A NEW CLASS OF CONSERVED DEFENSE-RELATED PROTEINS Michael H. Walter (1,2), Jianwei Liu (1), Claude Grand (2), Christopher J. Lamb (2) and Dieter Hess (1) ; (1) Universität Hohenheim, Institut für Pflanzenphysiologie 260, P. O. Box 70 05 62 D-7000 Stuttgart 70, FRG; (2) The Salk Institute, Plant Biology Lab, P. O. Box 85800, San Diego, CA 92138-9216, USA

We have searched for induced transcripts in a cDNA library derived from bean cell suspension cultures treated with fungal elicitor from *Colletotrichum lindemuthianum*. Six clones corresponding to rapidly induced small mRNAs have been classified into two groups from their DNA sequence. There is only very limited similarity in 5'- and 3'-untranslated regions but the deduced small acidic proteins (designated *Phaseolus vulgaris* (Pv)PR1 and PvPR2 are 89% identical. We have identified from published sequences homologs of these proteins also deduced from elicitor/pathogen-induced mRNAs in pea (pI49, 64% identity), in potato (pSTH2, 41% identity) and in parsley (PcPR1-1, 39% identity). An allergenic protein from birch pollen (BetvI) has 44% similarity to PvPR1. No relationship was found to the well-characterized PR1 protein class from tobacco. These similarities establish a new class of evolutionary conserved defense-related proteins and suggest a common but still unknown function. Southern blot analysis indicates that the bean genome contains a large gene family coding for variants of these proteins.

R 342 **RFLP ANALYSIS OF AN INTERSPECIFIC HYBRID BETWEEN SOLANUM BREVIDENS AND POTATO.** **Christie E. Williams and John P. Helgeson, USDA-ARS, Dept. of Plant Pathology, Univ. of Wisconsin, Madison, WI. 53706.**

Sexual incompatibility between wild species and crop plants often limits the use of wild germplasm in crop improvement. For example, Solanum brevidens has good resistance to potato leaf roll virus and Erwinia tuber soft rot but is incompatible with potato. Somatic hybridization between the species can circumvent this problem. The hexaploid somatic hybrids between diploid S. brevidens and tetraploid potato (S. tuberosum) express the disease resistances of both parents (Helgeson et al. 1986. Plant Cell Reports 3:212-214). Some of these hybrids are fertile (Ehlenfeldt and Helgeson. 1987. Theor. Appl. Genet. 73:395-402) and resistances can be conferred to sexual progeny of the resistant hybrid and susceptible potato cultivars (Austin et al. 1988. Phytopathology 78: 1216-1220). In this study we have analyzed restriction fragment length polymorphisms (RFLPs) in a hexaploid somatic hybrid and in sexual progeny that resulted from a cross with the cultivar Katahdin. By using RFLP markers for each of the 24 chromosome arms (n=12) we demonstrated that at least one copy of each parental chromosome was present in the genome of the somatic hybrid. The absence of a single band from one of the RFLP patterns of chromosome 8 suggests that the S. brevidens parent was heterozygous at that locus and that the somatic hybrid is deficient for that segment of one of the S. brevidens chromosomes. Non-random elimination of S. brevidens chromosomes was not detected among the sexual progeny of the somatic hybrid crossed with Katahdin, whereas segregation for disease resistance traits as well as RFLP markers was evident in the progeny population.

THE *ALTERNARIA* STEM CANKER DISEASE RESISTANCE LOCUS IN TOMATO.

Hanneke M.A. Witsenboer, Elga E. van de Griend, Karen M. Kloosterziel and Jacques Hille.
Free University, Department of Genetics, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

The fungus *Alternaria alternata* f.sp. *lycopersici* causes *Alternaria* stem canker in tomato. Host-specific toxins produced by the pathogen, AAL-toxins, play a major role in pathogenesis. One semi-dominant locus, the *Asc* - locus, involved in tomato resistance to the pathogen and insensitivity to AAL-toxins, was genetically mapped on chromosome 3 at 93 centiMorgan. By studying effects of AAL-toxins on different levels of plant differentiation of hosts and non-hosts, it was concluded that non-host resistance operates by a mechanism different from that operating in a resistant host. Different approaches to unravel the mechanism of resistance in tomato using near-isogenic lines for the *Asc*-locus are employed:

1) Effects of AAL-toxins are studied in leaf bioassays. Three parameters are demonstrated to play an important role in development of symptoms in leaves: the light quantum, AAL-toxin concentration and toxin exposure time.
2) Effects of AAL-toxins are studied in protoplasts. Genotype specific differences are observed at the protoplast level, so a cellular target site for AAL-toxins is apparent. Fluorescent labeling of AAL-toxins is carried out to study the fate of AAL-toxins in protoplasts.
3) To look for genotype specific differences between the near-isogenic lines 2D gel analysis, following in vitro translation of purified RNA, is performed.

These studies give insight into functioning of the *Asc*-locus, and may finally lead to elucidation of the mechanism of *Alternaria* stem canker resistance in tomato.

R 344 TISSUE SPECIFIC ACTIVITY OF A SOYBEAN LIPOXYGENASE-3 PROMOTER-β-GLUCURONIDASE GENE FUSION IN TRANSGENIC TOBACCO. Richard L. Yenofsky, Miriam Fine, and John W. Pellow, Phytogen, 101 Waverly Drive, Pasadena, CA 91105
Lipoxygenases are a ubiquitously distributed group of nonheme iron-containing enzymes that catalyze the oxygenation of polyunsaturated fatty acids or their esters containing cis, cis pentadiene systems to yield hydroperoxides and a range of secondary products. At least three lipoxygenases have been identified in soybean embryos. We had prevously isolated and characterized a soybean lipoxygenase-3 gene. In order to study factors which affect the expression of the gene, we have constructed a chimeric gene consisting of approximately 5 kilobases of 5' upstream soybean DNA linked to the reporter gene encoding β-glucuronidase (GUS). This construct has been introduced into tobacco via Agrobacterium mediated transformation and the expression of the gene monitored in various tissues.

R 345 A NOVEL PROTEIN KINASE GENE FROM *ARABIDOPSIS THALIANA*
Shaohui Zhang, Tony Hunter*, Christopher J. Lamb and Michael A. Lawton, Plant Biology Laboratory and *Molecular Biology and Virology Laboratory, Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138.

Phosphorylation and dephosphorylation of specific proteins play an important role in signal transduction in animal systems. Emerging evidence indicates that similar strategies also operate in plants. For example, calcium controls many physiological and biochemical processes in plants and in some instances this may reflect the involvement of calcium-dependent protein kinases. A DNA fragment was generated by the polymerase chain reaction using oligonucleotide primers designed according to the conserved features of the animal protein kinase C family with *Arabidopsis thaliana* genomic DNA as template. This PCR product was used as a probe to isolate the corresponding genomic clone. The gene contains several small introns ranging from 80 to 150 bp. A 1.9 kb mRNA from this gene has been identified by Northern blot analysis.This gene encodes a deduced protein containing the conserved features of the catalytic domains of animal serine-protein kinases. The derived amino acid sequence indicates that the gene product is a member of the plant protein kinase C and S6 ribosomal protein kinase family. Studies on the expression and function of this gene will be described.

R 346 DEVELOPMENT OF SEROLOGICAL AND NUCLEIC ACID ASSAYS TO DISTINGUISH BETWEEN VIRULENT AND AVIRULENT VIRUSES OF AGARICUS BISPORUS.
Thomas M. Zinnen and Thomas P. Conway, Plant Molecular Biology Center, Northern Illinois University, DeKalb, IL 60115. The yield of commercial mushroom and the development of improved mushroom varieties are hampered by several double-stranded RNA viruses. Infection by avirulent viruses confounds currently available tests for virulent viruses. Initial attempts to produce polyclonal rabbit antiserum specific for a selected virulent isolate were unsuccessful because the antiserum also reacted with an avirulent isolate. Now we are pursuing two alternatives to produce specific assays. First, the characteristics of monoclonal antibodies targeted for the virulent virus are described. Second, 5'-labelled RNAs from both the virulent and the avirulent isolates are used to locate RNA segments specific for the virulent virus that will be suitable for cloning in transcription vectors. This will allow assays based on nucleic acid hybridization to distinguish between the two isolates.

Gene Regulation and Plant Quality Improvement

R 400 ISOLATION AND CHARACTERIZATION OF POLLEN-SPECIFIC GENES FROM Brassica napus, Diego Albani and Steven F. Fabijanski, Department of Biochemistry, University of Ottawa, 40 Marie Curie priv., Ottawa., Ont., Canada, K1N 6N5 and Paladin Hybrids Inc., P.O. Box 219, Brampton, Ont., Canada, L6V 2L2.

A Brassica napus genomic library constructed in Charon 4A was differentially screened with ^{32}P-labeled cDNA made from poly(A)$^+$ RNA obtained from developing pollen and from leaf, stem, silique and flower tissues. Several pollen-specific clones were isolated. Corresponding cDNA clones were obtained from a cDNA library made from poly(A)$^+$ RNA isolated from B. napus developing microspores. The clones so far characterized correspond to genes which are specifically expressed during pollen development. Their transcripts are not accumulating in mature pollen indicating a possible involvement in microspore development but not in microspore germination. The complete nucleotide sequence of these genomic and cDNA clones has been determined and the promoter regions have been localized by primer extension and direct sequencing of the mRNAs. Some of these genes have been introduced into tobacco plants via Agrobacterium tumefaciens-mediated transformation. These B. napus pollen-specific genes are expressed in the transgenic plants and show a spatial and temporal regulation which is consistent with the one shown in the original plant.

R 401 **Cloning and sequence analysis of a sugarcane gene encoding phosphoenolpyruvate carboxylase (PEPC)**, Henrik H. Albert and Sam M. Sun, Department of Plant Molecular Physiology, University of Hawaii, Manoa, Honolulu, HI, 96822. The C-4 carbon fixation pathway confers an important adaptive advantage on plants growing in a tropical environment, and PEPC is the key enzyme in this pathway. We have constructed a genomic library and isolated a gene encoding PEPC from sugarcane (*Saccharrum* hybrid var. H32-8560), one of the most efficient carbon fixing plants, and of major agronomic importance in the tropics. In several C-4 plants, expression of this gene has been shown to be light-regulated and cell-type specific. We have identified the approximate region of transcription initiation by a novel and simple technique. Partial sequence from the coding region shows approximately 70% homology to a C-4 specific PEPC gene from maize. Sequence elements potentially conferring light and/or cell-type specific regulation are under study and will be discussed.

R 402 REGULATION OF THE EARLY AUXIN INDUCIBLE GENE, IAA4/5, IN PEA PROTOPLASTS[1].
Nurit Ballas-Citovsky, Lu Min Wong and Athanasios Theologis, Plant Gene Expression Center, USDA -ARS, 800 Buchanan St, Albany, CA 94710 and Department of Plant Biology, U. C. Berkeley, Berkeley, CA 94720.

One of our major goals is to delineate the molecular mechanism(s) by which auxin regulates gene expression. A first step toward this goal is to define the *cis* acting element(s) responsible for auxin inducibility and the *trans* acting factor(s) that interact with them. We have isolated and characterized several pea genes that are induced very rapidly in response to auxin[2]. One of the genes, PS IAA4/5, is transcriptionally induced, within 5 minutes, by low auxin concentration (20μM). It is also rapidly induced (15-20 min) by various protein synthesis inhibitors *e.g.* cyclohexamide, anisomycin.

To begin to define *cis* acting element(s), we have isolated protoplasts from the third internode of 7-day old etiolated pea seedlings. The protoplasts show the same mRNA induction characteristics to auxin as the intact epicotyl tissue. For example, protoplasts treated with 20 μM IAA, 2,4-D or NAA for 3 hrs accumulate IAA 4/5 mRNA to the same extent as intact pea segment. Furthermore, induction of the IAA4/5 gene by cycloheximide is also similar to that observed in intact tissue. The results demonstrate that protoplasts can be used as an experimental tool for defining the auxin responsive elements in the IAA4/5 promoter.

A plasmid carrying a reporter gene, chloroamphenicol acetyl transferase (CAT), driven by the IAA4/5 promoter has been introduced into pea protoplast. A dramatic increase in CAT activity is detected in response to 10 μM IAA , NAA and 2,4-D after 12 hrs of incubation. The auxin analogs PAA and pCIB are ineffective. Other plant hormones such as GA, ABA and BA do not induce CAT activity. Furthermore, auxin does not affect CAT activity when driven by the 35S CaMV promoter. We are currently testing various 5' and 3' deletions of the IAA4/5 promoter to localize sequences that are required for auxin inducibility.

1. This work was supported by a grant to A. T. from NIH (GM-35447).

2. Theologis, A., Hunyh, T. T. and Davis, R. (1985) *J .Mol. Biol.* 183, 53-68.

R 403 IDENTIFICATION OF A HISTONE H2A cDNA FROM TOMATO

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The cDNA clone pLEH2, selected from a tomato cell culture cDNA library, contains an open reading frame of 417 bp encoding a protein of calculated molecular weight 14.6 kDa. This protein is highly homologous to a minor variant histone H2A type identified in several animal species. These H2A variants, including tomato, exhibit distinct regions of consensus, and differ primarily at the amino- and carboxy-terminal ends of the proteins. The tomato protein has 85% identity with that of the chicken variant (H2af), and 68% nucleotide homology within the coding region. RNA extracted from cell cultures at different time points was probed with pLEH2. Peak expression of this sequence appears to occur during early log phase, prior to the peak accumulation of RNA. This sequence was also hybridized to polyA RNA from tomato leaf, root and stem. The coding region included in pLEH2 has been inserted in a bacterial expression vector. *In vitro* transcription and translation data will be presented.

R 404 ISOLATION AND CHARACTERIZATION OF BRASSICA GENES, PROMOTERS AND REGULATORY SEQUENCES,

C.L. Baszczynski [1], L. Fallis [1], R. Boivin, [1]A. Nantel, [1]G. Bellemare, E. Barbour, V. Armavil, [2]B. Huang, M. Arnoldo, G. Brown, G. Rayner, D. Macrae and R.J. Kemble, Allelix Crop Technologies, 6850 Goreway Dr., Mississauga, Ont., L4V 1P1, [1]Dept. de Biochimie, Universite Laval, Ste Foy, Que., G1K 7P4, and [2]Plant Research Centre, Agriculture Canada, Ottawa, Ont. K1A 0C6.

We are identifying and isolating genes from Brassica species for use as sources of gene, promoter and regulatory sequences in constructing plant transformation vectors. We have used both homologous and heterologous cloned sequences as probes, and novel screening techniques to identify various tissue-specific clones of interest. Included among these are cDNA and genomic clones for the Brassica napus RUBISCO SSU, Cab, napin and acetolactate synthase genes, and a few as yet incompletely characterized leaf, flower and root specific cDNA clones. Some of the genes have been completely sequenced, extensively characterized, and important regulatory regions have been identified. Promoter and various length upstream regions from several genes have been recovered by subcloning and PCR amplification, introduced into direct and binary transformation vectors, and are being tested for expression levels and tissue-specificity in transgenic Brassica and tobacco. In one study, constructs containing the Brassica RUBISCO SSU promoter region (about 500 nucleotides) yielded 5-10 times more GUS activity than those with a CaMV 35S promoter. This expression appears to be tissue-specific; light-inducibility is now being assessed. Assembly of other promoter-gene constructs and subsequent transformations are continuing.

R 405 TRANSIENT EXPRESSION OF GUS IN IMMATURE COTYLEDON PROTOPLASTS: ITS USE TO STUDY *cis*-ACTING ELEMENTS OF THE PHASEOLIN PROMOTER AND UPSTREAM REGION,

Mauricio M. Bustos, Michael J. Battraw, Fatima A. Kalkan and Timothy C. Hall, Department of Biology, Texas A&M Univ., College Station, TX 77843-3258.

We have developed a rapid transient expression assay to study the function of the phaseolin promoter in developing bean cotyledons. The assay utilizes cell protoplasts obtained from staged developing cotyledons and electroporation of supercoiled plasmid DNA containing the phaseolin upstream regulatory region (Bustos et al., 1989, The Plant Cell, 1:839-853), fused to a bacterial ß-glucuronidase (GUS) gene. Several parameters that affected the level of reporter enzyme expression were optimized, such as the developmental stage of the cotyledons used as a source of protoplasts, the pulse parameters (i.e. voltage), the amount of input DNA and the time of incubation after electroporation. Optimized conditions were used to test the effect of progressively deleting sequences from the phaseolin upstream region on GUS expression. Several elements were identified that either enhanced or decreased the activity of the promoter. These elements were also tested for their ability to modulate the activity of a heterologous CaMV-35S promoter. We concluded from this analysis that the phaseolin 5'-gene flanking region can be divided into at least five distinct functional domains. These include three Upstream Activating Sequences (UAS), two of which are likely to be functionally equivalent, one Negative Regulatory Sequence (NRS) and a minimal promoter region. These results were verified in tobacco plants indicating that transient expression analysis can be a powerful tool for the study of gene regulation in species not amenable to *A. tumefaciens* mediated transformation, or as a faster alternative to more time consuming stable transformation procedures.

R 406 IN-VIVO ANALYSIS OF 5' UNTRANSLATED LEADER SEQUENCES ON mRNA TRANLATIONAL EFFICIENCY USING MAIZE AND TOBACCO PROTOPLASTS, Nadine Carozzi and Susan Jayne, Department of Plant Molecular Biology, CIBA-GEIGY Biotechnology Research, P.O. Box 12257, Research Triangle Park, NC 27709-2257.

While it is known that the 5' untranslated leader of an mRNA plays an important role in gene expression, the basis of this regulation remains unclear. The role of 5' untranslated leader sequences in regulating translational efficiency was studied by making various constructs with heterologous leader sequences fused to a luciferase reporter gene driven by the CaMV 35S promotor. Plasmid DNA was introduced into maize and tobacco protoplasts by PEG-mediated DNA transfer and the expression of both the chimeric luciferase constructs as well as a CaMV 35S-GUS transformation control were measured. We have examined leader sequences from plant viruses (BMV, AMV, TMV, MSV) and from highly expressed plant genes (maize zein, maize glutelin, maize PEP-carboxylase, maize rbcS). The relative efficiency of these leader sequences will be discussed, as well as a comparison of their effects in maize and tobacco protoplasts.

R 407 **EVIDENCE FOR THE PRESENCE OF CELL WALL GLYCINE-RICH PROTEINS IN DIVERSE CROP SPECIES**, Shu-Hua Cheng, Beat Keller* and Carol M. Condit, Departments of Plant Science and Biochemistry, University of Nevada, Reno, NV 89557, U.S.A. and Swiss Federal Research Station for Agronomy, CH-8046 Zürich, Switzerland. We have examined a number of diverse crop species, including three monocots (wheat, barley and maize) and five dicots (rape, turnip, soybean, crabapple and tomato) for the presence of cell wall glycine-rich proteins (GRPs). An antibody (anti-ptGRP1) which is specific for the aminoterminal portion (amino acids 22 to 36) of the mature petunia GRP1 protein and a second antibody (anti-fbGRP1.8) which was raised against the glycine-rich regions of french bean GRP1.8 were used in Western analysis. Each of the antibodies recognized a single different polypeptide in wheat, barley, rape, turnip, crabapple and tomato. Both antibodies recognize two polypeptides in maize with an ~14 Kd protein in common. In soybean anti-ptGRP1 reacted with a single polypeptide, while anti-fbGRP1.8 recognized a number of polypeptides.

In Northern hybridization analysis, using petunia GRP1 anti-sense RNA as a probe, we found one highly expressed transcript and two to four less abundant transcripts in four dicots, and two or three fairly equally expressed transcripts in the three monocots examined. These results suggest that GRPs are not unique to french bean and petunia, but are likely ubiquitous among plant species.

R 408 Cis-ACTING ELEMENTS AND Trans-ACTING FACTORS INVOLVED IN Sesbania rostrata EARLY (Enod2) AND LATE (LEGHEMOGLOBIN; lb) NODULIN GENE EXPRESSION, Frans J. de Bruijn, Peter Welters, Laszlo Szabados, Pascal Ratet, Anoraj Goel and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, 5000 Köln 30, F.R.G. Nodulins are temporally expressed plant proteins specifically produced during nodule formation (early; Enod2) and in mature nitrogen fixing nodules (late; Lb), induced by rhizobia on leguminous plants. We are investigating the role of cis-acting nodulin promoter elements and trans-acting DNA binding factors in nodule specific gene expression. Moreover, we are examining rhizobial signals which may play a role in this process. We have chosen the unusual symbiotic interaction between the diazotrophic bacterium Azorhizobium caulinodans and the stem-nodulated tropical legume Sesbania rostrata for these studies. Here we will present a functional analysis of the S. rostrata leghemoglobin glb3 gene 5'upstream region in transgenic Lotus corniculatus plants, leading up to the identification of nodule specificity and general enhancer elements. Secondly we will describe the interaction of tissue-specific, conserved trans-acting factors with the glb3 5'upstream region and the discovery of a rhizobial DNA-binding protein specifically interacting with a glb3 5'upstream element. Thirdly, we will present evidence suggesting that the S. rostrata early nodulin Enod2 gene is induced by cytokinin. The possible implications of these observations for the signal-transduction pathway involved in tissue-(nodule)-specific gene expression in this highly evolved plant-bacterium interaction will be discussed.

R 409 ANALYSIS OF A CORTEX SPECIFIC GENE IN BRASSICA NAPUS.

R.A. Dietrich and J.J. Harada. Botany Dept., Univ of California, Davis CA. A gene showing cortex specific expression has been identified in Brassica napus. In in situ hybridization experiments, this gene, designated AX92, was most abundantly expressed in developing cortex cells of the seedling axis. AX92 was shown in mRNA dot blot experiments to be expressed during early embryogenesis as well as during germination. Genomic Southern blots indicate that AX92 is a member of a small gene family (about 4 copies), while northern analysis showed that all four genes are expressed both in embryogenesis and germination. Current research addresses two questions related to the observed expression patterns; 1, are the regulatory elements that control the expression of the gene the same in embryogenesis and germination, or are there seperate sets of regulatory elements in the two developmental stages, and 2, is expression of AX92 in the embryo limited to the cortex cells as it is in the seedlings. To address these questions, a chimeric gene was constructed linking the AX92 flanking sequences to the B-glucuronidase (GUS) reporter gene. Transgenic Brassica plants were made using this construct and will be analyzed to determine where and when GUS activity accumulates in the developing embryo. In addition, a series of promoter deletions have been made in the chimeric gene. The expression of the deletions in transgenic plants will be analyzed during embryogenesis and germination to determine the cis-acting sequences essential for proper regulation of AX92 in the two developmental stages.

R 410 MODIFICATION OF TOMATO DEVELOPMENT BY THE INTRODUCTION OF ROL-GENES OF AGROBACTERIUM RHIZOGENES.

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The Ri-plasmid of agropine strains of A.rhizogenes contains two distinct regions which can be transferred to the plant genome: TL-DNA and TR-DNA. Ri-Transformed plants show developmental abnormalities that are consistently observed in a variety of plant species. The transformed phenotype has been correlated with the presence of TL-DNA. Four TL-DNA loci have been localized: rol A, rol B, rol C and rol D.

The binary plasmids used for transformation contained different loci (Rol ABC, A, B) of the TL-DNA of pRi-plasmid pRi 1855 in combination with the NPT II-gene. Transgenic tomato plants (Lycopersicon esculentum, cv. Moneymaker) were regenerated from callus induced by inoculation of cotyledon segments. The plantlets were selected on kanamycin. Most of the transformed plants were tetraploid, probably due to the long duration of the callus phase. The phenotypic traits of the plants were determined both in vitro and in the greenhouse. Plants with rol A had small, dark green and serious wrinkled leaves, causing an open foliage. The rol B transformed plants had wider and shorter leaves and a reduced apical dominance. Rol ABC plants only showed a slight deviation from the morphology of the control plants. Rooting response was tested on medium with different levels of auxin. Southern blot analysis was carried out to investigate the integration of the genes. The expression of the rol loci will be studied to gain insight in their effects on plant growth and development.

R 411 PROTEINS ASSOCIATED WITH THE TRANSITION FROM SHOOT REGENERATION COMPETENCE TO CALLUS DETERMINED GROWTH IN INTERNODAL STEM EXPLANTS OF *Populus deltoides*,

Stephen G. Ernst and Gary D. Coleman, Department of Forestry, Fisheries and Wildlife, University of Nebraska, Lincoln, NE 68583-0814 USA

The competence status of internodal stem explants of 16 *Populus deltoides* genotypes was manipulated and monitored *in vitro*. The transition from shoot regeneration competent to callus determined growth when in the presence of the shoot inducer zeatin was the focus of this study. Competence status was manipulated by transferring internodal stem explants from callus inducing medium (CIM) to shoot inducing medium (SIM) at 1, 2, 4, 6, 8 and 10 day intervals, plus SIM and basal controls, based on the number of regenerated shoots per explant. The competence state transition from high levels of shoot regeneration to callus determination was very marked for genotypes which were initially competent or competence enhanceable. Within two days shoot regeneration decreased to almost zero and the explants became determined for callus growth (i.e., high levels of shoot regeneration for 2, 4 and 6 day treatments, and callus determined growth for the 8 and 10 day treatments). A 32 kD basic protein was strongly associated with shoot induction treatments (2, 4, and 6 day transfers from CIM to SIM), and a 35 kD basic protein was strongly associated with callus determined growth treatments (8 and 10 day transfers).

R 412 DEVELOPMENT OF A SYSTEM FOR SEED SPECIFIC HYPEREXPRESSION AND VACUOLAR TARGETING OF FOREIGN PROTEINS IN PLANTS, Leigh B. Farrell*, Federico L. Sebastiani*, Ganesh Kishore†, and Roger N. Beachy*, *Department of Biology, Washington University, St. Louis, MO 63130, †Monsanto Company, St. Louis, MO 63198. To date only mammalian, yeast and bacterial systems have been developed for hyperexpression of foreign proteins. We propose to extend this approach to plants by expressing foreign proteins under the control of a highly active promoter from the α-subunit of the soybean seed storage protein β-conglycinin. In addition, hyperexpressed proteins will be targeted to seed protein bodies using the N-terminal targeting sequences from the α-subunit of β-conglycinin. The first stage of this investigation requires the delineation of the minimal length sequence required to direct a passenger protein to tobacco protein bodies. To this end a series of C-terminal deletions have been constructed proximal and distal to conserved regions of the α-subunit precursor which were revealed by extensive amino acid comparisons with other related and non-related seed storage proteins. These C-terminally deleted sequences have been fused to an E. coli β-glucuronidase (GUS) gene that was modified to be resistant to N-glycosylation in the endomembrane system. At present chimeric genes are being transformed into tobacco in binary vectors carrying either the seed specific α'-subunit or the "constitutive" CaMV 35S promoters and into yeast on an autonomous centromeric vector carrying the Gal 1 promoter sequence. The intracellular location of GUS in seeds and yeast will be determined by subcellular membrane fractionation studies.

R 413 TISSUE-SPECIFIC EXPRESSION OF A PARSLEY 4-COUMARATE:CoA LIGASE GENE IN TRANSGENIC TOBACCO, Karl D. Hauffe and Carl Douglas, University of British Columbia, Department of Botany, Vancouver, B.C., Canada.
The 4-coumarate: CoA ligase (4CL), encoded by two genes in parsley (4CL-1 and 4CL-2), represents a key enzyme of the phenylpropanoid pathway. This metabolic pathway is essential for the biosynthesis of both defense-related and developmentally-required phenylpropanoid derivatives. As well as being accumulated upon stresses, such as pathogen infection, u.v. irradiation and wounding, phenylpropanoid derivatives are required in various tissues for the development of the plant. For instance lignin, a widely distributed phenylpropanoid polymer, is synthesized in the xylem during the differentiation of the vascular system. Other phenylpropanoids, such as anthocyanins and flavonoids, occur as pigments, which are produced during the development of flowers and seeds. Here we present evidence that regulatory sequences of the 4CL-1 gene upstream of the transcription start site are sufficient to confer tissue-specific expression upon the E. coli β-glucoronidase (GUS) gene in transgenic tobacco plants. This tissue-specific expression is temporally regulated during leaf and flower development. A series of 5' and internal deletion derivatives of the 4CL-1 promoter have been constructed to localize cis-acting elements required for this expression and to correlate expression with in vivo footprints within the promoter.

R 414 PRODUCTION OF CORRECTLY PROCESSED HUMAN SERUM ALBUMIN IN TRANSGENIC PLANTS, André Hoekema, Ben M.M. Dekker, Barbara Schrammeijer, Theo C. Verwoerd, Peter J.M. van den Elzen and Peter C. Sijmons, MOGEN NV, Einsteinweg 97, 2333 CB Leiden, The Netherlands
The production, processing and secretion of human serum albumin (HSA) in transgenic plants was demonstrated. A modified CaMV 35S promoter was utilized to direct the expression of chimaeric genes encoding HSA in transgenic potato and tobacco plants. To secrete the protein either the human prepro-sequence or the signal sequence from the extracellular tobacco protein PR-S was used. We demonstrate secretion of HSA with both types of signal sequences in transgenic leaf tissue and in suspension cultures. HSA produced in transgenic potato plants was purified to homogeneity. N-terminal amino acid sequence analysis revealed that the processing of the precursor protein was dependent on the type of signal sequence. Expression of the human preproHSA gene lead to partial processing of the precursor and secretion of proHSA. Fusion of HSA to the plant PR-S presequence resulted in cleavage of the presequence at its natural site and secretion of correctly processed HSA that is indistiguishable from the authentic human protein.

R 415 CHARACTERIZATION OF ABA-INDUCED cDNA CLONES IN BARLEY ALEURONE LAYERS, Bimei Hong, Scott J. Uknes, Rivka Barg and David T-H. Ho, Department of Biology, Washington University, St. Louis, Mo 63130

The addition of ABA to barley aleurone layers induces the expression of many proteins. Over a dozen ABA-inducible cDNA clones have been isolated by differential screening of a λgt 10 cDNA library with mRNA isolated from barley aleurone layers treated with or without the hormone. Several clones have been sequenced and further characterized. Clone pHVA1 mRNA encodes a 23 kD protein containing nine imperfect repeats with a sequence of: Thr-Glu-Ala-Ala-Lys-Gln-Lys-Ala-Ala-Glu-Thr, which is also found in similar proteins in cotton, carrot and rape. The mRNA is not readily detected in normal barley seedlings, but it can be induced in the roots of seedlings by ABA as well as by water stress. Polyclonal antibody against this protein has been raised. Transgenic tobacco plants with pHVA1 cDNA driven by 35S promoter have been regenerated and characterized. Clone pHVA22 is induced within 30 minutes by ABA, and is superinduced when both ABA and cycloheximide are present. This feature along with the deduced amino acid sequence suggestes that this clone encodes a protein which may be similar to zinc requiring DNA binding proteins. Other interesting clones will also be discussed.

R 416 THREE PENTAMER REPEATS, FUNCTION AS ENHANCER AND SILENCER, ARE RESPONSIBLE FOR THE LIGHT AND DARK EXPRESSION OF CAB-M5 GENE IN MAIZE,Hai Huang and Jenq-Yunn Sheen.Department of Molecular Biology,Massachusetts General Hospitol,Boston,02114

Unlike their counterparts that are strictly regulated by light in other plant species, some maize chlorophyll a/b binding protein genes are expressed at high level in the dark as well as in the light. We have studied the promoter of one of these cab genes(cab-m5, Sheen & Bogorad, 1987 or cab-1, Sullivan et al, 1989) in detail. At least six different cis-acting regulatory elements have been revealed by 5'-end deletion, internal deletion and site-directed mutagenesis of the 891 bp promoter of cab-m5 or cab-1 gene. Three different duplicated pentamer repeats, designate as CA1, nCA and CA2 boxes, are acting as upstream regulatory elements. They interact synergisticlly with three other downstream cis-acting elements, CCAAT box, dyad pentamer and TATTTA box. The nCA box(negative CA) interacts with both CA1 and CA2 boxes. In the absence of CA1, nCA acts as a silencer which inhibits the enhancer function of CA2 by a quenching mechanism, but only in a short distance. Same regulatory sequences are responsible for the light and dark expression of the cab-m5 or cab-1 gene, but give higher expression under the light. A G box (8 of 10 match) was found right next to the CA1 box, however contributes little function. More detailed analysis of these six cis-acting regulatory elements will be presented.

R 417 IDENTIFICATION AND CHARACTERIZATION OF IAA BINDING PROTEINS IN MAIZE: POSSIBLE ROLE IN LIGHT-REGULATED GROWTH, Alan Jones, Department of Biology, University of North Carolina, Chapel Hill 27599

[^{3}H],5-azidoindole-3-acetic acid was synthesized and used to photolabel three IAA-binding proteins in a solubilized microsomal fraction from maize shoots. The subunit molecular masses are approx. 43, 24, and 22 kilodaltons. These IAA-binding proteins were characterized with respect to their pI's, native molecular masses, subcellular location, and binding specificity. Antibodies were prepared against the 22-kDa subunit protein and used to quantitate changes in the abundance of this IAA-binding protein after various light treatments and it was found that red light causes the abundance in the mesocotyl tissue to rapidly decrease. Removing the source of endogenous auxin in the mesocotyl by decapitation of the coleoptile causes a similar decrease. In all cases, the change in abundance of this protein correlated with changes in growth capacity defined as the maximum amount of auxin-inducible cell elongation. This suggests that this protein is involved in mediating auxin-induced cell elongation. Furthermore, it raises the possibility that, in some instances, the level of auxin receptor may limit plant growth. Thus, one strategy for manipulating plant growth may be to alter the cellular level of one or more of these IAA-binding proteins.

R 418 BARLEY STRIPE MOSAIC VIRUS GENOME MEDIATED EXPRESSION OF A FOREIGN GENE IN MONOCOT- AND DICOTYLEDONOUS PLANT CELLS. Joshi, R. L. [1], Joshi, V. [1], Jackson, A.O. [2] & Ow, D.W. [1,2], [1]Plant Gene Expression Center-USDA/ARS, Albany CA 94710; [2]Department of Plant Pathology, University of California, Berkeley, CA 94720.

Barley stripe mosaic virus (BSMV; a hordeivirus) possesses a tripartite plus strand RNA genome composed of RNAs α, β, and γ. The genomic RNAs have recently been sequenced and cloned into in vitro transcription vectors from which infectious in vitro transcripts can be obtained (Petty et al., Virology 171: 342-349, 1989). Using these constructs, we have engineered a BSMV genome based vector to express foreign genes in plant cells. For this purpose, an in frame deletion in the ORF2 in RNA β has been introduced (leading to RNA β2) and the firefly luciferase reporter gene inserted into the deleted ORF2 in RNA β2 (leading to RNA β2-luc). The luciferase fusion was expressed when RNA β2-luc was used to transfect protoplasts derived from both monocot- and dicotyledonous plant cells, but only in the presence of RNAs α and γ. Replication of RNA β derivatives (RNA β2 and β2-luc) in infected protoplasts were confirmed by Northern analysis performed to examine minus strand synthesis. To our knowledge, these results constitute the first plant RNA virus genome based vector in which a foreign gene has been expressed upon insertion into a non-structural protein gene. A possible strategy permitting 'virus containment' for the use of plant viral expression vectors will be discussed.

R 419 REPLICATION OF PLASMIDS IN MITOCHONDRIA OF SUGARBEET CELLS: A MODEL SYSTEM FOR MITOCHONDRIAL TRANSFORMATION
S.B. Kaliappan[1], C. Paszty[1], B. Nielsen[2] and H. Daniell[1], [1]University of Idaho, Moscow, ID 83843, [2]Auburn University, Auburn, AL 36849. **Expression of chloramphenicol acetyltransferase (cat) by autonomously replicating vectors in chloroplasts of cultured tobacco cells following delivery by high velocity microprojectiles has been reported recently (Daniell et al, PNAS '89, in press). We now report an analogous system of plasmid replication in mitochondria of cultured sugarbeet cells. There are three endogenous plasmids of similar size (1.6 kb) inside sugarbeet mitochondria that have been characterized and sequenced (C.M. Thomas, Nucleic Acids Res. 14, 9353-9370). Our experiments show that these plasmids are labeled when they are incubated with [^{32}P]-thymidine triphosphate, after permeabilizing cultured cells with LPC (L-lysophosphatidylcholine). Autoradiograms of labeled plasmid DNA, isolated from intact mitochondria and cut with appropriate enzymes, reveal the correct size fragments. Currently, work is in progress to introduce the following replicons inserted into puc 19, using the gene gun, to observe foreign DNA replication in mitochondria of cultured cells: 1.6 kb sugarbeet plasmid replicon, pMCa18; a genomic mitochondrial replicon isolated from soybean, pMP30; a genomic chloroplast replicon isolated from pea, pCS 4.1. This work is supported in part by NSF-EPSCoR Grant RII-8902065 to HD.**

R 420 EXPRESSION OF BLASTICIDIN S RESISTANT GENE IN PLANT,
Takashi Kamakura, Katuyoshi Yoneyama and Isamu Yamaguchi, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01 Japan.
A potent fungicide of microbial origin, Blasticidin S (BS), is used practically for the control of rice blast disease. It has broad antimicrobial activity but occasionally exhibits an adverse phytotoxic effects on some sensitive plants such as tobacco and eggplant. An inactivating enzyme named BS-deaminase (aminohydrolase, EC 3.5.4.23) was discovered in *Aspergillus terreus* and *Bacillus cereus* K55-S1. This enzyme converts BS to inactive form by catalyzing the hydrolytic deamination of cytosine nucleus in BS. From the bacterium, a plasmid pBSR8 was isolated and correlated with the enzyme production, and a structural gene, *bsr*, of the enzyme was successfully cloned in *Bacillus subtilis* and *Escherichia coli*. Analysis of the gene resulted in its application as a selectable marker for microorganisms such as *Eschetichia coli* and *Saccharomyces cerevisiae*. Further, *bsr* gene was successfully introduced into tobacco plant by using Ti plasmid vector and expressed in the plant to afford resistance against blasticidin S. Thus it is further promising as a new selectable marker for plant gene engineering.

R 421 PROMOTER ANALYSIS OF A VASCULAR TISSUE-SPECIFIC GENE ENCODING A CELL WALL PROTEIN. Beat Keller, Swiss Federal Research Station for Agronomy, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland.

A bean glycine-rich cell wall protein is specifically synthesized in vascular tissue. Its gene has been shown earlier to be developmentally expressed in vascular tissue of transgenic tobacco plants. A set of GRP 1.8 promoter deletions starting at position -500 was made and the resulting fragments were fused to the ß-glucuronidase reporter gene. The activity of these constructs was analyzed in stably transformed tobacco plants. A histochemical assay for expression of the GUS reporter gene showed that deletions up to 205 base pairs in front of the transcription start site do not alter vascular specific gene expression in stems and leaves. However, expression in the vascular tissue of roots is lowered if only 205 bp of the promoter are present. Interestingly, meristematic regions of the root stained stronger in this deletion than with the complete promoter present. A deletion at position -76 was completely inactive in 17 independently transformed plants. This suggests a crucial role in vascular tissue-specific gene expression for a cis-acting element within a promoter fragment of 129 base pairs. A 10 bp sequence within these 129 bp is also found in a second gene specifically expressed in vascular tissue. Both these elements of 10 bp are partially or completely embedded in a short inverted repeat.

R 422 GIBBERELLIC ACID-INDUCED CYSTEINE PROTEINASES OF BARLEY ALEURONE : cDNA SEQUENCING, POST-TRANSLATIONAL PROCESSING, AND HORMONAL REGULATION, Koehler S.M., Ho T-h. D., Department of Biology, Washington University, St. Louis, MO 63130

Four full-length cDNA clones prepared from gibberellic acid (GA)-induced aleurone mRNA were isolated. One of these appears to be a full length clone of pHV14, which was first isolated by Chandler et al. (Plant Molec Biol 3: 407-418, 1984) and whose sequence is homologous to cysteine proteinases (Chandler, personal communication). Three of the four clones designated pHVEP1, 4, and 7, encode 2 cysteine proteinases whose amino acid sequences are 98% similar to each other and 90% similar to pHV14. pHVEP 1, 4, and 7 contain large prosequences followed by the amino acid sequence described as the mature N-terminus of a 30 kD cysteine proteinase (30 kD EP) we purified from GA-induced barley aleurone. Antibody against the purified 30 kD EP immunoprecipitates the *in vitro* transcription/translation products of these three clones. Post translational processing of 30 kD EP was found to occur in multiple steps. The *in vivo* synthesis of 30 kD EP as well as another GA-induced cysteine proteinase (37 kD EP) were compared to the accumulation of their respective translatable mRNA in response to hormone. Accumulation of total mRNA for each member of the 30 kD EP family, pHV14, and aleurain (another GA-induced putative cysteine proteinase [Rogers et al. Proc Natl Acad Sci USA 82: 6512-6516, 1985]) in response to hormone was compared by Northern Blot analysis using clone specific probes.

R 423 EXPRESSION OF MAIZE Adh-1 PROMOTER AND SPLICING OF INTRON 1 IN TRANSGENIC RICE PLANTS. J.Kyozuka, T.Izawa, H.Fujimoto, M.Nakajima and K.Shimamoto, Plantech Research Institute, 1000 Kamoshida, Midori-ku Yokohama, 227 Japan

To examine expression of maize Adh-1 promoter and stimulating effect and splicing of intron 1 of the Adh-1 in rice, we have introduced a chimeric genes consisting of the promoter, intron 1 and the GUS coding sequence.

In protoplasts and stably transformed calluses, GUS expression is increased by the presence of the intron located between the promoter and the GUS gene. However, examination of the transcript by Northern hybridization and the PCR method revealed that the intron is not efficiently spliced and that incorrect splicing of the intron takes place.

Histochemical GUS analysis of various tissues of transgenic plants showed that the Adh-1 promoter is predominantly expressed in root cap, floral tissues, pollen and seed. In contrast, little expression is observed in floral tissues and pollen of plants carring the CaMV35S promoter. From these, it is concluded that the expression of the maize Adh-1 promoter is correctly regulated in transgenic rice plants.

R 424 THE CELL-SPECIFIC ACCUMULATION OF MAIZE PHOSPHOENOLPYRUVATE CARBOXYLASE IS CORRELATED WITH DEMETHYLATION AT THE EXTREME 5' END OF THE GENE, Jane A. Langdale and Timothy Nelson, Biology Dept., Yale University, New Haven, CT 06511. Development of the C4 photosynthetic pathway relies upon the cell-specific accumulation of photosynthetic enzymes. Although the molecular basis of this cell-specific gene expression is not known, regulation appears to be exerted at the level of transcript accumulation. We have investigated the relationship between gene expression patterns and DNA methylation for genes encoding C4 photosynthetic enzymes. In the case of phosphoenolpyruvate carboxylase (PEPCase), demethylation of a specific site 5' to the PEPCase gene is strictly correlated with the light-induced, cell-specific accumulation of PEPCase mRNA. This differentially methylated site is positioned at a striking distance (over 3kb) from the start of transcription. This observation is made more interesting by the fact that the immediate 5' region of the gene, and some of the coding region, represents an unmethylated CpG island. Such islands are normally only associated with constitutively expressed genes.

R 425 INVESTIGATION OF THE ROLE OF <u>CIS</u>- AND <u>TRANS</u>- ACTING FACTORS IN REGULATING EXPRESSION OF β-CONGLYCININ GENES FROM SOYBEAN, Philip A. Lessard, Toru Fujiwara, Randy D. Allen, François Bernier and Roger N. Beachy, Department of Biology, Washington University, St. Louis, MO 63130. β-conglycinins are 7S seed storage proteins in soybeans and are composed of three types of subunits, α, α' and β. The genes encoding these subunits are differentially regulated. Though there are apparently fewer genes encoding the α' subunit (2-3 copies) than are encoding the β subunit (8-12 copies) in the soybean genome, transcripts from the α' subunit genes accumulate to a higher level than those from the β subunit genes. The timing of accumulation and spatial distribution of these gene products also differ. In addition, expression of the genes encoding the β subunit is strongly influenced by such factors as sulfur availability and abscisic acid concentration. These factors have no significant effect on expression of the α' subunit genes. Our lab has previously identified <u>cis</u>-acting regulatory regions which lie within the 5' flanking sequences of the α' subunit gene. We have also identified a number of proteinaceous <u>trans</u>-acting factors which bind to sequences within these regions. We have now extended these studies to include 5' flanking sequences from a gene encoding the β subunit. We have cultured immature soybean cotyledons in the presence or absence of methionine or abscisic acid. Using nuclear extracts from these cotyledons we have been able to examine the effects of sulfur and abscisic acid on DNA binding activities within the upstream regions of these genes. With this information we hope to determine the mechanisms underlying the differential regulation of the genes encoding the different subunits of β-conglycinin.

R 426 LOCATING AND SEQUENCING OF CHLOROPLAST DNA FRAGMENTS RELATED TO POLLEN FERTILITY IN RAPE AND RADISH, Li Jigeng, Wang Hong, Sun Wei, Zhang Lu, Zhang Jing and Kong Fanrui, Institute of Genetics, Academia Sinica, Beijing, China. Restriction analysis of chloroplast DNA showed that only one different DNA fragment(3.2kb) was observed between sterile line and its maintainer in EcoR1 pattern of rape. In radish five fragments about 2.0kb were different in migration in BamH1 pattern between sterile line and its maintainer. From them four recombinant clones carried the specific fragment A20, A21, B21, B22 respectively were obtained. Using the rRNA gene probe from maize ctDNA to hybridize with ctDNA of rape digested by EcoR1 andrecombinant DNAs digested by BamH1 of radish respectively, results indicated that fragment E3.2 from rape and fragments A21, B21, B22 from radish are homologous to rRNA gene probe. This means that these fragments are located in the inverted repeat region of ct DNA. Using the fragments E3.2 of rape and B21 of radish as gene probes to hybridize with operon rRNA, results showed that these two fragments are located at 4.0-2.0kb in front of 16S rDNA gene. Results of sequencing of partial fragment B21(474bp long) showed that it encodes 7 amino acid residues of ribosomal protein S12(exon3) and 93 amino acid residues of ribosomal protein S7 and includes a segment showing the figure of intron B of rps12-rps7 operon. This implies that genes of chloroplast ribosomal proteins S7 and S12 may be related to the formation of pollen fertility in some higher plants.

R 427 CHARACTERIZATION OF CIS ACTING SEQUENCES INVOLVED IN THE MODULATION OF GENE EXPRESSION BY THE PHYTOHORMONE ABSCISIC ACID - TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL EVENTS, William R. Marcotte, Jr. and R. S. Quatrano, Department of Biology, University of North Carolina, CB# 3280, Coker Hall, Chapel Hill, NC 27599-3280.
Various stimuli have been shown to result in alterations in the level of gene expression. In the cases of several of these stimuli, including cold stress and drought stress, the phytohormone abscisic acid (ABA) has been implicated in these changes. However, the molecular events involved in the alteration or modulation of gene expression by ABA are not well understood. We have described the preliminary identification of ABA-responsive sequences of the Em gene from wheat using a rice protoplast transient assay (Marcotte, et al. (1989), The Plant Cell 1:969-976). The sequences involved were shown to reside in transcribed and non-transcribed regions, suggestive of the presence of transcriptionally-activated sequences and a post-transcriptional component. A detailed analysis of these sequences and their contribution to ABA-modulated gene regulation will be presented. Supported by a grant from the U.S. Department of Agriculture Competitive Grants Program (89-37262-4456) to R.S.Q.

R 428 Expression of Heterologous and Homologous Small Subunit genes of Rubisco (SSU) in the genus *Flaveria*. David Marsh and Tim Nelson, The Department of Biology, Yale University, PO Box 6666, New Haven, Ct, 06511
Several species of *Flaveria* have been developed as transgenic hosts for the study of C4 and C3 photosynthetic genes. The *Flaveria* genus contains species that ultilise C4, intermediate C4/C3 and C3 photosynthetic pathways. Four species have been chosen as transgenic hosts and transformed using an Agrobacterium-mediated method similar to that described by Bevan *et al* (1984) for tobacco.
The cellular localisation of the expression of the marker gene GUS driven by the SSU promotor of tobacco (C3) will be discussed with respect to the hosts *F. trinervia* (C4), *F. brownii* (C4/C3), *F. linearis* (C4/C3), and *F.pringlei* . In addition the isolation and expression of the SSU genes from *F. trinervia* will be presented and discussed with respect to reintroducing these regions in the above transgenic hosts.

R 429 INSERTIONAL MUTAGENESIS AND MOLECULAR GENETIC STUDIES OF THE *P* LOCUS OF MAIZE, Maria A. Moreno-Gomez, Jychian Chen, Irwin M. Greenblatt, and Stephen L. Dellaporta, Department of Biology, Yale University, New Haven, CT 06511.
The *P* locus of maize, located on the short arm of chromosome one, is a complex locus that appears to be involved in the regulation of flavonoid biosynthesis. To understand the genetic organization of this locus, a genetic and molecular analysis has been undertaken using a transposon-mediated insertional mutagenesis approach. The transposable element *Activator (Ac)* was used as the transposon mutagen. Over 200 alleles of *P (P-VV:R)* containing single or double insertions of *Ac* have been generated. With the exception of two, all insertion alleles disrupt pigmentation patterns on the pericarp and cob tissues of the maize ear suggesting that the *P* locus is monocistronic. In addition, *Ac* insertions appear to occur randomly throughout the locus, and the variegation pattern observed seems to be a reflection of position rather than *Ac* dosage. Genomic blot analysis of several *P-VV:R* alleles has been used to localize *Ac* insertions in the*P-RR* region. These results indicate that *Ac* reinserts into multiple sites within *P-RR.* However, this analysis has not determined the number of target sites within a particular restriction fragment nor the orientation of the *Ac* insertions.

R 430 CHARACTERISTICS OF THE POLYPEPTIDES ENCODED BY THE EARLY AUXIN REGULATED GENE, PSIAA4/5, IN PEA EPICOTYL TISSUE[1], Paul W. Oeller and Athanasios Theologis, Plant Gene Expression Center, USDA-ARS, 800 Buchanan Street, Albany, CA 94710 and Department of Plant Biology, University of California at Berkeley, Berkeley, CA 94720.
Indole acetic acid (IAA) causes rapid transcriptional activation of at least two genes, PSIAA4/5 and PSIAA6 in etiolated pea epicotyls[2]. The function of the polypeptides encoded by these genes is unknown. In order to elucidate their role in auxin mediated cell elongation, we have focused upon the polypeptide(s) encoded by the most rapidly auxin activated gene PSIAA4/5. A full length cDNA (pIAA4/5) has been expressed in *E. coli* using a T7 based expression system and a high titer antiserum has been produced for the polypeptide encoded by this cDNA. The antiserum does not recognize an auxin-inducible polypeptide in total pea extracts by Western analysis; however it recognizes two auxin inducible polypeptides MW 26 and 27 kd by *in vitro* translation and subsequent immunoprecipitation. Furthermore, polypeptides of identical mobility are immunoprecipitated from *in vivo* labelled pea tissue treated with 20μM IAA for 2 hrs. A detailed time course of induction of the *in vivo* synthesized polypeptides and their turnover rate (half-life) is currently in progress. The data suggest that the protein(s) encoded by the PSIAA4/5 gene are of low abundance and their immunolocalization will be problematic.
1. This work was supported by a grant to A.T. from NIH (GM-35447).
2. Theologis et al (1985) J. Mol. Biol. 183, 53-68.

R 431 SOYBEAN VSP GENE STRUCTURE AND EXPRESSION, Yoon Rhee, Jingfeng Huang and Paul Staswick, Department of Agronomy, University of Nebraska, Lincoln, NE 68583
Soybean vegetative storage protein (VSP) genes are developmentally regulated; Their expression declines as leaves mature and the pattern of expression can be modulated by several factors including plant nitrogen nutrition, demand for mobilized leaf reserves (i.e. sink size), leaf wounding, and treatment with jasmonic acid (JA). The interaction between these factors was investigated by analyzing steady state VSP mRNA levels. Elevated N nutrition delayed the normal decline in expression as leaves matured, whereas N deficiency essentially abolished expression in all leaves. Expression was inducible by JA in plants grown with low as well as high levels of N. Two genes, each encoding a different VSP subunit, have been isolated and sequenced. Conserved sequences in the promoter regions have been identified. These genes are being transferred to tobacco and preliminary data on their expression in this system will be reported.

R 432 *NUCLEAR CONTROL OF MAIZE MITOCHONDRIAL OPEN READING FRAME-25 TRANSCRIPTION*, Torbert R. Rocheford and Daryl R. Pring, USDA-ARS and Plant Pathology Dept., Univ. of Florida, Gainesville, Fl, 32611. ORF25 is transcribed in all four maize cytoplasms (N,C,S,T), exhibiting complex transcriptional patterns. Northern analyses of genetic stocks with probes internal to and 5' to ORF25 determined that presence of specific ORF25 transcripts was under nuclear control. For lines Wf9 and W182BN in C cytoplasm, transcripts of ca. 3.4, 3.2, and 1.35 kb were evident, but other lines (A619, A632) carried an additional major transcript of 2.1 kb. We have determined that the nucleus confers this additional transcript, in that the 2.1 kb transcript appears in the F1 of W182BN(C) X A619(C). The 2.1 kb species was the only transcript to change in abundance. The line A188(N) exhibits transcripts of 3.1, 1.7 and 1.3 kb. However, the F1 of A188(N) X 187-2(N) exhibits additional 3.5 and 2.1 kb transcripts. The 3.5 kb transcript is the highest MW mRNA species detected. The tissue culture mutant A188(T7) has lost T-urf13 which is normally cotranscribed 5' to ORF25. A188(T7) displays an ORF25 transcript pattern identical to A188(N) whereas the progeny of A188(T7) X W64A exhibits a major 2.1 kb transcript which has not been detected in lines with T-urf13. Collectively, these data are consistent with modification of transcription initiation by nuclear encoded gene products that enhance initiation of specific ORF25 transcripts. Sequences 5' to ORF25 in B37(N) revealed similarity with the region 160 bp 5' to the presumed wheat atp6 leader sequence. The amino termini of these two open reading frames shared 13 of the initial 18 amino acids.

R 433 ANALYSIS OF RBCS AND PEPC GENES BY TRANSIENT EXPRESSION IN MAIZE

Anton R. Schäffner and Jen Sheen, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

The mutually exclusive and high expression of two carboxylases, phoshoenolpyruvate-carboxylase (PEPC) in mesopyll cells (M) and ribulose-1,5-bisphosphate-carboxylase/oxygenase (RBCS) in bundle sheath cells around the leaf veins (B) plays an important role for the efficient photosynthesis in C_4 plants. Promoter regions of the C_4 PEPC and two RBCS genes from maize have been fused to the CAT reporter gene and introduced via electroporation into maize mesophyll protoplasts, that had been freshly isolated from leaves of etiolated, greening and green seedlings to study gene expression at different developmental stages.

In all cases less than 500 bp confer the same expression pattern as constructs with several kb of 5'-sequences. The cell type specificity of RBCS (M vs.B) is not reflected in the transient system, but both RBCS promoters show light inducibility, to a different extent, and highest level of expression in cells from greening leaves as found *in vivo*. The expression of PEPC differs from that of RBCS as 5'-sequences alone do not confer light and developmental regulation.

Separate regulatory elements in these promoters revealed by deletion analysis are further characterized in detail by *in vitro* mutagenesis.

R 434 EXPRESSION OF BRASSICA SEED STORAGE PROTEIN GENES IN TRANSGENIC TOBACCO, Charles H. Shaw, Anderson J. Ryan and Chris Royal. Department of Biological Sciences, University of Durham, Durham, DH1 3LE, England.

Genomic clones encoding the major seed storage proteins of *Brassica napus* have been isolated and sequenced. A 12.4Kb SalI fragment, encoding the *napB* gene was introduced into tobacco, using the binary vector pBin19. Northern blots upon tissues from the regenerated transgenic plants demonstrated the production of an 800bp napin transcript only in developing seeds. ELISA revealed the appearance of napin in mature seeds to a level of 0.5% of total soluble protein. The napin precursor in transgenic tobacco seeds was correctly processed to the 9KDa and 4KDa mature subunits. Within the napin upstream region several conserved elements and a potential Z-DNA forming sequence were identified, and their role in tissue-specific regulation addressed.

R 435 TISSUE SPECIFIC EXPRESSION OF NADP-ME ISOFORMS IN MAIZE IS CORRELATED WITH CHANGES IN THE PATTERN OF METHYLATION. Virginia Stiefel, Beverly A. Rothermel and Timothy Nelson, Biology Department, Yale University, New Haven, CT 06511.

Two genes for the NADP-dependent malic enzyme from maize have been cloned. One of these corresponds to the chloroplastic form which is expressed only in the bundle sheath cells of the leaf. The other corresponds to a cytosolic form which is expressed in the roots. Changes in methylation of the 5' regions flanking these genes are correlated with their distinct pattern of expression. Both genes are hypomethylated when active, although some sites are methylated in the active gene and demethylated when inactive. These results, along with similar patterns for PEPCase genes are consistent with a model in which methylation plays a role in maintaining inactive expression states of tissue-specific genes.

R 436 RECOMBINANT *BACILLUS THURINGIENSIS* CRYSTAL PROTEIN GENES AND THEIR ENTOMOCIDAL HOST RANGE, Wim Stiekema, Bert Visser, Guy Honee and Wim Vriezen, Research Institute Ital, P.O. Box 48, 6700 AA Wageningen, The Netherlands

Two truncated *Bacillus thuringiensis* crystal protein genes, belonging to the classes cryIA (b) and cryIC respectively, and both coding for insecticidal N-terminal fragments of the corresponding crystal proteins, were translationally fused. Expression of the gene fusion in *Escherichia coli* showed a biologically active protein. The fusion product was as toxic to *Heliothis virescens* as the cryIA(b) product and as toxic to *Spodoptera exigua* as the cryIC product, suggesting that the toxicity spectrum overlaps with those of the two constituting crystal proteins. From strain *kenyae* 4F1 a new crystal protein gene not described before was isolated. Sequences located at the 5'-end of the putative gene only showed homology to a class-specific cry1A(b) probe, whereas internally located sequences showed exclusive homology to a cry1C-specific probe, suggesting a relatively recent recombination event between the two different gene classes. The gene was expressed in *E. coli* and *E. coli* lysates were shown to be toxic against *S. exigua*, but not to *Mamestra brassicae*, *Piaris brassicae* and *H. virescens*. Presently the nucleotide sequence of the gene is determined and a larger number of insects is tested for sensitivity against the gene product.

R 437 TARGETING OF LacZ INTO PLASTIDS AND THE EFFECT OF THE PEA rbcS GENE ss3.6 FIRST INTRON ON REPORTER GENE ACTIVITY, Teemu H. Teeri and Sirpa Kurkela, Molecular Genetics Laboratory, Department of Genetics, University of Helsinki, SF-00100 Helsinki, Finland. We have studied the effects of the pea rbcS gene ss3.6 (Cashmore 1983) transit peptide coding sequence (tp) and first intron (including the tp) on the expression of lacZ in transgenic tobacco. All constructs were expressed from the CaMV 35S promoter. The expression level of the 35S-lacZ in tobacco root tissue is twice as high as in leaf. The 35S-tp-lacZ, on the other hand, expresses as strongly as the 35S-lacZ in the leaf tissue but does not have higher activity in roots than in leaves. The gene product of the tp containing construct, but not of the control construct, can be found in plastids where it is protected from proteases. Adding the first intron of the rbcS gene to lacZ was originally expected to lead to increased reporter activity. However, we found that plants transformed with this gene fusion contained only small amounts of LacZ protein (which was found to be targeted to the plastids as expected). The level at which the intron affects gene expression will be discussed.

Reference: Cashmore, A.R. (1983) In: Kosuge, T., Meredith, C.P. and Hollaener, A. (eds.), Genetic Engineering of Plants, Plenum Press, New York, pp. 29-38.

R 438 COMPARISON OF THE REGULATION OF NATIVE AND INTRODUCED RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE SMALL SUBUNIT RNAs IN TRANSGENIC PETUNIA, D.M. Thompson, M.M. Tanzer, B.W. Shirley* and R.B. Meagher, Department of Genetics, University of Georgia, Athens, GA 30602, * Department of Molecular Biology, Massachusetts General Hospital, Boston, MA. The small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase, an enzyme involved in photosynthetic carbon fixation, is encoded by a small nuclear gene family in both soybean and petunia. It has been shown that the stability of SSU RNA is regulated differently in different plant species and even developmentally within a single species. In other systems where RNA turnover has been examined (*e.g.* tubulin, histone) differences in RNA stability have been linked to changes in translation of particular RNAs. In soybean seedlings SSU RNAs are preferentially loaded onto polysomes with respect to other polyadenylated messages. We focused our study on one of the eight soybean SSU genes, SRS4. In order to determine whether this pattern of preferential loading of SSU RNAs onto polysomes was maintained in transgenic hosts, SRS4 SSU genes were introduced into mature petunia plants under the control of a constitutively expressed promoter. Neither the native petunia SSU RNA (SSU301) nor the SRS4 RNA was preferentially loaded onto polysomes in these transgenic plants. Polysomal loading of SRS4 RNA is now being studied in mature soybean to determine if developmental and/or species differences account for the different results obtained from soybean seedlings and mature petunia plants. In order to further study the degradation of SSU RNAs in soybean and in petunia the putative degradation products of the native SSU301 RNA and the introduced SRS4 RNA in these same transgenic petunia are being identified (by a combination of primer extension and S1 analysis). These are being compared to the native SRS4 RNA products in soybean. These putative cleavage sites will be mapped to computer generated models of the secondary structure of these RNAs to determine possible sequence and/or structural constraints on cleavage sites of SSU RNAs. This work should lead to a better understanding of the roles of RNA structure and turnover on SSU gene expression.

R 439 **MOLECULAR WEIGHT DISTRIBUTIONS OF CELL WALL POLYMERS FROM FOUR NEAR ISOGENIC COTTON FIBER MUTANTS.** Judy D.Timpa and Barbara A. Triplett, USDA, ARS, Southern Regional Research Center, New Orleans, LA 70179.
Cotton fibers differentiate from single epidermal cells on the developing cotton seed. Fiber cells elongate to >2.5 cm in 3 weeks. In 7-8 weeks, fiber cells are encased in a thick secondary cell wall. Cotton fiber quality is determined by fiber strength, length, and fineness, properties which are determined largely by cell wall composition, polymer molecular weight and wall extensibility. Cotton fiber wall polymers from Gossypium hirsutum, Texas Marker 1 and four near isogenic fiber mutants have been analyzed by gel permeation chromatography of LiCl/DMAC-soluble components. This procedure solubilizes fiber cell wall components directly without prior extraction or derivatization, processes that lead to degradation of high molecular weight components. Fiber from mutants and the wild-type control were analyzed at several developmental stages from 8 to 35 days post-anthesis (DPA). The molecular weight of cellulose from normal fibers younger than 16 DPA (primary cell wall stages) was lower than the cellulose molecular weight from older fibers (secondary wall stages). Cellulose produced during the secondary wall stages in all of the mutants was identical in molecular weight to the cellulose produced by the wild type. This suggests that the mutants are not defective in their ability to produce cellulose characteristic of the genotype. Two mutants started producing high molecular weight cellulose earlier than the wild type, indicating an alteration in the normal developmental switch from elongation growth to secondary wall synthesis.

R 440 **HORMONAL AND TISSUE-SPECIFIC REGULATION OF CELLULASE GENE EXPRESSION:** Mark Tucker, Roy Sexton, Susan Baird, Stephen Milligan; USDA, ARS, PSI, Plant Molecular Biology Lab, Beltsville, MD

We have identified and sequenced a cDNA clone of a bean abscission cellulase mRNA. Cellulase mRNA accumulates in the fracture plane of abscising leaves. Experiments with 2,5-norbornadiene, a competitive inhibitor of ethylene action, shows that ethylene is required not only to initiate cellulase gene expression in abscission but also to maintain its expression. Auxin applied prior to an ethylene treatment blocks abscission and cellulase mRNA accumulation. Moreover, an ethylene evoked accumulation of cellulase mRNA in the fracture plane can be reversed by an application of the auxin analogs αNAA and 2,4-D directly onto the separation layer cells. The auxin induced reversal of the accumulation of cellulase mRNA was observed in the continued presence of 5 µl/l ethylene. The tissue and cell-specific accumulation of cellulase mRNA is examined using RNA gel blots, tissue prints, and *in situ* hybridization.

R 441 IDENTIFICATION OF UPSTREAM REGULATORY ELEMENTS INVOLVED IN THE EXPRESSION OF TWO POLLEN-SPECIFIC GENES. David Twell, Judy Yamaguchi and Sheila McCormick.
To investigate the regulation of gene expression during male gametophyte development we have analysed the promoters of two genes from tomato (LAT52 and LAT59) that show pollen-specific expression when introduced into tomato, tobacco and Arabidopsis plants. We have defined for both genes the minimal promoter length required for pollen-specific expression by introducing a series of 5' promoter deletions into transgenic plants. We have also developed a transient assay for gene expression in pollen using microprojectile bombardment of tobacco pollen. This has enabled a more detailed promoter mutational analysis than is practical in stably transformed plants, and has led to the identification of a short (97 bp) region of the LAT52 promoter that is capable of pollen-specific activation of a truncated CaMV35S promoter.

R 442 MAIZE INTRONS DIFFER IN THEIR RELATIVE ENHANCEMENT OF REPORTER GENE ACTIVITY WITHIN MAIZE, WHEAT, AND CARROT PROTOPLASTS,

Thomas H. Ulrich[1], David R. Korman[1], Michael Wach[1], Jim Wong[1], Anne Fennell[2], and Randal M. Hauptmann[2], Jeffrey Labovitz[1], [1]Sogetal,Inc., 3876 Bay Center Place, Hayward, CA 94545, [2] Plant Molecular Biology Center, Northern Illinois University, Montgomery Hall, Dekalb, IL 60115. The enhancement of gene expression by the maize (*Zea mays*) Adh1-1S introns 1 (I_1) & 6 (I_6) and the maize sucrose synthase intron 1 (SS_1) was compared among two monocots, maize and wheat(*Tritcum aestivum*), and a dicot, carrot (*Daucus carota*). Each intron was inserted into an identical position within the 5' mRNA leader sequence of the β-glucuronidase (GUS) reporter gene driven by the CaMV 35S promoter. Plasmid DNA with and without introns was delivered to plant protoplasts by electroporation and transient GUS activity was measured. Each intron construct differed in its ability to enhanced gene expression at the level of GUS activity. In maize and wheat cells, GUS activity was enhance up to 50-fold over that of the CaMV 35S intronless construct. Relative expression levels were greater in maize BMS protoplasts than in wheat protoplasts. The relative order of enhancement was $I_6 > SS_1 > I_1$. In contrast, the presence of the maize introns in our chimeric GUS constructs inhibited GUS gene expression in carrot protoplasts. Therefore, even though enhancement of gene expression was not species specific in the two monocots tested, some important part of the mechanism contributing to the enhancement of gene activity is not shared by a member of the dicots.

R 443 LOCALIZATION AND CHARACTERIZATION OF ELONGATION FACTOR-1α IN DEVELOPING TOBACCO PLANTS, Virginia M. Ursin, Jonathan M. Irvine, William R. Hiatt and C. K. Shewmaker. Calgene Inc. 1920 Fifth Street, Davis, Ca. 95616. Protein synthesis in eukaryotes requires activity of elongation factor EF-1 alpha (EF-1α) which catalyzes the binding of aminoacyl-tRNA to the aminoacyl site on the ribosome. In rapidly growing cells, EF-1α protein is highly abundant-a reflection of active protein synthesis. We have shown that in tomato (Lycopersicon esculentum), EF-1α is encoded by a small, multigene family and mRNAs corresponding to EF-1α are most highly expressed in the meristematic regions of the plant.(Pokalsky et al., 1989). Genomic clones for 2 expressed members of this gene family have been isolated and appear to correspond to a highly active gene, as well as a relatively inactive gene.

To further analyze the regulation and expression of the EF-1α gene in plants, we have transformed plants with a fusion construct containing the promoter of an active EF-1α gene and the coding region of the B-glucuronidase (GUS) gene. Transgenic tomato and tobacco plants containing the EF-1α-GUS fusions were analyzed qualitatively (localization of GUS activity in situ) and quantitatively (MUG assays) for GUS activity. Our results show that the patterns of GUS localization closely follow that observed for native EF-1α mRNA (Pokalsky et al., 1989), with highest expression levels observed in regions of rapid cell division within the plant. In addition, we have observed striking localization of GUS activity in developing reproductive structures of transgenic tobacco lines.

(Pokalsky et al., 1989. Nucleic Acids Research 17:4661-4673)

R 444 Efficient complementation of acetohydroxyacid synthase (AHAS) deficient bacteria by a modified *Brassica napus* AHAS gene. **Paul A. Wiersma, John E. Hachey, William L. Crosby*, and Maurice M. Moloney**, Dept. Biological Sciences, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4, and *Molecular Genetics Section, National Research Council Canada, Plant Biotechnology Institute, Saskatoon, Sask., Canada S7N 0W9.

The enzyme acetohydroxyacid synthase (EC 4.1.3.18) is the first common step in the pathway to the synthesis of the branched-chain amino acids in plants and bacteria. It has been demonstrated to be the target site for the action of three classes of herbicides (sulfonylureas, imidazolinones, and triazolopyrimidines). It would be advantageous to be able to test the effects of these herbicides on the plant gene product in the rapidly growing environment of a bacterial culture. This would permit mutations of the plant gene which produce a herbicide resistant phenotype to be rapidly identified. We have developed a system in which a modified *Brassica napus* AHAS gene is expressed at high levels using a bacterial promoter in an AHAS-minus bacterial host. The complemented bacteria grow in minimal media at approximately two-thirds the rate of wild-type bacteria and produce more AHAS enzyme activity than the wild-type. Introduction of mutations which produce resistance to the herbicide chlorsulfuron into this plant gene confer on the bacteria the resistant phenotype. Bacteria grown overnight on plates or in liquid culture allow rapid analysis of the characteristics of the plant gene. The potential of this system for saturation mutagenesis studies of AHAS is under investigation.

R 445 ROLE OF GLYCAN IN PROCESSING AND TRANSPORT OF CHITIN-BINDING LECTIN OF BARLEY IN TRANSGENIC TOBACCO, Thea A. Wilkins[1,2], Sebastian Y. Bednarek[1] and Natasha V.Raikhel[1], [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312; [2]*Present Address*: Department of Agronomy and Range Science, University of California, Davis, CA 95616.

There is increasing evidence that the chitin-binding lectins of the Gramineae, which accumulate in vacuoles, are plant defense-related proteins. We are interested in understanding the mechanism of this defense response by expressing and altering the distribution and subcellular site of accumulation of these lectins in vegetative tissues. To initially evaluate the role of Gramineae lectins in the plant defense response, the expression and targeting of barley lectin has been investigated in tobacco under the transcriptional control of the CaMV 35S promoter. Mature barley lectin is a dimeric protein comprised of two identical 18 kd polypeptides. The subunits of barley lectin are initially synthesized as glycosylated proproteins which are post-translationally processed to the mature protein preceding or commensurate with deposition of barley lectin in vacuoles. Thus, the synthesis, assembly, post-translational processing and targeting of barley lectin in tobacco leaves is analogous to expression in barley embryos. Since only the barley lectin proprotein is glycosylated, the sole N-linked glycosylation site residing within the COOH-terminal propeptide of barley lectin was altered by site-directed mutagenesis to determine if barley lectin could be redirected in the cell by removing the glycan. Barley lectin processed from the glycosylation-minus (*gly*') proprotein is correctly targeted to vacuoles of tobacco leaves. Localization of barley lectin in vacuoles processed from the nonglycosylated *gly* proprotein indicates that the high mannose glycan of the barley lectin proprotein is not essential for targeting barley lectin to vacuoles. However, pulse-chase labeling and monensin experiments demonstrated that the glycosylated wild-type proprotein and the nonglycosylated *gly* proprotein are differentially processed to the mature protein and transported from the Golgi complex with distinctive and characteristic kinetics. These results implicate an indirect functional role for the glycan in post-translational processing and transport of barley lectin to vacuoles.

R 446 TISSUE-SPECIFIC AND WOUND-RESPONSIVE EXPRESSION OF A HYDROXY-PROLINE-RICH GLYCOPROTEIN GENE PROMOTER IN TRANSGENIC TOBACCO, Keith L. Wycoff, Patricia A. Powell, Richard A. Dixon[§], and Christopher J. Lamb, Plant Biology Laboratory, Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138 and the [§]Plant Biology Division, Noble Foundation, Ardmore, OK 73402.

Hydroxyproline-rich glycoproteins (HRGPs) are the major structural proteins of plant cell walls and also accumulate in response to pathogen infection, wounding and exposure to fungal elicitor. Three closely related genes encoding apoproteins of HRGPs have been cloned from bean. We have constructed promoter-GUS fusions using the promoter of one of these genes, HRGP4.1, and expressed them in transgenic tobacco. The pattern of expression in response to wounding in tobacco is similar to that in bean. In addition to the response to environmental signals, an interesting tissue-specific pattern of GUS enzyme expression was observed in transgenic tobacco. The presense of GUS activity was highly localized to root tips, stem-petiole junctions and stigmas. Functional analysis of promoter deletions in transgenic tobacco indicated that all the sequences required for tissue-specificity and wound responsiveness are located within a region of 250 bp 5' of the transcription start site and that sequences further upstream facilitate an enhancement of promoter activity.

R 447 *IN VITRO* AND *IN VIVO* ACTIVITY OF RIBOZYMES DIRECTED AGAINST CAT mRNA AND PATHOGENIC TARGETS

Mark Young, Rhonda Perriman, Lisa Kelly,
Linda Graf, Danny Llewellyn and Wayne Gerlach

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Ribozymes are catalytic RNAs which direct the site-specific cleavage of RNA molecules. *In vitro* cleavage of targeted RNAs has been previously demonstrated in a number of ribozyme systems. Our laboratory has demonstrated that ribozymes derived from the satellite of tobacco ringspot virus system are capable of efficient, site-specific cleavage of CAT mRNA *in vitro*. Recent efforts in our laboratory have concentrated on optimizing ribozyme design for increased activity *in vitro* and testing ribozyme activity *in vivo*.

We are currently assessing both *in vitro* and *in vivo* ribozyme activity directed against CAT mRNA and two plant pathogenic RNA targets. The two pathogen model systems are the 6000nt ss RNA genome of tobacco mosaic virus (TMV) and the 371nt circular ssRNA of citrus exocortis viroid (CEV). *In vitro* kinetic studies indicate that the secondary structure of the target RNA greatly influences ribozyme activity. Studies will also be discussed which describe the *in vivo* activity of ribozymes directed against CAT mRNA and TMV infection in transgenic plants.

Plant Quality Improvement

R 500 CHARACTERIZATION AND MODIFICATION OF GENES ENCODING QUALITY-RELATED WHEAT PROTEINS OF THE GLUTEN MATIRX. Olin D. Anderson[1], Jody Steffen[1], Brandt Cassidy[2], Gad Galili[3], Jan Dvorak[2], Frank C. Greene[1]. [1]U.S. Dept. of Agriculture, ARS, Western Regional Research Center, Albany, CA. [2]Dept. of Agronomy and Range Science, University of California, Davis, CA. [3]Weizmann Institute, Rehovot, Israel. One important aspect of wheat flour quality derives from the physical characteristics of specific flour-water mixtures. These can interact to form an insoluble gluten matrix with elatiscity and strength properties that are generally correlated with the presence of specific classes of wheat storage proteins, particularly the high- (HMW) and low-molecular-weight (LMW) glutenins. Variations of dough properites among cultivars correlate with the presence and expression of specific members of the multigene families that encode the gluten matrix proteins. We are investigating the molecular basis of protein-quality relationships with the eventual goal of engineering cereal grains with a broader spectrum of utilization, and seek an understanding of how structural modifications will affect flour quality characteristics. We have isolated and sequenced all six HMW-glutenin genes from the hard red winter wheat Cheyenne, a cultivar known for good bread-making quality, and are also characterizing the LMW-glutenin gene family from this cultivar. Individual genes are being expressed in heterologous systems to allow physical study of both native and modified polypeptide structures.

R 501 TRANSGENIC GRAPEVINE: REGENERATION OF SHOOTS EXPRESSING β-GLUCURONIDASE, T. J. Baribault, K. G. M. Skene, P.A. Cain and N. Steele Scott, CSIRO Division of Horticulture, GPO Box 350, Adelaide, SA 5001, Australia

Genetic transformation has been studied in fragmented shoot apex cultures of Vitis vinifera L. following cocultivation with Agrobacterium tumefaciens. Transgenic shoots of the cultivar Cabernet Sauvignon tolerant to low levels of kanamycin have been produced. Proliferation of transgenic cells in the presumptive bud forming area of cultured fragments has been observed using the enzyme activity of β-glucuronidase (GUS) as a marker. The distribution of GUS stained cells both in this tissue and in transgenic shoots, the presence of low copy numbers of the neomycin phosphotransferase II gene in transgenic shoots and the relatively low levels of kanamycin resistance suggest that these shoots contain both transformed and untransformed cells.

R 502 DIFFERENTIAL EXPRESSION OF *Globulin* GENES IN MAIZE EMBRYOS, Faith C. Belanger, Nancy M. Houmard, and Alan L. Kriz, Department of Agronomy, University of Illinois, Urbana, IL 61801

During normal maize embryo development, the products of the distinct genes *Globulin-1* (*Glb1*) and *Globulin-2* (*Glb2*), which encode storage proteins of *Mr* 63,000 and *Mr* 45,000, respectively, accumulate to high levels. Since these proteins are degraded during the early stages of seed germination, *Glb* gene expression provides an excellent system for the study of gene regulation during maize embryo development. Northern blot analysis of embryo RNA indicates that *Glb1* and *Glb2* transcripts exhibit different patterns of accumulation during seed development and maturation. In addition, naturally-occurring null alleles for both *Glb1* and *Glb2* have been identified. Analysis of these null alleles may provide insight to the mechanisms involved in transcriptional and/or post-transcriptional regulation of gene expression in developing seeds. To further characterize *Glb* genes and aspects of their regulation in the developing seed, we have determined the nucleotide sequences of genomic clones corresponding to both functional and null alleles of *Glb1*. Experiments are in progress to investigate transcriptional activity of functional and null *Glb* alleles in nuclei isolated from maize embryos. These and related studies should allow for identification of promoter regions which are important for high levels of embryo-specific activity observed for these genes *in vivo*. Such information may allow for utilization of *Glb* promoters in efforts to improve the nutritional quality of maize grain protein.

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R 503 THE REGULATION OF GLUTAMINE SYNTHETASE : A KEY ENZYME IN NITROGEN METABOLISM. Malcolm J. Bennett, Ranjan Swarup and Julie V. Cullimore, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, U.K.

Higher plants obtain nitrogen from primary sources, such as nitrate, and for legumes, dinitrogen, initially reducing them to ammonium before assimilation. In addition, ammonium is released from a number of other metabolic pathways such as photorespiration and amino acid catabolism. All these sources of ammonium appear to be assimilated by glutamine synthetase (GS). GS is present as a number of isoenzymes, located in both plastid and cytosolic subcellular compartments of higher plants. In Phaseolus vulgaris, the GS isoenzymes are encoded by a small gene family of four expressed nuclear genes (glnα, glnβ, glnγ and glnδ) which encode three cytosolic GS polypeptides (α, β, γ) and a precursor to the chloroplast GS polypeptide (δ). Data will be presented using techniques developed to specifically and quantitatively study the regulation of expression of the individual genes and their gene products.

R 504 CLONING OF A cDNA EXPRESSED IN LATICIFERS OF RUBBER TREE CONTAINING HEVEIN AMINO ACID SEQUENCE. Willem Broekaert[1,3], Hyung-il Lee[1], Anil Kush[2], Nam-Hai Chua[2] and Natasha Raikhel[1], [1]MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing, MI 48824-1312; [2]Lab of Plant Molecular Biology, The Rockerfeller University NY, NY 10021-6399; [3]on leave from the F. A. Janssens Memorial Laboratory of Genetics, Katholieke Universiteit Leuven, Leuven, Belgium.

Hevein is a chitin-binding protein which is present in laticifers of the rubber tree (Hevea braziliensis). Evidence indicates that hevein possesses antifungal properties. We are interested in analyzing the function and expression of hevein at the molecular level. The DNA polymerase chain reaction (PCR) was applied to the isolation of a cDNA clone encoding hevein. Mixed oligonucleotide primers corresponding to two regions of hevein were used in conjunction with a hevein cDNA template in the PCR reaction. A cDNA clone (HEV1) was isolated and sequenced. HEV1 is 1021 nucleotides long and includes an open reading frame of 205 amino acids. The deduced amino acid sequence contains a putative signal sequence of 17 amino acids residues followed by a 188 amino acid polypeptide. This polypeptide has two striking features. The N-terminus of the protein (43 aa) has 100% identity to the known amino acid sequence of hevein (1) and shows homology to several chitin-binding proteins (2) and N-termini of wound-induced proteins in potato (67-72%) (3) and poplar tree (50%) (4). The C-terminal portion of the polypeptide (145 aa) revealed 70-72% homology to the C-terminus portion of wound inducible proteins in potato (3). In vivo and in vitro analysis of both portions of HEV1 are in progress.

1. Walujuno et al., (1976), in Proc. Internat. Rubber Conf.,518-531.
2. Raikhel and Wilkins (1987), PNAS, 84, 6745-6749.
3. Stanford et al., (1989), MGG, 215, 200-208.
4. Parsons et al., (1989), PNAS, 86, 7895-7899.

R 505 A CANDIDATE GENE FOR THE EXTENSION OF PEACH FRUIT SHELF LIFE, Ann M. Callahan, Peter H. Morgens, Paul Wright, and Ken Nichols, USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

One of the major problems in commercial peach fruit is that they soften too fast to be harvested at optimum quality. We have begun searching for genes that might affect this process without affecting quality. A cDNA clone, pch313, was isolated from a library constructed from ripe peach fruit RNA. The clone detects a RNA that is at very low abundance in fruit development until the fruit begin to soften. During this period there is a 400 fold increase in abundance of the RNA that correlates with softening as measured with a penetrometer. The insert of the cDNA was sequenced and found to have 78% sequence identity with pTom13 in the coding region. pTom13 represents a tomato gene that is expressed during fruit ripening and also upon wounding. Wounding experiments were undertaken to see if pch313 responded to wounding. In both leaf and fruit tissue, RNA homologous to pch313 was detected by 30 min after wounding and peaked in accumulation by 4 hours. Wound ethylene was measured at the same time and paralleled the accumulation of the RNA. Antisense constructions are in progress for use in peach and plum transformation/regeneration systems to see what effect variable blockage of the translation of this gene will have on wounding responses and fruit softening.

R 506 PEA LIPOXYGENASES Rod Casey, Claire Domoney, Paul Ealing and Helen North, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.
Lipoxygenases (LOXs) catalyse the hydroperoxidation of polyunsaturated fatty acids to various primary and secondary oxidation products, some of which affect the aroma of food and some of which may be of significance in plant growth and development and/or pest resistance. LOXs are also important in bread-making. We have been investigating the synthesis and structure of LOXs from pea (Pisum sativum) seeds and other organs. The two major seed LOX polypeptides are detected from 17 days after flowering (daf) onwards, and their corresponding RNAs are first detected at 16 daf; this is a relatively late stage of seed development. Using specific antibodies, we can also detect a LOX species that is predominant in early embryos; thus there is differential temporal regulation of pea seed LOX species. 'Western' blotting, and immunoprecipitation of cell-free translation products, indicates the existence of a range of LOX polypeptides of different apparent Mr in pea seed, flowers, stems and roots.

The deduced sequences of the two major seed LOX polypeptides show a high degree of homology and are similar to those from soybean, tobacco and man. Comparison of these sequences highlights two areas of conservation that may be important to the enzymes' activity; one of these shows similarity to a mammalian lipase and may be involved in substrate binding.

Molecular weight variants of the major pea seed LOX polypeptides have been used to map their genes, which are linked to markers on linkage group 4.

R 507 CLONING A MUTABLE ALLELE OF THE MAIZE *tassel-seed2* GENE, Alison DeLong, Jychian Chen and Stephen L. Dellaporta, Department of Biology, Yale University, New Haven, CT 06511. Maize is a monoecious plant that produces a terminal male inflorescence, or tassel, and axillary female inflorescences, or ear shoots. The *tassel-seed2* (*ts2*) gene is required for normal development of the tassel. The terminal inflorescence in homozygous *ts2* mutant plants develops as a female inflorescence, bearing fertile pistillate flowers rather than staminate flowers. We have isolated an unstable *ts* mutation (*ts2-m1*) through transposon tagging with the *Activator* (*Ac*) element. The phenotype of the homozygous mutant is identical to that of the known *tassel-seed2* allele, except for somatic and germinal instability. Homozygous *ts2-m1* plants frequently display sectors of fertile male florets on a background of female tissue. The *ts2-m1* phenotype is tightly linked to the *P* locus, and segregates with a unique *Ac* element detected by Southern analysis. These data are consistent with the hypothesis that the mutation constitutes an *Ac*-induced *ts2* allele. We have cloned the unique *Ac* and flanking genomic DNA from the homozygous *ts2-m1* mutant. Probes derived from this flanking DNA recognize unique sequences in genomic Southern blots and detect the inserted *Ac* in *ts2-m1* homozygotes. Germinal reversion of the *ts2-m1* phenotype is correlated with excision of the *Ac* element. We are currently examining the transcriptional activity of this locus in the developing tassel.

R 508 ESTIMATION OF IMPROVEMENT IN MAIZE GRAIN QUALITY WITH SPECTRAL METHODS. Vladimir S. Fedenko, Victor S. Struzhko. Department of Molecular Biology, Research Institute of Biology, State University, Dniepropetrovsk 320625, Ukraine, USSR.
When using the genetic methods of grain quality improvement the neccesity of an effective technique of a selectional estimation arises. The spectral methods of a rapid choice of the maize mutant forms having the economically useful features are proposed. The peculiarities of the protein complex formation are found to connect with the changes of the biochemical characteristics determined with the use of absorbtion spectra. According to the near infrared diffuse reflectance spectroscopic data the maize grain with o2/o2 mutation is characterized by the dicreased content of proteins, lipids, and starch as compared to its ordinary analogues. The o2/o2 su2/su2 mutation action is found to removes the grain quality negative tendency caused by the introduction of o2 mutant gene into the genotype. The grain chemical composition dependence of the genotype nature is confirmed with the reflectance spectra of the maize flour.

R 509 MOLECULAR ANALYSIS OF RYE B-CHROMOSOMES, John W. Forster(+), Michael J. Sandery(o), Richard Blunden(+), Simon R. McAdam(+), R. Neil Jones(+) and Steven D.M. Brown(x), Dept. of Agric. Sciences, UCW Aberystwyth, Wales, UK(+), Centre for Plant Biotechnology, Bond University, Australia (o) and Department of Biochemistry, St. Mary's Medical School, Paddington, London, UK(x). Supernumerary B-chromosomes are a common form of numerical chromosome polymorphism. In rye, the Bs are additional to the basic complement of 2n = 14. The rye Bs fail to pair with the A-chromosomes and show irregular modes of inheritance. In high numbers, the Bs have deleterious effects on many phenotypic characters. They can influence other characters of relevance to breeders such as frequency and distribution of chiasmata during meiosis. Comparisons of the properties of 0B and +B DNA using such methods as the analysis of reassociation kinetics have shown no significant differences between the As and Bs. Hence little is known of the composition of B-chromosome DNA. By comparing restriction digests of DNA from closely related 0B and +B plants, we have identified a highly repetitive sequence family which is present only on the rye B. The repeat sequence has been used as a hybridisation probe against multiple digests in order to assess its abundance, structure and relationship to previously characterised rye A-chromosome repeats. The repeat sequence has been cloned in the vector λgt10. We believe this to be the first report of a plant B-chromosome specific DNA sequence. The significance of this sequence for models of the evolution of Bs will be discussed. We have also applied the techniques of microdissection and microcloning to the isolation of molecular clones from B-chromosomes. Recent results on the isolation of intact Bs and their subsequent manipulation will be presented.

R 510 **ISOLATION AND CHARACTERIZATION OF MALE FLOWER-SPECIFIC GENES AND PROMOTER SEQUENCES FROM MAIZE.** Andrew J. Greenland, Philip Bell, Marie-Marthe Suner and Susan Y. Wright, Plant Biotechnology Group, ICI Seeds, Bracknell, Berks, RG12 6EY, UK. We are interested in the mechanisms which control expression of male flower-specific (MFS) genes during early development of maize tassels. We have isolated six unique MFS cDNAs by differential screening of libraries. These cDNAs show strong hybridization to RNA isolated from developing tassels bearing meiotic anthers but not to RNA from pollen. They are either absent or present at considerably lower levels in RNA isolated from leaf, cob and root.

We have selected three of these clones (pMS10, pMS14 and pMS18), which are highly specific for developing tassels, for detailed analysis and isolation of genes. Genomic Southern blots show that pMS14 and pMS18 are present as single copies and pMS10 as 2-3 copies per genome. Isolation of the genes from genomic libraries has been has proved problematic as the cDNAs, which contain repeated sequences with high G-C content, bind at high frequency to genomic clones which on analysis show only limited sequence identity with the cDNA. To overcome these difficulties and to isolate the genes we have used either high specific activity probes generated from 100 base specific oligonucleotides (pMS10 and pMS18) or the radiolabelled cDNA at high stringencies (pMS14). Currently we are isolating and characterizing promoter sequences.

R 511 INHIBITION OF CINNAMYL ALCOHOL DEHYDROGENASE SYNTHESIS IN STABLY TRANSFORMED TOBACCO PLANTS EXPRESSING ANTISENSE RNA , Jacqueline Grima-Pettenati*, Mary Knight**, Claude Grand*, Alain Boudet* and Wolfgang Schuch**, * Centre de Physiologie Végétale, URA CNRS 241, Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse cedex, France, ** ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell, Berkshire RG 12 6 EY, UK - Cinnamyl alcohol dehydrogenase (CAD) is an enzyme specifically involved in lignification process producing the second most abundant biopolymer after cellulose, the lignins. These biopolymers, highly resistant to biodegradation, decrease the digestibility of forage crops and represent waste products in the pulp industry. Thus, decreasing the amount of lignins would be useful to improve forage and woody plants. To reach this goal, we have developed the antisense RNA strategy which has recently been shown to be very efficient in plant systems. We have decided to target the CAD gene because of previous work done in our laboratory showing that the use of specific chemical inhibitors of CAD can lead to reduction in lignin content of plants without affecting their overall metabolism. Firstly the isolation of a bean full length cDNA encoding the CAD , allowed us to generate several antisense constructs. We have used vectors based on a derivative of the binary vector, BIN 19 pJR1, confering kanamycine resistance to the Ti-transformed tissues. Transformed Tobacco plants have been analysed for CAD enzyme activity and lignin content.

R 512 THE CATALASE GENES OF TOMATO: MOLECULAR ANALYSIS AND SEQUENCE, Gordon S. Inamine and James E. Baker, Plant Hormone Laboratory USDA/ARS, BARC-West, Beltsville, MD 20705.
Catalase cDNA clones from tomato fruit have been isolated and sequenced as part of a study on enzymes that are involved in oxidative processes in plants. As a means of obtaining probes for both antigen and nucleic acids, a catalase was purified to homogeneity from tomato fruit and polyclonal antibodies raised against it for use in immune-blotting and screening of a cDNA expression library . Several immunologically-positive cDNA clones have been isolated and partially sequenced. Preliminary data support the identification of these clones as being derived from catalase mRNA. Two forms of catalase have been observed in developing tomato seedlings by means of immunological blotting and both appear to be regulated at the level of antigen amount during development. Analysis of transcript size and levels will be presented.

R 513 **EXPRESSION OF SIX MALE FLOWER-SPECIFIC mRNAS DURING EARLY DEVELOPMENT OF MAIZE TASSELS.** Ian Jepson, Philip Bell, Marie Marthe-Suner, Susan Y. Wright and Andrew J. Greenland, Plant Biotechnology Group, ICI Seeds, Bracknell, Berks. RG12 6EY, UK. We are interested in genes which are expressed specifically in developing male flowers of maize. We have made two cDNA libraries from tassels bearing either early meiotic (EM) anthers (most meiocytes in early prophase) or late meiotic (LM) anthers (diad and tetrad stages). We have characterized five male flower-specific (MFS) cDNAs from the EM library (pMS1, pMS2, pMS4, pMS10 and pMS18) and a single MFS cDNA from the LM library (pMS14). The EM mRNAs show a very similar pattern of expression, accumulating very early in tassel development, persisting through meiosis and declining as pollen grains mature. The EM mRNAs are not wholly specific to male flowers and are detected at considerably lower levels in leaves (pMS10 and pMS18) or in leaves, cobs and roots (pMS1, pMS2 and pMS4). In contrast the LM mRNA, pMS14, is not detected in very young tassels, increases dramatically as the sporogenous cells undergo meiosis and declines abruptly as pollen grains mature. pMS 14 is not detected in leaves, cobs and roots. *In situ* hybridization studies with pMS14 show that it is localized to the single layer of tapetal cells surrounding meiotic sporogenous cells. Currently we examining the localization of the LM mRNAs by *in situ* hybridization and are using RNAase protection assays to quantify expression levels of all the mRNAs during male flower development.

R 514 *THE DESIGN, CONSTRUCTION, CLONING, AND INTEGRATION OF A SYNTHETIC GENE ENCODING A NOVEL POLYPEPTIDE TO ENHANCE THE PROTEIN QUALITY OF PLANTS*, JaeHo Kim*[1], M. Selim Cetiner[2], William J. Blackmon[2], and Jesse M. Jaynes[1], Departments of Biochemistry[1] and Horticulture[2], Louisiana State University, Baton Rouge, LA 70803.
A novel storage protein (HEAAE II, High Essential Amino Acid Encoding), rich in essential amino acids (~75% of total), was designed and constructed in our laboratory. The monomeric form of the protein consists of 20 amino acid residues with an additional 4 amino acids comprising a potential ß-turn. Circular dichroism and size exclusion analyses demonstrate that the monomer exists in a very stable α-helical conformation and self-aggregates in solution to form higher ordered multimeric structures (this is very reminiscent of natural plant storage proteins). The DNA, encoding this amino acid sequence, was synthesized and from this monomeric gene fragment, a tetrameric form of the gene was generated. The resultant DNA sequence was then introduced into the binary vector pBI 121 under the control of the cauliflower mosaic virus 35S promoter. *Agrobacterium tumefaciens*, strain LBA 4404, was subsequently transformed with this new construct. *Nicotiana tabacum* var. Xanthi tissue was then infected and transgenic plants obtained. Southern analysis confirms the presence of single copy gene integration. Northern and western analyses are currently underway to determine the level of expression and stability of this new protein.

R 515 REGULATION OF RICE GLUTELIN GENE EXPRESSION AND INTRACELLULAR PROTEIN TARGETING.

Soo-Young Kim, Eunpyo Moon, Jun Cao, Helen M. Moore, Isaku Tanida, and Ray Wu, Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, NY 14853. We have cloned a genomic DNA sequence encoding rice glutelin (pGL), the major seed storage protein of rice. The 5' flanking sequences of pGL were fused to the GUS reporter gene and introduced to rice immature seed tissue by particle gun bombardment. GUS assays showed that the pGL promoter sequence was active in the bombarded tissue. The binding of pGL promoter sequences to nuclear protein extracts of immature rice seed has been investigated in gel-shift and footprinting assays, and positive results obtained. A 24 amino acid putative signal sequence and additional N-terminal sequences are likely to be important to the processing and targeting of glutelins to the protein bodies of rice seed. We have constructed a deletion series of DNA sequences coding from 1 to 50 amino acids of the mature glutelin, fused in frame to the GUS reporter gene. The deletion constructs are being tested for correct targeting to rice protein bodies in in vitro packaging experiments and in rice transformation experiments. Hybrid storage protein genes (glutelin/patatin and glutelin/zein) are being used in rice transformation experiments to investigate the correct targeting and accumulation of foreign proteins using the promoter and N-terminal amino acid sequences of rice glutelin.

R 516 CHALCONE SYNTHASE AND CHALCONE-FLAVANONE ISOMERASE PROMOTERS ARE ACTIVE IN PIGMENTED AND UNPIGMENTED CELLS OF *PETUNIA HYBRIDA*, Ronald E. Koes, Arjen J. van Tunen, Rik van Blokland, Leon A. Mur, Stephan Brekelmans, Francesca Quattrocchio, and Joseph N.M. Mol. Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands (Fax: 31-20-429202).
Flavonoids are plant secondary metabolites which function in flower pigmentation, protection against environmental stress and induction of nodulation genes. The first two enzymes of the flavonoid pathway are chalcone synthase (CHS) and chalcone-flavanone isomerase (CHI). *Petunia hybrida* contains about 12 *chs* genes, four of which are active genes (*chsA, chsJ, chsB, chsG*) and two *chi* genes (*chiA* and *chiB*), which are both transcriptionally active We have fused the promoters of these six genes to the GUS reporter gene and analysed their activity at the cellular level. Surprisingly, we found *chs* and *chi* promoter activity not only in pigmented cell-types (e.g. epidermal cells of the flower corolla and tube and (sub)epidermal cells of the flower stem), but also in several unpigmented cell types (mesophylic cells of the flower corolla, several cell types in the ovary and the seed coat). Comparison of *chs*-GUS expression and pigmentation patterns in anthers suggests that intercellular transport of flavonoids occurs. The *chiA* gene is preceded by a tandem promoter. Expression of the GUS gene driven by the downstream *chiA* promoter exhibits the same spatial and temporal control as the *chs*-GUS fusions. The upstream *chiA* promoter is expressed in pollen grains only. The promoter of the *chiB* gene is active in anthers only.This makes these *chi* promoters attractive candidates to drive expression of genes that interfere with anther and pollen development.
The activity of *chs* and *chi* promoters in the (unpigmented) ovary is related to the synthesis of colourless flavonols. In seeds simple anthocyanins are accumulated and no further modifications occur. Only a small subset of the genes that control flavonoid flavonoid biosynthesis in floral tissues operates in seeds, which implies that the (genetic) control of flavonoid biosynthesis is highly tissue-specific.

R 517 HIGH FREQUENCY EMBRYOID AND PLANTLET FORMATION FROM TISSUE CULTURES OF THE FINGER MILLET (ELEUSINE CORACANA), KOTHARI S.L. Sivadas, P. and Chandra, N., Department of Botany, University of Rajasthan, Jaipur, India.
Compact nodulated callus with numerous embryoids differentiated from cultured seeds of Eleusine coracana (Finger millet) on MS medium with 2,4-D (1.0, 3.0 mg l^{-1}). This embryogenic callus was as maintained on a medium with lower level of 2,4-D (0.2 mg l^{-1}). At every subculture the embryogenic callus had some preexisting embryoids in it. No loss of morphogenitic potential occurred for 4 years if the callus subcultured had preexisting embryoids in it. Following transfer to media with BAP, KN, IAA, IBA, NAA, GA_3, 2,4-D the embryogenic callus bearing embryoids showed varied pattern of growth and morphogenesis. On a medium with GA_3 embryoids germinated in profusion to form plantlets which could be transferred to field and developed seeds. On a medium with BAP shoot buds differentiated from the whole surface of the embryoids or the flattened meristemoids.

R 518 MECHANISM OF THE PROTEIN QUANTITY AND QUALITY REGULATION IN MAIZE GRAINS OF THE HIGH LYSIN MUTANTS. Nadezhda P. Kotsubinskaya, Valentina S. Bilchuck. Departmemt of Molecular Biology, Research Institute of Biology, State University, Dniepropetrovsk 320625, Ukraine, USSR.
The presence of the biochemical effect of the opaque-2 gene dose has been found. Albumine, globuline and glutelin contents were increased when the depression of zeins and the general protein in the grain of maize heterozigotic mutants was absent. The absence of a negative correlation between quantity and quality of protein was demonstrated with the use of different genetic maize forms containing the mutant genes o2, wx, su2. In homozigotic maize mutants, the zein level decrease correlated with the hightened activity of proteolytic enzymes when grain maturating. Proteases isolated from the high lysin maize grains, broked up actively the zein during in vitro experiments. The increased sensitivity of opaque-2 maize zein to the exogenic protease action was shown.

R 519 MOLECULAR BIOLOGY OF SUCROSE STORAGE IN SUGARCANE (SACCHARUM OFFICINARUM). A. Suresh Kumar and S. Ramagopal, Agricultural Research Service, U.S. Department of Agriculture, Experiment Station, Hawaiian Sugar Planters' Association, Aiea, HI 96701. Normally small amount of sucrose accumulates in various parts of the plant in many species, but a distinct feature of the sugarcane crop is that sucrose can accumulate in massive quantity, as much as 500 mM or higher, in the vacuolar compartment of stalk parenchyma cells. The biochemical and molecular mechanisms leading to the storage of sucrose in stalk tissue are presently unknown. Two enzymes of sucrose metabolism, sucrose synthase, and invertase, are thought to play key roles in the transport and storage of sucrose in vacuoles. The aim of the present project is to elucidate the role of sucrose synthase (EC 2.4.1.13), an enzyme that catalyses the reversible cleavage of sucrose into UDP-glucose and D-fructose. Preliminary experiments have established that the sugarcane enzyme protein cross reacts with antibody to maize sucrose synthase, and that sugarcane mRNA also hybridizes with cDNA probes from maize. Therefore, we are analyzing the expression of sugarcane enzyme in the various source (leaf) and sink (stem) tissues by Western and Northern techniques. In a parallel approach, we have also constructed a sugarcane genomic library in EMBL3 vector to isolate the sugarcane genes. After screening a million plaques with maize cDNA probes, seven positive genomic clones were selected. The genomic clones are being grouped into different classes by screening them differentially with two cDNA probes of the maize enzyme, SS1 and SS2. The findings on gene isolation and gene expression in sugarcane will be presented. The results are expected to contribute to a better molecular understanding of this important agronomic trait in sugarcane.

R 520 CHARACTERISATION OF THE HYPERSENSITIVE RESPONSE INDUCED IN SOYBEAN LEAVES BY A BACTERIAL PATHOGEN AND A SPECIFIC ELICITOR, Diane M. Lawrence and Mark M. Stayton, Department of Molecular Biology, University of Wyoming, Laramie, WY 82071. Avirulence gene D was isolated from Pseudomonas syringae pv. tomato by virtue of its ability to confer avirulence on strains of Pseudomonas syringae pv. glycinea (1). Over-expression of this gene in both Pseudomonas syringae and E. coli leads to the production of a cultivar-specific elicitor (SE) of the soybean defence response (2). The pattern of plant gene expression induced by the bacterial pathogen has been characterised and compared to that induced by the SE. The characterisation is based on the timing and the extent of mRNA accumulation for two enzymes of the phenylpropanoid pathway, phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS) and also a pathogenesis-related (PR) protein. In addition, the accumulation of glyceollin, the major soybean phytoalexin, has been monitored. The results are consistent with the SE as mediator of the gene-for-gene interaction observed in vivo.

1. Kobayashi, D.Y. et al (1989) PNAS 86 157-161.
2. Keen, N.T. et al (1990) MPMI In press.

R 521 ANALYSIS OF AGRONOMIC TRAITS AND INHERITANCE OF TRANSGENIC RICE PLANTS.
Zhijian Li and Norimoto Murai, Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803-1720.

We have established an efficient procedure for protoplast transformation and regeneration of fertile transgenic plants in rice (Oryza sativa L.) cultivar Nipponbare and Taipei 309. Protoplasts were mixed with a plant-expressible hygromycin resistance gene and treated with 25 %(w/v) polyethylene glycol (PEG). A hygromycin phosphotransferase coding sequence was placed under the nos or CaMV 35S promoter in pTRA131 and 132, respectively. Stringent selection of transformed colonies were applied to 14-day old protoplasts in the presence of 50 mg/l of hygromycin for 12 days. After selection, 500 and 200 resistant colonies were recovered per million treated Taipei 309 and Nipponbare protoplasts, respectively. Southern hybridization analysis of hygromycin-resistant cell lines and regenerated plants indicated that one to ten copies of transferred DNA were integrated at one to four loci of the rice genome. The DNA analysis suggests that the introduced plasmid DNA may form concatemers by intermolecular recombination prior to the integration.

Four Taipei 309 transgenic plants were regenerated from three transformed cell lines and grown to maturity in the greenhouse. One transformed line, T132-4, regenerated two phenotypically normal plants. Both plants set flowering panicles and produced abundant viable seeds. Fifty nine Nipponbare transgenic plants were grown to maturity and set flowering panicles in the greenhouse. Eighteen plants were transformed with pTRA131 and forty one plants with pTRA132. After selfing almost all plants produced viable seeds. Analysis of seed fertility and other agronomic traits of these transgenic plants and inheritance of the hygromycin-resistance gene will be reported.

R 522 EXPRESSION OF HEPATITIS B VIRUS SURFACE ANTIGEN IN TRANSGENIC TOBACCO PLANTS, Bai-Ling Lin*, Sue J. Jiang, Jing-Shu Huang, Yu-Liang Kuo, I-Ping Cheng*, Chao-Jung Tu* and Shih-Tung Liu, *Institute of Molecular Biology, Academia Sinica, Nankang, Taipei 11529, Taiwan, Republic of China, and Department of Microbiology and Immunology, Chang-Gung Medical College, Kwei-Shan, Taoyuan 33332, Taiwan, Republic of China.

We have explored the possibility of producing hepatitis B vaccine in plant cells. The coding sequence of hepatitis B virus surface antigen (HBsAg) plus the pre-S1 and pre-S2 regions from a locally isolated *adw* serotype of hepatitis B virus (HBV) was fused to the cauliflower mosaic virus 35S promoter in plasmid pBI 121.1 (Jefferson, J. A. et al. 1987. EMBO J. 6:3901-3907). The chimeric gene was introduced into tobacco leaf segments using *Agrobacterium*-mediated transformation. The transformants were regenerated in selection medium and grown in soil to set seeds. The incorporation and the expression of HBV gene were demonstrated in the transgenic tobacco plants.

R 523 ISOLATION AND ANALYSIS OF RIPENING STAGE SPECIFIC GENES IN TOMATO, Andrew D. Lloyd, Ann Callahan, and Peter Morgens, USDA, ARS, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV 25430. cDNA clones of mRNAs that are expressed during different stages in the ripening of tomato fruit (Lycopersicon esculentum Mill. var. Pixie) have been isolated by differential hybridization. Corresponding genomic clones from an EMBL3 tomato genomic library were isolated using these cDNA clones. Expression of clone 102 increased during ripening, while clone 204 showed peak expression in green fruit, with mRNA levels decreasing as ripening progressed. Insert DNA from genomic clones 102 and 204 was transferred into a Bluescript plasmid vector and analyzed by restriction enzyme mapping. Southern blot analysis of the genomic clone of 204, pAL204, using the 204 cDNA as a probe, indicated that all genomic sequences complimentary to the cDNA were present in a 2.0 kb Eco R1 fragment. The DNA sequences of this genomic fragment, as well as that of the cDNA clones for both 102 and 204 is being determined.

R 524 APPROACHES FOR IMPROVING PREHARVEST SPROUTING RESISTANCE IN CEREALS USING GRAIN SPECIFIC PROMOTERS, A.Lönneborg, S.S.Klemsdal, R.Aalen, K.Jakobsen, W.Hughes and O.A.Olsen, PLANT MOLECULAR BIOLOGY LAB NLVF, P.O.B. 51 N-1432 ÅS-NLH NORWAY, Ph +47 9 948132, FAX + 47 9 941465
Economic loss due to preharvest sprouting is a serious problem particularily in wheat, but also in barley. One approach to reduce the effect of the prematurely induced hydrolytic and proteolytic enzymes causing the damage involves the production of transgenic plants containing either antisense transcripts for the unwanted transcripts or inhibitors of the corresponding proteins. Key elements in this strategy are promoters driving expression in the relevant tissues (aleurone and scutellum) of the maturing grain. We have isolated 4 barley cDNAs representing mRNAs that are expressed by genes likely to contain promotors fulfilling these requirements. Their pattern of expression is as follows: B22E) aleurone and scutellum of developing grains pluss scutella of germinating grains, ABA supressible in developing embryos. B11E and B14D) exclusively in the aleurone layer of developing grains. B32E) aleurone and embryo of developing grains, ABA inducible in young embryos, absent from germinating grains. The cDNA sequences checked are not homologous to known sequences. We are currently expressing cDNAs in tobacco to study functional aspects of the sequences. Genomic clones have been isolated, and in one case (B22E) also sequenced. Promoter studies are underwa in tobacco and rice.

R 525 ISOLATION OF GENES WITH ENHANCED EXPRESSION IN XYLEM TISSUE OF LOBLOLLY PINE, Carol A. Loopstra and Ronald R. Sederoff, Department of Forestry, North Carolina State University, Raleigh, NC 27695. The isolation of clones for tissue specific genes is useful for studying the regulation of genes involved in the formation of wood. Associated regulatory elements may be valuable in attempts to alter wood properties by genetic engineering. Differential screening of a loblolly pine cDNA library yielded clones of 2 different genes with enhanced expression in xylem. Northern blot analyses show these genes to be expressed at high levels in newly differentiated xylem tissue removed from the trunks of 10-year-old trees, at considerably lower levels in needles, and hardly at all in embryo and megagametophyte tissues. One of the 2 genes is highly expressed in the stems of 6-week old seedlings. Genomic Southern blots probed with the 3' ends of each gene indicate they are probably present as single copies.

R 526 TRANSIENT ASSAY OF MONOCOT ENDOSPERM GENE EXPRESSION. Gale F. Lorens, Ann E. Blechl, Olin D. Anderson and Frank C. Greene, Western Regional Research Center, Agricultural Research Service, USDA, Albany, CA 94710. We are exploring the potential of transient assay as a means of rapidly delineating sequence elements that control expression of wheat seed storage protein genes in the developing endosperm. Putative promoter regions from two wheat storage protein genes and the maize sucrose synthase gene *Sh1* have been examined. Hybrid constructions were prepared by fusion of 5'-gene flanking regions to the Chloramphenicol Acetyl Tranferase (CAT) gene coding region, and introduced by electroporation into protoplasts derived from cell suspension culture lines of maize endosperm (inbred line A636) or *Triticum monococcum* root origin. CAT activity was taken as a measure of gene expression. In the maize endosperm system, a segment (-442 to -1) from the wheat High Molecular Weight Glutenin subunit (HMW) Glu1-Dx5 enhanced CAT reporter gene activity approximately 40-fold relative to promoterless constructs, or constructs containing the HMW segment in opposite orientation. An α-gliadin gene upstream segment (-666 to -41) stimulated reporter activity marginally but consistantly, and a segment (-1210 to +40) of the *Sh1* gene exhibited promoter activity intermediate between that of the storage protein gene sequence elements. None of these upstream regions exhibited detectable promoter activity in *T. monococcum* protoplasts. *Adh1* promoter constructs (which included the first intron) exhibited high activity in both protoplast systems. These results are consistant with those obtained in transgenic plants, and demonstrate that the maize cell line is suitable as a system for analysis of the control of genes which exhibit endosperm-specific or endosperm-enhanced expression.

R 527 **THE LATEX OF THE RUBBER TREE, *HEVEA BRASILIENSIS*, CONTAINS BOTH CHITINASES AND LYSOZYMES**, Melinda N. Martin, Dept. of Biol. Sciences, Rutgers University, Newark, NJ 077102

The level of the putative defense protein chitinase is low or undetectable in leaves and most other tissues of young, unstressed dicotyledonous plants, but rapid induction is often observed following treatment of these plants with ethylene or various biotic or abiotic elicitors. Consistent with other dicotyledons, chitinase activity is very low in the leaves of young plants of the commercial rubber tree, *Hevea brasiliensis*. In contrast, little or no increase in chitinase activity is observed following exposure to the ethylene releasing compound, ethephon. However, in the latex of young *Hevea brasiliensis*, chitinase appears to represent from 12-25% of the total protein and shows a specific activity as high as 3 nkat/mg of protein. Equally striking is the high lysozyme activity (10,000 units/mg of protein) found in crude latex. Most plant chitinases characterized to date have very low associated lysozyme activity. The chitinase/lysozyme activities in latex were resolved by anion exchange FPLC into acidic and basic proteins. The acidic protein(s) had chitinase activity and no lysozyme activity. Six basic proteins, having both chitinase and lysozyme activities in varying ratios, were resolved by cation exchange FPLC. One of the basic proteins exhibited a high lysozyme activity comparable to those reported for animal lysozymes. Since ethephon has been reported to stimulate latex flow and yield of rubber in *Hevea brasiliensis*, the effect of ethephon treatment on the protein composition of latex and specifically on the individual chitinase/lysozymes was analyzed using two-dimensional gel electrophoresis and FPLC.

R 528 DIFFERENTIAL EXPRESSION OF GLYCININ GENE IN TRANSGENIC PLANTS. Momma T.[1], Toguri T.[1], Okada K.[1], Fukazawa C.[2] and Ohtani T.[1] (1: Plant Laboratory, Kirin Brewery , Kitsuregawa, Shioya, Tochigi 329-14, Japan. 2: Genetic Engineering Laboratory, National Food Research Institute, Tsukuba, Ibaraki 305, Japan.) Glycinin, the 11S seed storage protein of soybean, is processed through the following steps; 1. Co-translational removal of a signal peptide, 2. Formation of a disulfide bond between acidic and basic chain of proglycinin, 3. Post-translational cleavage of proglycinin into the acidic and basic chain. A maturing enzyme is assumed to be involved in the cleavage step of proglycinin. Our interest is to know the processing of glycinin polypeptides made in the anatomically distinct tissues. We constructed a chimaeric glycinin gene from a 35S promoter of CaMV and a cDNA copy of glycinin A_2B_{1a} subunit. We transformed the construct into tobacco and potato by Agrobacterium Ti plasmid. Synthesis of the glycinin was detected immunologically in the leaves (tobacco, potato), tuber (potato) and seed (tobacco). The cleavage into acidic and basic chain was evident in the proteins extracted from the seeds of the transgenic tobacco plants, but the glycinin was not processed in the other tissues. Experiments to sequence N-terminal amino acids of the processed and the non-processed glycinin are in progress.

R 529 ANALYSIS OF REGULATORY GENES AFFECTING MAIZE STORAGE PROTEIN SYNTHESIS, Mario Motto, Natale Di Fonzo, Hans Hartings, Massimo Maddaloni, Jaume Palau, Francesco Salamini, Richard Thompson, Istituto Sperimentale per la Cerealicoltura, Sezione di Bergamo, 24100 Bergamo, Italy; Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, Fed. Rep. of Germany.

Further progress in understanding of the normal control of maize zein genes is expected from the molecular cloning and analysis of the regulatory loci affecting the accumulation of zein polypeptides in developing kernels. To begin to analyze by which manner the product of these loci could affect zein expression we have cloned and sequenced two of these genes, i. e. the b-32 and the Opaque-2 (O2) gene.

The cDNA coding for the b-32 protein, an albumine expressed in maize endosperm cells under the control of the O2 and O6 loci, has been cloned and the complete amino acid sequence of the protein derived. The deduced protein is composed of 303 amino acids (mol wt 32,430). From the structural analysis of the b-32 protein it was possible to predict the existence of an acid central domain separated by two compact domains, that by folding may give rise to a globular shape of the molecule; it is attractive to postulate that a similar structure might permit this albumine-like protein to accomplish a regulatory role by interacting with the transcriptional machinery at the 5' flanking region of zein genes. In addition, three b-32 genes from two inbreds have been cloned and sequenced indicating that the b-32 genes form a small gene family showing polymorphism.

The structure of the zein regulatory gene O2 has been determined by sequence analysis of genomic and cDNA clones. The main ORF comprises 1362 bp and is composed of six exons ranging in size from 465 to 61 bp and five introns of 678 bp to 83 bp. The opaque-2 protein contains a putative domain similar to the leucine zipper motif identified in DNA binding proteins of animal proto-oncogenes and in transcriptional regulators of yeast. Adjacent to this sequence, towards the amino terminus, a conserved cluster of basic residues is present similar to those adjacent to the leucine zipper of transcriptional activators, such as c-myc, v-jun, v-fos and GCN4. Moreover, in the carboxy terminal region of the 50 kD O2 protein a motif closely resembling a metal binding domain is present. The molecular mechanism by which the activation of zein genes expression occurs, in relation to the b-32 and O2 loci, is currently under investigation and the results of these experiments will be presented.

R 530 INTRODUCTION OF A CHALCONE SYNTHASE TRANSGENE RESULTS IN REVERSIBLE CO-SUPPRESSION OF THE ACTIVITY OF BOTH THE TRANSGENE AND ITS HOMOLOGUE, C. Napoli, C. Lemieux, and R. Jorgensen, DNA Plant Technology Corp., 6701 San Pablo Ave, Oakland, CA 94608
In an attempt to test whether chalcone synthase (CHS) is rate-limiting to anthocyanin biosynthesis in petunia we introduced a 35S/CHS gene fusion to several genotypes. Twenty of 48 transgenotes (42%) displayed white or patterned flowers. The remainder were identical to the control transgenic flowers, which have never shown altered color phenotypes among hundreds of transgenotes. RNase protection analysis of RNA from white flowers showed that the level of message from the endogenous gene was suppressed 50-fold throughout development. Pigmented somatic revertant flowers which were phenotypically parental were found to have normal levels of endogenous CHS message as well as 50-fold higher levels of introduced CHS message. This indicates that the homologous introduced and endogenous CHS genes are coordinately suppressed in white flowers. This reversible co-suppression of homologues must be accounted for in the mechanism of CHS gene regulation. The similarities between co-suppression and other phenomena such as paramutation and transvection will be discussed.

R 531 IN VITRO GROWTH PROMOTION OF POTATO BY PSEUDOMONAD BACTERIUM, J. Nowak,[1] M. Frommel[2] and G. Lazarovits[2], [1]N.S.A.C., Truro, N.S., Canada, [2]Agriculture Canada, London, Ontario, Canada
Pseudomonad bacterium isolated from onion roots caused considerable stimulation of rooting and stem growth in micropropagated potatoes. Both endophytic and epiphytic populations were found. Significantly higher activity of phenylalanine ammonia lyase (PAL), higher lignin content and lower levels of free phenolics were determined in the inoculated plantlets compared to the non-inoculated control. Inoculated plantlets were able to withstand transplant stress better than the uninoculated controls. Three hundred fifty mutants by Tn5 transposon mutagenesis were produced. Each mutant exhibited different degree of resistance to streptomycin and kanamycin. Six randomly selected mutants were evaluated for in vitro growth promotion of potato. All of them stimulated growth of the inoculated plantlets. Both, the wild strain and the mutants, have a single plasmid of approximately 120 Kd. The role of the plasmid in the growth stimulation is under study. The plasmid has been transferred to Pseudomonas syringae which does not normally have growth stimulatory activity. We are also attempting to cure the wild strain from the plasmid and to use strain cc118 of Escherichia coli as the donor of a plasmid which confers antibiotic resistance and codifies for alkaline phosphatase activity (AP). The AP could be a good indicator of plant colonization by the bacterium.

R 532 PURIFICATION AND CHARACTERIZATION OF CINNAMYL ALCOHOL DEHYDROGENASE FROM DEVELOPING XYLEM OF LOBLOLLY PINE, AND ITS ROLE IN STRATEGIES TO MODIFY THE LIGNIN CONTENT OF WOOD, David M. O'Malley and Ronald R. Sederoff, Department of Forestry, North Carolina State University, Raleigh, NC 27695. Reduction of lignin content in loblolly pine would result in a large improvement of pulp yield in the manufacture of paper. Cinnamyl alcohol dehydrogenase (CAD) is the last enzyme in the pathway which synthesizes coniferyl alcohol, the monomeric precursor of lignin. CAD was purified to homogeneity from developing xylem stripped from young loblolly pine trees. The native enzyme is a dimer with an apparent molecular weight of 82,000 and a subunit apparent molecular weight of 44,000. CAD is genetically variable. Electrophoretic variants showed simple Mendelian inheritance suggesting that CAD is encoded by a single gene. Monoclonal antibodies raised against CAD and/or protein sequence information will be used to screen a cDNA library for CAD clones.

R 533 CLONING OF MAIZE viviparous 7, A GENE IN THE ABSCISIC ACID/CAROTENOID BIOSYNTHETIC PATHWAY, BY TRANSPOSON -TAGGING, Oishi, K.K., Department of Molecular and Cellular Biology and Department of Plant Sciences, University of Arizona, Tucson, AZ 85721. During seed development the zygote goes through two distinct developmental phases, cell differentiation and division (embryogenesis), followed by inhibition of growth and desiccation (dormancy). There is a great deal of biochemical and genetic evidence which implicates the growth hormone, abscisic acid (ABA), as the regulator of induction of seed dormancy. In maize there are eight genetically characterized viviparous mutants which exhibit precocious germination. Seven of these, vp2, vp5, vp7, vp8, vp9, al, w3, are ABA deficient mutants and one, vp1, is an ABA insensitive. Viviparous mutants were isolated from maize lines containing the family of transposons, Mutator. Ten of these mutations were allelic to an ABA deficient mutation, vp7. A single Mu1-containing restriction fragment was found to segregate with one allele, vp7-KO183. By restriction mapping of the cloned HindIII fragment, the Mu1 was determined to have inserted into single-copy DNA. The single-copy DNA was used to analyze six other vp7 mutant alleles. Two of these had detectable insertions, vp7-KO844, vp7-KO189, and one had a deletion, vp7-KO835, which segregated with a 12 kbp DNA region and the mutant allele.

R 534 THE TRANSITION FROM DEVELOPMENTAL TO POST-GERMINATIVE METABOLISM IN MAIZE ALEURONE TISSUE IS ELICITED BY DRYING, Maurice Oishi and J.D. Bewley, Botany Department, University of Guelph, Guelph, Ontario, Canada, N1G 2W1. α-Amylase is a starch-hydrolysing enzyme synthesized by maize aleurone tissue following germination of mature grains. It is not synthesized in abundance during grain development. However, a drying treatment enables developing (21 and 35 DAP) tissue to synthesize it after 4-days rehydration.

This response is independent of changes in ABA levels caused by drying. Endosperm from 35 DAP kernels treated with fluridone, an inhibitor of ABA biosynthesis, and from 35 DAP dried kernels contain similarily reduced levels of ABA relative to fresh control tissue. Yet the fluridone-treated aleurone tissue, incubated for 4 days, produces only 10% of the enzyme found in dry/rehydrated tissue. Endogenous levels of GA are not a limiting factor in the fluridone-treated tissues since exogenous GA has relatively little effect on enzyme production. Thus, drying, whether prematurely imposed or as occurs normally at the end of grain development, acts beyond merely altering hormone levels within the aleurone tissue to effect a transition from development to post-germination.

R 535 THE TOMATO ACC SYNTHASE GENE INVOLVED IN FRUIT RIPENING[1]. William H. Rottmann, Gary F. Peter, Julie Keller and Athanasios Theologis. Plant Gene Expression Center, USDA-ARS, 800 Buchanan St., Albany, CA 94710 and Department of Plant Biology, UC Berkeley, Berkeley, CA 94720.

A λgt10 cDNA library constructed with polyA+-RNA from climacteric tomato fruit was screened at low stringency with the zucchini ACC synthase cDNA pACC1[2]. Numerous cDNAs with overlapping restriction patterns were isolated, and the largest, TACC1 (1775 bp), was characterized. The authenticity of this clone was confirmed by its ability to convert [1-^{14}C]methionine to [carboxyl ^{14}C]ACC in *E. coli* that expresses the TACC1 cDNA in the correct orientation. Sequence analysis revealed an open reading frame coding for a polypeptide of 57 kDa. The amino acid identity between the tomato and zucchini ACC synthases is 68%. The TACC1 cDNA hybridizes to an mRNA 1900 nucleotides long that is undetectable in mature green (MG) stage fruit, but is greatly induced during ripening. The majority of the mRNA increase is detected between the MG and breaker stages. Ethylene accelerates fruit ripening with the concomittant induction of the ACC synthase mRNA.

Hybridization of tomato genomic libraries with TACC1 allowed the isolation of overlapping genomic clones. Reconstruction experiments show that the ACC synthase involved in fruit ripening is represented by a single copy gene. The complete gene structure and its transcription initiation site have been determined.

1. This work was supported by grants to A.T. from NSF (DCB-8819129 and -8916286), NIH (GM-35447) and USDA (5835-23410-D002).

2. T. Sato and A. Theologis (1989). PNAS 86, 6621-6625.

R 536 TRANSGENIC MEDICINAL PLANTS: INTRODUCTION OF GENES FOR DRUG METABOLIZING ENZYMES. Kazuki Saito, Mami Yamazaki, Hiroaki Kaneko, Masaaki Noji, Yoshio Imai*, and Isamu Murakoshi. Faculty of Pharmaceutical Sciences, Chiba University, Chiba 260, Japan, *Faculty of Agriculture, Osaka Prefecture University, Sakai 591, Japan.

The transgenic technique based on a Ti or Ri-*Agrobcaterium* system is becoming important for genetic manipulation of secondary metabolism of medicinal plants. Genes of drug metabolizing enzymes from microorganisms and animal tissues are interesting to introduce into pharmaceutically important plants for modification their useful secondary metabolites. β -Glucuronidase gene (*Gus*) on a mini Ti plasmid pGSGluC1 together with NPT-II gene was co-introduced by Ti or Ri binary vectors into *Nicotiana tabacum, Atropa belladonna, Datura stramonium, Hyoscyamus niger, Capsicum annuum, Digitalis purpurea* and *Glycyrrhiza uralensis*. Transformed states were confirmed by opine assay and/or Southern blot analysis. Transformed hairy roots of Solanaceae plants produced each their own alkaloids. The cytochrome P-450 cDNA (pAHF3) from phenobarbital-treated rabbit livers was cloned into pGSH160 to construct a chimeric gene pSN002 flanking with Tr2' promoter and 3' end of T7. We have obtained several tobacco transformants with pSN002. The expression of the rabbit P-450 gene will be reported.

R 537 GENETICS AND BIOCHEMISTRY OF IN-VITRO SELECTED LYSINE MUTANTS, G. W. Schaeffer, USDA, ARS, Plant Molecular Biology Lab, Beltsville, MD 20705, USA.
Lysine is an important and limiting amino acid for optimal nutritional quality of rice. Experiments were designed to provide bridge technologies between biochemical selections in established cultivars to characterize progeny from these in-vitro selections. Lysine mutants were recovered by growing anther-derived callus with inhibitors affecting the lysine pathway, namely inhibitory levels of lysine plus threonine and s-(aminoethyl)cysteine an analog of lysine. Plants regenerated from inhibitor resistant cells and progeny, crosses, F_2's, F_3's, F_4's and backcrosses were analzyed for percent lysine in endosperm proteins, for chalkiness and seed weight. Endosperm proteins were fractionated into solubility classes, amino acid levels determined and high lysine proteins identified. Mutants conditioning modified lysine are recessive and increased lysine is correlated with decreased seed size, although segregants are now available with normal seed size. The mutants have higher endosperm lysine in all protein solubility fractions but the largest shift occurred in the salt soluble globulin fraction. Some hydrophobic amino acids, particularly tyrosine were decreased in the mutant. The work shows that in-vitro selections produce complex but predictable phenotypes for lysine level. β-glucanases and endochitinases are expressed more actively in the mutant than the control.

R 538 RESTORAGE OF GENETICALLY ENGINEERED MALE STERILITY BY ANTISENSE mRNA, Thomas Schmülling, Angelo Spena and Jeff Schell, Max-Planck-Institut für Züchtungsforschung, D-5000 Cologne 30, FRG.
Expression of the *rolC* gene of the TL-DNA of *Agrobacterium rhizogenes* A4 under the transcriptional control of the Cauliflower mosaic virus 35S promoter causes in transgenic tobacco plants an altered phenotype consisting of i) a strongly reduced apical dominance, ii) lanceolate leaves with a pale green appearance, due to a reduced pigment content, and iii) small flowers which are male sterile. These phenotypic traits of P_{35S}-*rolC* transgenic plants are inherited in a dominant fashion, cosegregating with the kanamycin resistant marker. Restorer lines have been built by expressing *rolC* antisense mRNA under the control of different promoters in tobacco plants. Restoration of male fertility was attempted by pollinating P_{35S}-*rolC* transgenic tobacco plants with pollen from plants expressing the *rolC* antisense mRNA. Results obtained in the progeny of such crosses will be presented.

R 539 CHARACTERIZATION OF SELF-INCOMPATIBILITY AND STYLE-SPECIFIC GENES OF PETUNIA, Thomas L. Sims, K. Reed Clark, John J. Okuley and Pamela D. Collins, Department of Molecular Genetics, Ohio State University, Columbus, OH 43210.

Self-incompatibility is a genetic barrier to inbreeding, governed by a single locus (the S-locus), and characterized by the ability to discriminate between self and non-self pollen tubes. To investigate the molecular basis of self-incompatibility, we have isolated cDNA and genomic clones for three S-alleles of the gametophytically self-incompatible species, Petunia hybrida. Analysis of these alleles demonstrates that their expression is style-specific and increases over the course of floral development, with the maximum increase in expression occurring during the transition from compatibility to self-incompatibility. DNA blot hybridization shows that Petunia S-locus genes are single copy and that distinct RFLP bands can be associated with each S-allele. DNA sequence analysis indicates that Petunia S-locus genes are homologous to those isolated from other Solanaceous species, with discrete blocks of sequence homology interupted by blocks of non-homology. To test whether these cloned sequences confer allelic specificity, we are using the S1 and the S3 alleles in experiments to transform both incompatible and compatible lines of Petunia. To identify additional genes that may be important in pollination interactions and for making comparisons of regulatory sequences governing style-specific gene expression, we have isolated random cDNA clones representing mRNAs preferentially expressed in styles. These genes are not homologous to our S-locus clones, hybridize to unique style mRNAs, are expressed in various developmental patterns, and show no RFLP differences among different S-genotypes.

R 540 ISOLATION OF A GENOMIC DNA CLONE FROM A MUTABLE ALLELE OF THE MAIZE *BRITTLE-1* LOCUS USING *SPM* SEQUENCES AS PROBES, Thomas D. Sullivan, Lisa I. Strelow and Oliver E. Nelson, Jr., Department of Genetics, University of Wisconsin, Madison, WI 53706 Mutations in the *Brittle-1* (*Bt*) gene result in decreased starch synthesis in the endosperm of maize kernels, but the enzymatic defect in plants homozygous for the recessive *bt* allele has not been definitively determined. To learn more about the function of the *Bt* locus, we have isolated DNA clones from the locus using the approach of Cone *et al.* for the cloning of mutable genes with *Spm* insertions (PNAS 83:9631, 1986). The methylation-sensitive restriction enzyme *SalI* was used to digest DNA from a *dSpm*-containing *bt-m* allele. A 4.2 kbp *Spm*-sequence containing fragment that is absent in wild-type revertants of *bt-m* and that segregates with the *bt-m* allele was enriched by glycerol gradient centrifugation, and a sub-genomic library was contructed in λZap. The library was probed with *Spm* sequences, and three clones were obtained that contain 4.2 kbp inserts with identical restriction maps. A 125 bp fragment that lacks *Spm* sequences was isolated from one of these genomic clones and used to probe a λgt11 endosperm cDNA library, and several positive clones were obtained. One of the cDNA clones was used to probe genomic southern blots of DNA from plants in families segregating for different *Bt* alleles, as well as from wild-type revertants of *bt-m*, and to probe northern blots of RNA from various *Bt* and *bt* alleles. These analyses indicate that the isolated genomic and cDNA clones are derived from the *Bt* locus. Further experiments, starting with the analysis of both the DNA and inferred amino acid sequence, should help to elucidate the enzymatic function of the *Bt* gene.

R 541 STUDY OF THE GENETIC LESION IN THE CHS MUTANT 'RED STAR': INVOLVEMENT OF THE CHS STRUCTURAL SEQUENCE IN THE REGULATION MECHANISM, Ingrid M. van der Meer, Maike Stam, Cees E. Spelt, Joseph N.M.Mol, Antoine R. Stuitje, Department of Genetics, Free University, Amsterdam.

In most plants flower pigments derive from the flavonoid biosynthesis pathway. The expression of chalcone synthase (CHS), the key enzyme of flavonoid synthesis in *Petunia hybrida*, is developmentally regulated in flower tissue and can be induced in other tissues under (UV) stress conditions. In *P. hybrida* one CHS mutant has extensively been studied, the flowers of which exhibit alternating white and red coloured sectors ('Red Star'). CHS protein and mRNA levels are strongly reduced in the white sectors as compared to red sectors or flowers from wild type varieties . Elucidation of the genetic lesion in this mutant can give insight in the regulation mechanism of CHS gene expression of *P. hybrida*. This mutation is also of interest because the same sectorization pattern was found when CHS expression was inhibited by antisense technology .To study the regulation mechanism of this mutation a chimeric gene, consisting of the CHS(A) gene of *P. hybrida* (cv. V30) in which the structural region was replaced by the chloroamphenicolacetyltransferase (CAT) structural sequence, was introduced in the 'Red Star' phenotype and its expression analysed. Surprisingly, the reporter gene driven by the CHS (V30) promoter was expressed in both red and white sectors, as analysed on enzymatic and RNA level. In contrast, primer extension experiments demonstrate that CHS gene(s) derived from different petunia varieties follow the same expression pattern (Star type) within the flower tissue as the authentic 'Red Star' CHS gene(s). Moreover, the CHS gene from *Petunia hybrida* V30 (of which the cloned CHS gene originates) also respond to the Star type regulation when crossed into the Red Star phenomenon. These results suggest that trans-acting regulatory gene(s) are involved in the 'Red Star' CHS mutant and that the cis-acting element(s) responding to the trans-acting factors may be located within the CHS structural sequence. New constructs have been made to test this hypothesis.

R 542 EXPRESSION OF LIPOXYGENASES IN BARLEY SEEDS. Jan R. v. Mechelen, Anneke C. Douma, André W. Schram, Freek Heidekamp en Betty E. Enger-Valk, Center for Phytotechnology RUL-TNO, Department of Molecular Plant Biotechnology TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.
Up to 5% of the dry weight of barley seeds consists of lipids, which are mainly stored in lipid bodies in aleurone and embryo. Upon germination, the lipid content decreases but for barley seeds the fate of this storage material is yet unknown. In principle, such compounds may be metabolized via two different pathways, namely a lipoxygenase (lox) and a ß-oxidation route. Therefore, our current interest is to study these pathways as well as their possible interrelation upon germination of seeds. The approach includes experiments for obtaining information on the synthesis of lox enzymes in seeds. Both localization of lox gene-products as well as regulation of expression upon germination are subjects of research.
As a first step of this study we have initiated experiments to clone lox-specific sequences from barley seeds. Both lox-cDNA fragments from pea and soybean as well as mixed synthetic oligonucleotides probes were used in southern- and northern blot experiments. The data indicate that different fragments hybridize with the mentioned probes indicating that barley seeds may contain various lox-genes. The cloning of these fragments as well as an analysis of cloned sequences are underway and the results of these experiments will be discussed.

R 543 EXPRESSION OF ABA-INDUCIBLE GENES IN IMBIBED EMBRYOS FROM DORMANT WHEAT SEEDS, M.K. Walker-Simmons, Craig F. Morris and Robert J. Anderberg, USDA-ARS, Washington State University, Pullman, WA 99164-6420

No consistent phenotypic characteristic associated with seed dormancy has yet been identified, and plant scientists who attempt to manipulate dormancy by selective breeding have seen only moderate success. Because of this difficulty major germination problems occur in wheat including preharvest sprouting.

Our research is aimed at obtaining molecular markers for cereal seed dormancy. Once identified, these molecular markers can be used to develop wheat cultivars more resistent to preharvest sprouting damage. We have established previously that the phytohormone, ABA, is an effective germination inhibitor of dormant seed embryos. Responsiveness to ABA is lost as grains lose dormancy. For the work presented in this report, we prepared a cDNA library from poly(A)$^+$RNA isolated from ABA-treated embryonic axes of highly dormant grain. cDNA clones associated with ABA-responsiveness and seed dormancy were selected by differential screening. Utilizing the clones we find that active accumulation of specific mRNAs occurs when dormant grain axes are imbibed in water. Many of the mRNAs are ABA-responsive. The temporal regulation of these mRNAs in dormant grain embryos during the early stages of imbibition will be described.

R 544 PHENYLALANINE-AMMONIA LYASE IN LOBLOLLY PINE, Ross Whetten, David O'Malley and Ronald R. Sederoff, Department of Forestry, North Carolina State University, Raleigh, NC 27695. Phenylalanine-ammonia lyase, the enzyme that catalyzes the first step of the phenylpropanoid pathway, has been purified to homogeneity from developing xylem of loblolly pine trees. The pine enzyme is similar in size and tetrameric structure to PAL isolated from a wide variety of other organisms. Kinetic studies suggest the presence of a single isozyme, in contrast to the multiple isoforms found in suspension cultures of angiosperm cells. We are raising polyclonal and monoclonal antisera, and obtaining protein sequence data from the purified enzyme. The next step will be isolation of cDNA clones and immunohistological determination of the cell types in which PAL is expressed in developing xylem.

R 545 APOPLASTIC EXPRESSION OF A YEAST-DERIVED INVERTASE IN TRANSGENIC PLANTS LEADS TO DRAMATIC CHANGES IN HABITUS AND GROWTH, Lothar Willmitzer, Antie von Schaeven and Uwe Sonnewald, Institut für Gebiologische Forschung Berlin GmbH, Ihnestr. 63, 1000 Berlin 33
Growth and development of a plant is dependent on the energy gained by fixing carbon dioxide. Primary places for photosynthesis are leaf and to a much lesser extent stem tissue, whereas other organs such as roots, seeds or tubers do not contribute to carbon assimilation but rather totally depend on supply by photosynthetically active organs. Thus there is a net flo of energy from photosynthetically active tissue (source) to photosynthetically inactive parts of the plant (sink).
Plant development is accompanied by continuous changes with respect to an organ representing a sink or a source. Essentially all plant organs at some stage act as a sink. During plant development however the relative sink strength of different organs changes.
Sink-source relationships are central with respect to crop yield.One possible enzymatic activity involved in regulating sink-source relationships is represented by invertase.
In order to get a first insight into the possible role of invertase we decided to express a yeast derived invertase in both sink and source tissue of transgenic plants. Furthermore we targetted the invertase into the apoplast . The constitutive expression of the yeast-derived invertase in both sink and source tissues of transgenic tobacco plants results in dramatic changes of these plants with respect to habitus, growth and the development of sin and source organs.

R 546 CONSTRUCTION OF A TOMATO YAC LIBRARY TO CLONE MALE STERILE GENES, Rod A. Wing and Sheila McCormick, Plant Gene Expression Center, United States Department of Agriculture, Agricultural Research Service, 800 Buchanan St., Albany, CA 94710

The ability to clone large DNA fragments in yeast artificial chromosomes (YAC) has allowed for the resolution gap between classical and molecular genetic maps to be filled. We will present molecular analysis of an expanding tomato-YAC library being constructed from megabase tomato DNA. Preliminary analysis of this library indicates an average insert size of 140 kb. A more detailed analysis of one clone containing 500 kb of DNA (a theoretical tomato genetic map unit) will be presented.

We plan to use this library to clone genes that are known to affect flower development such as male sterile genes. Chromosome 11 has 4 male sterile mutants (ms-3, ms-7, ms-12 and ms-14) mapped on it which are our target genes. The mutants are being interdigitated with the tomato RFLP map by linking chromosome 11 RFLPs to the ms mutants in F2 plants derived from self pollination of crosses between each male sterile mutant with L. pennellii. Once an RFLP is linked to a male sterile gene it will be used as a starting point to probe the tomato YAC library. Linkage analysis with ms-14 will be presented.

R 547 EARLY INTERACTIONS BETWEEN BACTERIAL AND FUNGAL PATHOGENS AND INDUCED HYPERSENSITIVITY IN ALFALFA AND TOBACCO. Nichole R. O'Neill, *C. Jacyn Baker, and James A. Saunders. Germplasm Quality and Enhancement Laboratory and *Microbiology and Plant Pathology Laboratory, USDA, ARS, Beltsville, MD 20705.
The bacteria-induced hypersensitive response in alfalfa and tobacco is characterized as a rapid, localized necrotic response to pathogenic bacteria. In whole plant tissues the interaction with an incompatible race of Colletotrichum trifolii is expressed as rapid necrotic flecking. We are investigating the nature of early interactions with compatible and incompatible bacteria and fungi and their association with defense gene activation and defense-related gene expression in suspension cells and whole plant tissues. The addition of bacteria which induce the hypersensitive response initiated a net uptake of extracellular H+ and net increase in extracellular K+. A transient and prolonged pH change was observed, and this response correlated with the evolution of active oxygen, determined by luminol-mediated chemiluminescense. Conductivity and bacterial cell death were also monitored. The physiological responses of various plant-bacteria combinations correlated with host-pathogen compatibility. The time course for changes in the activity of phenylalanine ammonia-lyase and chalcone synthase and their mRNAs related to the accumulation of alfalfa phytoalexins in whole plant tissues. These responses also correlated with differences between incompatible and compatible interactions.