CROP IMPROVEMENT VIA BIOTECHNOLOGY: AN INTERNATIONAL PERSPECTIVE

Organizers: Robert Fraley, Marc Van Montagu, James Peacock and Charles Arntzen

April 10-16, 1992

Plenary Sessions Page

Poster Sessions

New Developments in Gene Transfer Technology

Y 001 GENETIC ENGINEERING OF WHEAT THROUGH MICROPROJECTILE BOMBARDMENT USING IMMATURE ZYGOTIC EMBRYOS, Kutty K. Kartha, Ravindra N. Chibbar, Narender S. Nehra, Nick Leung, Karen Caswell, Monika Baga, Cliff S. Mallard and Lee Steinhauer. Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Place, Saskatoon, S7N 0W9, SK, Canada.

For development of a pragmatic genetic transformation system in wheat, the availability of an efficient tissue culture regeneration system and a successful gene delivery method are of paramount importance. We have used the high velocity microprojectile bombardment method of gene delivery into immature zygotic embryos with the aim of obtaining transgenic wheat. The choice of immature zygotic embryos as a recipient system was based on the establishment of a genotype independent plant regeneration system from such explants by manipulation of various tissue culture parameters.

The immature zygotic embryos were bombarded with the constructs containing *cat* and *gus* marker genes driven by cauliflower mosaic virus (CaMV35S) promoter for optimization of gene delivery parameters. The transient expression of DNA delivered into the scutellar tissue of immature zygotic embryos was confirmed by the enzyme assays. The comparison of DNA expression vectors with and without an alcohol dehydrogenase (*Adh1*) intron 1 cloned in between the CaMV35S promoter and the coding region of the marker genes indicated that the presence of the intron enhanced expression of the respective marker gene. However, the constructs containing the actin promoter and its first intron isolated from rice (McElroy and Wu, Cornell University, N.Y) gave several fold higher expression of marker genes in transient assays as compared to other promoter intron combinations tested.

To achieve stable transformation of wheat, the constructs were assembled with *hph* gene encoding hygromycin phosphotransferase (HPH) as a selectable marker gene. Using these constructs for bombardment of immature zygotic embryos, the hygromycin resistant calli were recovered at a frequency of more than 40% in the presence of 100 mg/l hygromycin. Five to ten percent of these calli exhibited HPH activity in enzyme assays. In some of the selected calli, the presence of an internal fragment of *hph* gene was confirmed by Southern hybridization. However, the attempts to regenerate plants from the selected calli were unsuccessful because of the prolonged culture period.

In order to overcome the problem of plant regeneration from selected calli, we have recently developed an improved plant regeneration system via somatic embryogenesis for a number of wheat genotypes. In this system the regeneration frequencies of 80-90% have been achieved in about 3 weeks with an average of 10-15 somatic embryos per explant. The system is being optimized for production of transgenic wheat using microprojectile bombardment. The results of these experiments will be presented.

New Developments in Gene Expression Technology

Y 002 UNDERSTANDING THE DIVERSITY OF EXPRESSION OF PAL GENES. Michael Bevan[1], Diane Shufflebottom[1], Robert Sablowski[1], Mei-Hing Yung[1], Alfredo Cersosimo[2], Keith Edwards[3], and Wolfgang Schuch[3]. [1]Cambridge Laboratory, Norwich, Norfolk, UK; [2] Instituto Sperimentale per la Viticoltura, Conegliano (Treviso), Italy; [3] ICI Seeds, Bracknell, Berkshire, UK.

The genes encoding enzymes of phenylpropanoid metabolism in higher plants are regulated at the transcriptional level in response to the numerous developmental and environmental cues that activate the synthesis of phenylpropanoids. The first enzyme in the committed pathway of phenylpropanoid metabolism is PAL (phenylalanine ammonia-lyase), which catalyses the deamination of phenylalanine to cinnamic acid. As it is the first enzyme in the pathway, it is likely that the genes encoding this enzyme are subject to more levels of control than the genes encoding more specialised enyme activities further down the pathway. We have investigated the expression of two memebers of a PAL gene family from *Phaseolus* in transgenic tobacco, potato and *Arabidopsis*. A minimal active promoter fragment of 400bp from a gPAL3 gene is expressed in petals, the endodermis and pith tissue as well as in the epidermis and trichomes. In contrast, a minimal promoter of 250bp from a gPAL3 gene was strongly expressed in xylem tissue, shoot and root apices, the gland cell of trichomes and in the epidermis. Both genes were induced in tissues surrounding wound sites and in response to fungal elicitor preparations. These findings demonstrated that there is a division of labour between members of the PAL gene family, with some common modes of expression and some specialised modes. We then investigated the molecular basis for the complex pattern of expression of the gPAL2 promoter. Extensive deletion studies indicated that the tissue specific patterns of expression were associated with distinct regions of the promoter; for example, a regions confering expression in xylem and petal tissues could be separated from sequences conferring expression in wound periderm. Specific DNA motifs in each of these domains have been identified by methylation interference and EMSA experiments using nuclear protein extracts from developing woody stems, floral tissue and wounded potato tuber tissue. Sequence motifs conferring expression in wound periderm are found in chalcone synthase (CHS) promoters, where transient expression studies have shown that the same sequences are involved in elicitor- and light- mediated expression of CHS genes. A putative motif involved in petal-specific expression is a G box also shown to be important in elicitor and light regulated expression of a CHS gene in cell cultures. Sequences responsible for conferring expression in xylem cells have not been characterised previously. Future work aims to isolate the transcription factors involved in the signal transduction pathways leading to PAL gene activation.

Y 003 CHARACTERIZATION AND CLASSIFICATION OF PLANT G-BOX BINDING FACTORS, Takeshi Izawa, Randy Foster, Mary E. Williams, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, York Avenue 1230, New York, NY 10021

The G-box motif, CACGTG, is a highly conserved DNA sequence that has been identified in the 5' upstream region of several plant genes exhibiting regulation by a variety of environmental signals and physiological cues;i.e. light, the phytohormone abscissic acid (ABA), anaerobic stress, osmotic stress, and the desiccation of seed during normal embryonic development. Protein/DNA binding studies have illustrated that the G-box motif interacts with G-box binding proteins and that nucleotides outside the hexameric CACGTG core are important for binding. To investigate the role of sequences flanking the G-box motif in binding, we examined the binding activity of a panel of palindromic G-box oligonucleotides differing in their flanking sequences. Gel shift mobility assays employing cauliflower, tobacco and Arabidopsis extracts identified two conserved types of G-box binding activity, types A and B, which exhibit different mobilities. Type B protein factors interacted strongly with class II G-box oligonucleotides and weakly with class I elements while type A factors interacted with class I elements only under our assay conditions. The classification of G-box binding activity was used to predict the binding activity of all reported consensus plant G-box motifs. The binding activity classification was consistent with functional organization of some G-box containing genes;i.e. *rbcS* and *chs* gene families. Several different plant cDNAs have been isolated which encode bZIP proteins that interact specifically with DNA sequences containing the G-box motif. To investigate whether the binding activity of the bZIP proteins obeyed our classification rules, we examined the binding specificity of E.coli-expressed protein products of these bZIP cDNAs using our panel of palindromic G-box oligonucleotides. Our results have demonstrated that certain bZIP proteins exhibit a binding specificity similar to that of the type A factors. These results strongly suggest that type A activity may be partly derived from homologs of these cDNAs *in vivo*. Protein/DNA binding analysis allowed the classification of plant bZIP proteins according to their binding specificities. Physiological relevance of transcriptional factor binding specificity will be discussed.

Progress on Identifying New Multi-Genic Traits For Crop Improvement

Y 004 DEVELOPING A STRATEGY FOR CLONING GENES CONTROLLING VARIATION OF TRAITS WITH COMPLEX INHERITANCE, Tim Helentjaris, Rod Winkler, & Mary Ann F. Cushman, Dept. of Plant Sciences, University of Arizona, Tucson, Arizona.

Many of the most important traits in agriculture are controlled by the actions of multiple genes. Previous strategies to analyze the numbers and locations of these genes have relied upon statistical inference from data obtained from segregating populations. The use of molecular markers, in particular RFLPs, has provided a new tool that has allowed the systematic assay of individual chromosomal regions for the presence of genes with major effects upon phenotypic variation. Studies to date in corn have involved the analysis of many different traits, such as height, maturity, and even yield, and have determined a number of chromosomal regions which appear to contain genes with effective factors for these agronomically-important traits. Interestingly, many of these regions seem to be important across different genetic backgrounds and environments, suggesting they may be worth studying from a molecular genetic perspective. We are attempting to develop strategies that facilitate the further characterization of the gene(s) within these important regions, with the goal of providing to researchers a means for isolating, cloning, and perhaps even engineering genes involved in quantitative traits.

Our strategy is based in part upon the ideas of Robertson[1], who proposed that quantitative and qualitative variation might both represent different alleles of the same loci. If true, this might facilitate the cloning of a gene which is involved in quantitative variation by allowing the researcher to target an extreme variant which is more easily recognized. Initial results in maize suggest tantalizing positional correlations between quantitative trait loci (QTLs) and similar but more extreme mutant phenotypes. We have chosen plant height as our model system, as there are a number of well-defined height mutants in maize, which are amenable to both genetic and biochemical analysis. We have previously noted correlations between height QTLs and mapped height mutants. As the position of these QTLs is only approximately defined, other approaches are required to confirm any correlations such that a serious cloning effort could be justified. In one approach we are converting quantitative variation to qualitative variation at the target loci by using near-isogenic lines (NILs). Development of lines covering all areas of the genome is in progress; in the meantime we are using *waxy*-conversion lines to study a correlation between a height QTL and a GA-biosynthetic dwarf, both near the centromere of chromosome 9. High resolution mapping is being used to better define the position of both factors and to determine the number of genes which underlie the QTL. Treatment of these lines and their original recipient parents with exogenous GA is being used to determine if the QTL is controlling height variation by limiting the level of endogenous GA within these inbreds. Variation in GA response of these conversions, when compared to values for the original lines, may elucidate the function of this locus. Efforts are also underway to clone this and other candidate genes in maize that might be involved in controlling quantitative variation, both by transposon-tagging in maize as well as by finding maize homologues for genes cloned in other species.

---

[1] Robertson, D. S. 1985. A Possible Technique for Isolating Genic DNA for Quantitative Traits in Plants. *J. Theor. Biol.* 117:1-10.

Y 005 INTROGRESSION OF AGRICULTURALLY VALUABLE POLYGENIC TRAITS FROM WILD PLANTS, Andrew H. Paterson[1,2] and Rod A. Wing[1], [1]Texas A&M University, Department of Soil and Crop Sciences, Faculty of Genetics, and Faculty of Plant Physiology and Biotechnology, College Station, TX 77843-2474, [2]University of Delaware, Department of Plant and Soil Sciences, Newark, DE 19711.

Wild and feral relatives of cultivated plants harbor many traits of potential value for crop improvement. While classical plant breeders are well aware of this valuable resource, several difficulties have hindered exploitation of these traits in crop improvement. This has been particularly true of polygenic traits, influenced by many genes with small effects which are difficult to discern. The use of restriction fragment length polymorphisms (RFLPs) and related DNA markers provides the means for accessing this valuable reservoir of genetic variation. Of particular value are mapping and characterization of individual genes influencing complex traits, identification of rare recombinations between desirable and undesirable traits, development of high-resolution genetic maps near genes of interest, and map-based cloning of genes influencing agriculturally important phenotypes. This repertoire of technologies will facilitate classical breeding objectives which were previously difficult, and open new avenues of research into the genetic basis of crop productivity.

Genes and Gene Expression for New Traits - Pest Control

Y 006 FIELD PERFORMANCE OF INSECT RESISTANT CROPS, David A. Fischhoff, Monsanto Co., St. Louis.

Insect resistant crops demonstrating excellent field control of important agronomic pests have been developed by optimizing the expression of insecticidal protein genes from Bacillus thuringiensis (B.t.) in plants. Cotton plants resistant to lepidopteran insects and potatoes resistant to Colorado potato beetle have been the primary focus to date. As previously reported, initial expression of wild type B.t. genes in plants was poor and did not provide commercial levels of insect control in the field. B.t. expression was optimized by significant modification of B.t. structural gene coding sequences, which has led to up to 1000-fold increases in plant expression of these genes. This approach has been successfully applied to at least four distinct types of B.t. genes. Plants expressing the higher levels of B.t. proteins obtained with these modified genes have been field tested and show commercial levels of insect control.

Cotton plants expressing lepidopteran active B.t. genes from B.t. var. kurstaki were tested in the field in 1990 and 1991 at six locations across the U.S. cotton belt. At five of these locations the plants were subjected to either natural or artificial infestation by cotton bollworm and tobacco budworm (the Heliothis complex), the primary lepidopteran pests of U.S. cotton. At one location (Arizona) the plants were infested with pink bollworm, the primary lepidopteran pest in the Southwest. Plants were assessed for damage to squares and bolls throughout the season and for yield at harvest. Comparisons were made to cotton plants treated with a heavier than normal application of pyrethroid chemical insecticide, the current standard for lepidopteran control. In all locations in both years, the B.t. cotton plants performed as well as or better than control plants treated with pyrethroids when assessed for either damage or yield.

Given the commercial level of performance of B.t. cotton plants in the field, future field tests will focus on two issues. First, a total package of integrated pest management for insect pests in cotton centered around B.t. cotton will be developed. Second, appropriate insect resistance management strategies to delay or prevent the potential development of insect resistance to B.t. will be developed and implemented. The details of this resistance management strategy for B.t. cotton, including the identification and expression of a second insecticidal B.t. gene in cotton, will be discussed.

A second example of the production and development of insect resistant crops is Russet Burbank potato plants resistant to Colorado potato beetle through expression of a gene from B.t. var. tenebrionis These plants were field tested in multiple locations in the U.S. in 1991 and showed essentially complete control of Colorado potato damage in all locations. This control typically exceeded that provided by standard chemical insecticide treatments. In greenhouse and field tests B.t. potato plants controlled damage by both larvae and adult beetles and significantly reduced oviposition by adults, providing a premium level of beetle control.

Y 007 GENES IN PLANT-FUNGUS INTERACTIONS AND THEIR POTENTIAL USE IN CROP PROTECTION, Pappachan E. Kolattukudy[1], Royce Mohan[1], Bruce Sherf[1], [1]The Ohio State University Biotechnology Center, 206 Rightmire Hall, 1060 Carmack Road, Columbus, Ohio 43210.

Fungal attack of plants involves mutual triggering of gene expression. As soon as fungal spores contact with the plant surface, they begin to sense the environment using physical and chemical cues from the plant surface. Chemical components from avocado wax, for example, trigger germination of the fungal spore and differentiation into appressorium required for pathogenesis. A unique set of fungal genes are turned on by the plant surface components. Other plant signals trigger expression of fungal genes that allow the infection pegs to penetrate through the cuticular and wall barriers. The molecular mechanisms involved in the transcriptional regulation of the fungal genes by the plant signals are being elucidated. These early steps essential for infection are open for intervention to prevent infection of plants.

Fungal signals trigger expression of plant defense genes. Among them are genes involved in reinforcing cell walls to retard or prevent fungal ingress. One such mechanism involves deposition and cross linking of phenolics on the cell wall and further deposition of aliphatic polyester domains to complete suberization. A highly anionic peroxidase was found to be involved in the the deposition of phenolics on the wall. Two highly homologous genes, arranged in tandem in the tomato genome, were found to be coding for the anionic peroxidases. Transgenic tobacco plants containing the peroxidase promoter fused to GUS showed GUS expression only when induced by wounding or by fungal attack. Timely expression of this gene was found to be associated with resistance of a tomato line to *Verticillium albo-atrum* whereas a near isogenic tomato line susceptible to this fungus could not respond in this manner. When a construct that codes for antisense transcripts for both anionic peroxidase genes was introduced into tomato with a strong constitutive promoter, the transgenic plants accumulated antisense transcripts. Neither fungal attack nor abscisic acid caused production of the normal anionic peroxidase transcripts in the transgenic tomato plants whereas such treatments caused accumulation of transcripts in the nontransformed controls. These transgenic tomato plants were found to be susceptible to *V. albo-atrum* whereas the untransformed controls were resistant. The conclusion from these results that the anionic peroxidase is involved in fungal resistance was supported by the finding that transgenic tobacco plants constitutively expressing the tomato anionic peroxidase showed resistance to fungal attack. The effect of constitutive expression of the anionic peroxidase in other crop plants will also be discussed.

Y 008 THE MOLECULAR BIOLOGY OF SYSTEMIC ACQUIRED RESISTANCE. John Ryals[1], Eric Ward[1], Scott Uknes[1], Kay Lawton[1], Tom Gaffney[1], Danny Alexander[2], Robert Goodman[3], Jean-Pierre Métraux[4], Helmut Kessmann[5], and Patricia Ahl Goy[5]. [1]Agricultural Biotechnology, CIBA-GEIGY Corp., Research Triangle Park, NC; [2]Calgene, Inc., Davis, CA; [3]University of Wisconsin, Madison, WI.; [4]Université de Fribourg, Fribourg, Switzerland; [5]CIBA-GEIGY Limited, Basel, Switzerland.

Many plant species can be immunized to subsequent infection following an initial inoculation by a necrotizing pathogen. This acquired disease resistance was first documented in 1901 and is thought to "play an important role in the preservation of plants in nature" [Chester KS (1933) Quarterly Reviews of Biology 8: 275-234]. Particularly well characterized examples of plant immunity are the phenomenon of systemic acquired resistance (SAR) in tobacco [Ross AF (1961) Virology 14: 340-358] and induced resistance in cucumber [Kuc J (1982) Bioscience 32: 854-860]. In these systems, inoculation by a necrotizing pathogen results in systemic immunization to subsequent infections by a number of agronomically important bacterial, fungal and viral pathogens. In order to better understand this phenomenon, we have initiated the characterization of molecular and biochemical events associated with the induction and maintenence of the induced resistant state in tobacco, cucumber and *Arabidopsis thaliana*.

In tobacco, nine SAR-gene families are coordinately induced in leaves after treatment in tobacco mosaic virus (TMV), salicylic acid (SA) or methyl-2,6-dichloroisonicotinic acid (INA), a synthetic plant immunomodulator. The timing and amount of RNA expression correlates well with the onset and degree of resistance to further TMV infection. Each of the SAR-genes has been expressed at high levels in transgenic tobacco independently and in pairs. Several of the genes and gene combinations are resistant to various pathogens. Salicylic acid has been proposed as a putative endogenous signal molecule that mediates SAR in tobacco and cucumber. To test this hypothesis, plants were transformed with the gene encoding salicylate hydroxylase from *Pseudomonas putida*. This enzyme is capable of converting SA to catechol, a compound with no immunomodulating activity.

In cucumber, two types of biochemical markers are associated with the onset of resistance. One of these is the class III chitinase. The gene encoding this enzyme has been isolated and its developmental and environmental regulation has been characterized. Progress toward the identification of trans-acting factor binding sites will be discussed.

We have established an induced resistance system in *Arabidopsis thaliana*. In an attempt to develop a genetic approach to understand immunity, INA was used to induce resistance to both *Pseudomonas syringae* and *Peronospora parasitica*. Three molecular and biochemical markers associated with the onset of resistance have been identified and characterized. A genetic screen for the isolation of mutants in the SAR pathway will be discussed.

Genes and Gene Expression for New Traits-Food Quality-I

Y 009 MODIFICATION OF CARBOHYDRATE COMPOSITION IN PLANTS, G. Kishore[1], D. Stark[1], G. Barry[1], and J. Preiss[2], [1]Monsanto Agricultural Company, 700, Chesterfield Village Parkway, Chesterfield, Mo 63198, [2] Department of Biochemistry, Michigan State University, East Lansing, MI 48824

Regulation of starch metabolism in crops such as potato and corn is of significant scientific and commercial interest. In order to identify the rate limiting step in starch biosynthesis, we used the genes involved in glycogen biosynthesis in E. coli. The E. coli glgc16 gene was used as a source of the deregulated ADP glucose pyrophosphorylase (ADPGPP). In E. coli, expression of this gene is known to increase the rate and extent of glycogen accumulation compared to cells expressing the wild type glgC gene. To target the glgC16 gene product to amyloplast, we fused the translation initiation site of this gene to the nucleotide sequence encoding the transit peptide of a modified Arabidopsis small subunit of RUBPCase. The fusion protein was demonstrated to be imported and processed by isolated chloroplasts under *in vitro* conditions. Transient expression of this fusion gene in tobacco TxD cells resulted in production of catalytically active ADPGPP protein. Stable expression of the fusion gene in tobacco and tomato cells resulted in elevation of starch content by nearly 8 fold. Potato plants expressing the gene under the control of patatin promoter displayed an elevation in dry matter and starch content of the tuber. These experiments firmly illustrate that ADPGPP catalyzes the rate determining step in starch biosynthesis in plants and that levels of starch in plant cells is regulated by the activity of this enzyme.

Technology Transfer to Developing Countries

Y 010 ADAPTING COAT PROTEIN MEDIATED RESISTANCE FOR VIRUS DISEASES OF RICE AND CASSAVA, R.N. Beachy, P. Shen, R. Qu, A. deKochko, A. Sangare, C. Schöpke, L. Li, and C. Fauquet, Division of Plant Biology, The Scripps Research Institute, La Jolla, CA 92037.

Several years ago we initiated a research program to develop strategies to confer virus disease resistance in several crops that are important in developing countries, e.g. rice and cassava. The targets selected were rice tungro disease (RTV), African cassava mosaic geminivirus (ACMV), and cassava common mosaic potexvirus (CCMV). We have fully characterized by nucleotide sequencing the genomes of the two agents that are responsible for RTV; RTSpherical Virus contains a ss, (+)-sense genome of > 12 kb in size, and apparently belongs to a new group of plant viruses; RTBacilliform Virus contains ds, circular DNA, is similar in some ways to cauliflower mosaic virus, and has been grouped with newly named badnaviruses. ACMV and CCMV have been characterized by other research groups. We have constructed a variety of chimeric genes encoding various viral genes for expression in transgenic rice and cassava plants.

We are currently developing strategies for the transformation and regeneration of cassava, *Manehot esculenta*, a member of the family Euphorbiacae. Both *Agrobacterium* mediated, and particle bombardment mediated transformation are each being attempted on plant tissues that are, separately, capable of regenerating intact plants. To date we have selected tissues that are transgenic but are not, as yet, regenerated to produce whole plants. Our progress to develop transgenic *indica* and *japonica* rice cultivars that express CP genes of the agents of rice tungro disease has been more successful and will be described in this presentation.

Y 011 ISSUES SURROUNDING TECHNOLOGY TRANSFER TO DEVELOPING COUNTRIES, Clive James, Acting Executive Director of the International Service for the Acquisition of Agri-biotech Application (ISAAA), Lisboa 27, A.P. 6-641, 06 600 Mexico D.F., Mexico.

In the past, developing countries have had the privilege of accessing non-proprietary traditional technology freely from the public sector. Biotech is changing this situation because it is increasingly proprietary, owned primarily by private corporations in industrial countries To assist developing countries in the acquisition of proprietary biotech applications, a new organization, ISAAA, has been created to facilitate the sharing and transfer of proprietary agricultural biotech applications from the industrial countries, particularly from the private sector, for the benefit of the Third World. ISAAA is designed to be a small, responsive, non-bureaucratic, international network with 10 professional staff at three *Center*Nodes co-located at centers of excellence in North America (*Ameri*Center), Europe (*Euro*Center) and the Asian Pacific Rim (*Asia*Center), to monitor and evaluate availability of proprietary biotech for transfer to the Third World; similarly, three *Network*Nodes will be co-located in Africa (*Afri*Net), Asia (*Asia*Net) and Latin America (*Lati*Net) to assist national programs to identify priority needs that lend themselves for resolution through biotech applications. ISAAA has comparative advantage in providing unique services cost-effectively because its international activities operate at the crossroads of technology donors, recipients, and funding agencies. It is the only institution serving agricultural biotech in the Third World co-sponsored by the public and private sectors with funding from Europe, North America and the Asian Pacific Rim, through private foundations, bilateral/multilateral agencies and private corporations. ISAAA permits both the public and private sectors to work together as true partners in an international biotech program for the benefit of the Third World. The strategy which ISAAA pursues to assist the national programs in the Third World is: Focus on Near-Term Applications which have been tested in industrial countries and have high probabilities of success. These applications will demonstrate potential benefits and constraints of the technology; Emphasize Applications to Increase the Productivity of Food Crops and Contribute to a Safer Environment particularly non-commercial food crops grown by poor farmers, and make a contribution to income and the environment/sustainability through the development of alternatives to the use of toxic conventional pesticides; Concentrate on Three Classes of Plant Biotechnology Applications i.e. tissue culture, diagnostics, and transgenic plants, and assign high priority to horticulture (vegetables and fruit) and to forestry. Although ISAAA has only operated for a short period of time, two projects have already been brokered and another 30 potential projects are at different stages of development. This demonstrates the feasibility of sharing proprietary biotech applications with the Third World. The paper will share the ISAAA experience in technology transfer by specifically addressing the issues related to institutional and technical aspects, particularly those related to transgenic plants.

Genes and Gene Expression for New Traits-Food Quality-II

Y 012 GENETIC ENGINEERING OF OIL COMPOSITION IN RAPESEED, Vic C. Knauf Calgene, Inc., Davis, CA 95616.

Oil composition is largely determined by the kinds of fatty acids synthesized during triacylglycerol synthesis in developing seeds. The cloning of genes encoding fatty acid synthesis enzymes of *Brassica campestris*, *Carthamus tinctorius*, *Ricinus communis*, *Umbellaria californica*, *and Simmonsia chinensis* has allowed the directed modification of oil composition in transgenic *Brassica napus* rapeseed. The genes used in sense or in antisense configurations include stearoyl-ACP desaturase, β-ketoacyl-ACP synthase I, β-ketoacyl-ACP synthase II, and lauroyl-ACP thioesterase. This work demonstrates 1) the use of antisense gene control to lower an endogenous enzyme activity and thus redirect the profile of fatty acids made (high stearate rapeseed, currently in field trials); and 2) the introduction of novel enzyme activities into *B. napus* which results in novel fatty acids and oils (high laurate rapeseed). These applications require facile transformation of rapeseed and tissue-specific control of gene expression for initial success, but product development will also require field trials and a breeding program designed to generate well-adapted crop varieties.

Promising New Research Areas-I

Y 013 CHARACTERIZATION OF GENES THAT IMPROVE PROTEIN AND SEED QUALITY IN MAIZE, Brian A. Larkins, Mauricio A. Lopes, Koichi Takasaki, and Fumio Takaiwa, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

The *opaque*-2 mutation in maize reduces the synthesis of the lysine-poor storage proteins (zeins), resulting in a significant improvement in protein nutritional quality. But the value of using this mutation to improve maize protein quality is significantly diminished as a consequence of the soft, floury endosperm of the mutant kernels, which leads to lower kernel density and increased susceptibility to insects, pathogens, and mechanical damage. Recognition that in certain genetic backgrounds *opaque*-2 mutants have a harder, more vitreous phenotype led to the identification of *opaque*-2 "modifier" genes. In recent years, these genes have been used to develop Quality Protein Maize (QPM), which has both enhanced lysine content and excellent seed quality. Our recent studies have shown that QPM genotypes carry genetic factors that increase the synthesis of one particular type of storage protein, the gamma-zein. Although this protein contains no lysine, mechanisms associated with its increased synthesis appear to be responsible for doubling the lysine content of the seed, as well as increasing kernel hardness. We have used a combination of genetic and biochemical analyses to investigate the relationships between endosperm modification and gamma-zein content in QPM. The use of unmodified and modified mutants allowed us to demonstrate in reciprocal F1 hybrids and their F2 segregating progenies that the degree of vitreousness and increased synthesis of gamma-zein in modified endosperms are both dosage-dependent and directly correlated. We found that these same genetic factors are active in other soft endosperm mutants, such as *floury*-2, as well as in normal inbred lines. Increased synthesis of the gamma-zein protein correlates with enhanced amounts of mRNA. However, we do not yet have definitive evidence that this is a consequence of increased gene transcription. In the modified and unmodified genotypes that have been examined, there are one or two copies of gamma-zein genes; however, the amount of gamma-zein protein is not related to gene copy number. Using PCR technology, we cloned and sequenced the gamma-zein locus from one QPM variety. Comparison of these genes with those from non-modified genotypes did not reveal significant differences in nucleotide sequences. Experiments are in progress to characterize the regulatory regions controlling the transcription of these genes, and how their expression is altered through the action of "modifier" genes.

Y 014 APPLICATIONS OF SITE-SPECIFIC RECOMBINATION IN PLANT GENETIC ENGINEERING, David W. Ow, Emily C. Dale, Minmin Qin, Christopher Bayley and Michael Morgan, Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan Street, Albany, CA 94710.

Of the several simple site-specific recombination systems known, the Cre-lox system from bacteriophage P1 has been reported to catalyze intra- and intermolecular recombination in plant cells (Gene 91:79-85, 1990; MGG 223:369-378, 1990). Since then, we have examined the utility of this recombination system in a number of applications. For controlling gene expression, a site-specific deletion/fusion event was shown to exchange transcriptional activity from a promoter-proximal to a promoter-distal gene (PMB 18:353-362, 1992). For plant gene transfer, we have engineered selection-gene-free transgenic plants by the site-specific excision of lox flanked selectable markers followed by genetic segregation of the cre gene (PNAS 88:10558-10562, 1991). Removal of foreign sequences that are unnecessary should speed regulatory approval and consumer acceptance of transgenic plants. Another potential use for gene transfer is the site-specific integration of DNA into chromosomal lox sites, which might control position effects, permit the clustering of genetic information and enhance transformation efficiency. To overcome the reversibility of the Cre-lox reaction, which favors excision over integration events, mutant lox sites have been developed that are less reversible in the direction of recombination. Finally, by introducing lox sites in the same or in separate chromosomes, we have been investigating the use of this system to generate chromosomal deletions and translocations. These results along with current emphasis on chromosome engineering will be presented.

Genes and Gene Expression for New Traits-Yield Improvement

Y 015 COMMERCIALIZATION OF GLYPHOSATE HERBICIDE RESISTANT CROPS FOR IMPROVED WEED CONTROL, Gerard Barry, Ganesh Kishore, Stephen Padgette, Kathryn Kolacz, Diane Re, Marcia Weldon, Mary Taylor, Greg Parker, David Eichholtz, Maria Hayford, Harry Klee, Karen Fincher, Renee Rozman, Dan Didier, Xavier Delannay, David DeBoer, and Laurence Hallas, Monsanto Agricultural Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198

Roundup® herbicide is widely used as a post-emergent herbicide for the control of a wide range of weeds. The herbicide, while providing outstanding weed control, shows little selectivity on crop plants. This is primarily related to the fact that glyphosate, the active ingredient of the herbicide, inhibits the activity of the aromatic pathway enzyme EPSPS (enolpyruvylshikimate-3-phosphate synthase) of all plants to a similar extent.

Herbicide tolerance may be imparted to transgenic crops by employing two possible mechanisms: Target Site Modification - where the target of action of the herbicide is tolerant to inhibition, or Metabolic Inactivation - where the active ingredient is metabolized *in planta* to a non-herbicidal derivative. The former approach has been employed extensively and the factors that contribute to the success of this approach include the identification of glyphosate tolerant and efficient EPSPS enzymes and high level and uniform expression of the EPSPS gene in the crop. The realization of the former has been complicated by the decrease in EPSPS enzyme efficiency frequently observed in conjunction with tolerance to inhibition by glyphosate. A number of highly efficient EPSPS's that show normal EPSPS enzyme kinetics have been identified and their role in imparting *in planta* glyphosate tolerance will be presented. Glyphosate may be metabolized by two degradation routes and the details of these pathways have been elucidated in a number of microorganisms. The possibilities presented by these degradation routes in providing *in planta* glyphosate tolerance will be discussed.

Y 016 GENETIC ENGINEERING FOR FERTILITY CONTROL, Jan Leemans, Plant Genetic Systems N.V., Jozef Plateaustraat 22, 9000 Gent, Belgium

A dominant gene for male sterility

To engineer male sterility, we isolated the tobacco TA29 gene which is characterized by its extreme cell specificity in the tapetal cells of immature anthers. The 5' regulatory region of TA29 was used to target the expression of ribonucleases such as barnase, from *Bacillus amyloliquefaciens*, specifically to the tapetum of immature anthers. Barnase expression led to the precocious degeneration of the tapetum cells, the arrest of microspore development, and male sterility.

Fertility restoration

We have constructed genes which can restore fertility to the genetically engineered male sterility. These chimaeric genes consist of a tapetal specific promoter linked to the intracellular inhibitor of barnase, called barstar. When barnase and barstar are produced simultaneously in the same tapetal cells, they form a one to one complex which has no residual ribonuclease activity. Progeny of crosses between male sterile oilseed rape plants that express barnase and fertile pollinator plants that are homozygous for the barstar gene produce fully normal pollen and showed normal seed set upon selfing.

Maintaining male sterility

To maintain and multiply male sterile plants, the male sterility gene was linked to the herbicide resistance gene bar. The linkage of both genes allows the elimination of segregating fertile plants after backcrossing by herbicide treatment, thereby eliminating all herbicide sensitive, male fertile plants.

Applications

The new hybrid system has been successfully applied to various crops including corn, oilseed rape, Brassica vegetables and cotton. Field tests in N. America and Europe allowed to identify male sterile oilseed rape lines in which the sterility is stable under various environmental conditions and which show otherwise normal agronomic performances. The advantages of this system for F1 hybrid breeding and seed production in various crops will be discussed.

Y 017 GENES INVOLVED IN COLD ACCLIMATION, Michael F. Thomashow, Department of Crop and Soil Sciences, Michigan State University, East Lansing, MI 48824

Freezing temperature is an environmental factor that limits the geographical locations where crops can be grown and periodically accounts for severe crop loss. Many plants, however, have evolved mechanisms that enable them to acclimate to low nonfreezing temperature and in the process, become more freezing tolerant. This response is known as cold acclimation. Much effort has been directed at understanding the molecular basis of cold acclimation with the long term view of improving the freezing tolerance of agronomic plant species. Despite these efforts, the biochemical and genetic basis of freezing tolerance remains poorly understood.

Our primary interest is to understand better the genetics of cold acclimation. Previous studies have shown that cold acclimation is a quantitative trait and that changes in gene expression occur during the response. However, individual genes and/or gene products with roles in freezing tolerance have not been identified and the mechanism responsible for cold-regulated gene expression is unknown. To address these issues, we are studying cor (cold-regulated) gene expression in Arabidopsis, a plant that increases in freezing tolerance in response to low temperature. Studies to date have centered on four genes: cor160, cor47, cor15 and cor6.6. The transcript levels of these genes increase 10 fold or more in response to low temperature (4^{o}C). Nuclear run-on transcription assays indicate that cor15 is regulated largely at the transcriptional level while cor160, cor47 and cor6.6 are regulated primarily at the posttranscriptional level. All four genes are ABA responsive raising the question of whether ABA normally mediates the cold-regulated expression of cor genes. To address this issue, we examined the expression of cor160, cor47, and cor6.6 in the ABA-deficient (aba) and ABA-insensitive (abi) mutants of Arabidopsis isolated by Koornneef and colleagues. The data indicate that ABA-induced expression of all three genes was unaffected in the abi2, abi3, and aba-1 mutants, but was dramatically impaired in the abi1 mutant. Cold-regulated expression of these cor genes, however, was nearly the same in wild type and abi1 mutant plants. Thus, cold- and ABA-regulated expression of at least certain cor genes appears to be mediated through independent control mechanisms.

Studies directed at characterizing the polypeptides encoded by Arabidopsis cor genes have shown that many share the unusual property of remaining soluble upon boiling. Indeed, each of the Arabidopsis cor genes for which we have isolated a cDNA encodes a "boiling-stable" polypeptide. Consideration of these and other data has led us to speculate that the boiling-stable COR polypeptides might act as cryoprotectants. Consistent with this suggestion is the finding that one of the polypeptides, COR15, has cryoprotective activity in an in vitro assay. Whether COR15 acts as a cryoprotectant in planta remains to be determined. DNA sequence analysis of cDNA clones for cor47 and cor6.6 indicates that the former encodes a polypeptide that is related to Group II LEA (late-embryogenesis-abundant) proteins, polypeptides suggested to have roles in dehydration tolerance, while the latter encodes a polypeptide almost identical to KIN1, a cold-regulated Arabidopsis polypeptide reported to have sequence similarities with certain fish antifreeze proteins [Kurkela and Frank (1990) Plant Mol Biol 15:137]. The relationships between COR, LEA and antifreeze proteins and their potential roles in cold acclimation and water stress tolerance will be discussed.

New Developments in Gene Transfer Technology;
New Developments in Gene Transfer and Expression

Y 100 EXPRESSION OF CATALASE GENES IN SOYBEAN, Sibel H. Isin and Randy D. Allen, Department of Biological Sciences, and Department of Agronomy Horticulture and Entomology, Texas Tech University, Lubbock, TX 79409

Catalase is a peroxisomal enzyme that is responsible for scavenging hydrogen peroxide produced by oxidative reactions in this organelle. We are interested in understanding the factors that control the expression of plant catalase genes in response to developmental and environmental cues. We have isolated and characterized a soybean catalase gene that includes approximately 1.7 kb of 5' flanking sequence. Catalase coding sequences of this gene consist of 7 exons. The promoter and 5' flanking sequences of this gene have been fused to the ß-glucuronidase (GUS) gene and this reporter gene construct has been introduced into tobacco plants. Staining of leaf tissues of transgenic plants with x-gluc indicated that this catalase promoter sequence appears to direct GUS expression mainly in the vascular tissues. In order to begin to localize *cis*-acting transcriptional regulatory elements, 5' terminal deletions of the catalase upstream sequences were analyzed for transcriptional activity. Preliminary fluorometric assays suggest the presence of an activating sequence, necessary for maximal expression levels, between -604 and -269 bp from the cap site.

Y 101 **FUNCTIONAL ANALYSIS OF HETEROLOGOUS ANTHER/POLLEN- SPECIFIC PROMOTERS IN TRANSGENIC BRASSICA NAPUS** M. Arnoldo, C. Garnaat, L. Fallis, S. Rozakis, E.Barbour, C. Baszczynski, G. Huffman, B. Huang, R. Kemble. Allelix Crop Technologies, A Division of Pioneer Hi-bred Production Ltd., 6850 Goreway Dr., Mississauga, Ontario L4V 1P1 Canada.

The objective of this study was to test for the regulation of gene expression by heterologous anther- and pollen-specific promoters. Toward this end, constructs containing three anther-specific promoters (two from tobacco, one from maize) and one pollen-specific promoter (from tomato), fused to the GUS coding region, were introduced into B. napus via Agrobacterium-mediated transformation. At least 10 independent transgenic plants were produced for each vector, and were analyzed at different stages to determine tissue specificity and developmental regulation of GUS expression by the different promoters. The majority of the transgenic plants containing the dicot promoter - GUS constructs showed predicted tissue and cell specific expression based on the pattern of expression in the original host plant. Functionality of the maize anther-specific promoter in transgenic Brassica is being analyzed.

Y 102 GENETIC CONTROL OF THE *AGROBACTERIUM*-SOYBEAN INTERACTION, Matthew A. Bailey and Wayne A. Parrott. Department of Agronomy, University of Georgia, Athens, GA 30602.

Transformation of plants with *Agrobacterium tumefaciens* requires a compatible interaction between the bacterial strain and regenerable tissues of the host. Incompatibility between most soybean genotypes and *Agrobacterium* has limited the success of this mode of transformation. The objective of this study was to investigate genetic factors in soybean which influence susceptibility. First, a rapid assay was developed which consisted of wounding young, intact hypocotyls of several genotypes at multiple sites and inoculating with a fresh mucilage of the wild-type *Agrobacterium* strain A281. Seedlings were scored for gall frequency and size at 21 d. Second, the inheritance of host susceptibility was studied using the parents and F_2 generations of crosses between Peking, a susceptible genotype, and Century, a resistant genotype. The galling response of the F_2 population suggests that susceptibility of soybean to *Agrobacterium* is a quantitative trait. Heritabilities will be calculated by regressing F_3 progeny means on F_2 parental values. Finally, 30 soybean genotypes of agronomic importance were assayed for susceptibility. The results of these studies confirm that susceptibility to *Agrobacterium* in soybean is rare, but suggest that this limitation may be overcome through breeding and selection.

Y 103 ISOLATION AND CHARACTERIZATION OF A B. NAPUS MUTANT ALS GENE AND EXPRESSION IN TRANSGENIC PLANTS, Eric Barbour, Vigen Armavil, Bin Huang, Roger J. Kemble and Chris L. Baszczynski, Allelix Crop Technologies, A Division of Pioneer Hi-Bred Production Ltd., 6850 Goreway Drive, Mississauga, Ontario, Canada, L4V-1P1

Through microspore mutagenesis and selection we previously generated Brassica napus lines resistant to imidazolinone and sulfonylurea classes of herbicides (Swanson et al., 1989). Resistance at up to five times the normal field application levels was obtained in one line. Biochemical studies indicated that this line had altered acetolactate synthase (ALS) activity. Through screening of libraries and PCR amplification we obtained clones for four of the five ALS genes in this mutant line (PM2). Exhaustive sequencing of all four clones revealed only a single point mutation in the ALSIII gene resulting in a tryptophan to leucine substitution. We introduced this mutant gene into the binary vector pALLTKrep and transformed both B. napus and tobacco with this construct. Stable transformants showing high levels of herbicide resistance have been obtained in both species. In a number of cases the transgenics are showing resistance to 1000 times the levels which will kill normal explants in culture. Expression data comparing stable transgenics to our original mutant and wild type lines, both in culture and in vivo, will be presented.

Y 104 CEREAL SHOOT APICAL MERISTEMS AS A TRANSIENT GENE EXPRESSION SYSTEM

Roland Bilang, Shi-bo Zhang, John A. Simmonds*, Christof Sautter and Ingo Potrykus
Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland, and
*Plant Research Centre, Research Branch, Ottawa, Canada, K1A 0C6

The investigation of the expression of hybrid gene constructs in cereal meristems is difficult since the production of transgenic cereals is still rather unpredictable and inefficient. The ballistic micro-targeting system (Sautter et al., Biotechnology 9:1080 [1991]) proved to be suitable for delivering DNA-carrying micro-projectiles with high reliability and precision into shoot apical meristems of cereal seedlings. Gun parameters were optimized to direct particles into L1 and L2 cells without causing visible damage to the meristems. We present results of transient gene expression studies using meristems of various cereal species. This system should be useful to study patterns in plant development and presents a tissue culture-independent approach towards stable transformation of cereals.

Y 105 AN ANTHER CULTURE-DERIVED SYSTEM FOR TRANSFORMATION STUDIES IN WHEAT (*Triticum aestivum* L.) Yin-Fu Chang, Colleen Y. Warfield, Andrea A. Itano and James R. Wong. Sogetal Inc., 3876 Bay Center Place, Hayward, CA 94545, USA

A reliable tissue culture system is essential for plant transformation. In wheat, obtaining such a system is still difficult. We have routinely induced a friable and embryogenic type of callus from wheat anther cultures, and have established regenerable suspension cultures from such callus. This cell culture system, including the influence of genotype on the induction of friable callus from wheat anthers, will be discussed.

Y 106 AGROBACTERIUM-MEDIATED TRANSFORMATION OF ANTHURIUM AND REGENERATION OF TRANSGENIC PLANTS. Fure-Chyi Chen and Adelheid R. Kuehnle, Dept. of Horticulture University of Hawaii, 3190 Maile Way, Honolulu, HI 96822 USA

Transgenic Anthurium andraeanum plants expressing antibacterial genes have been produced using an Agrobacterium-mediated transformation system. Etiolated internodes of Univ. of Hawaii anthurium cultivar UH965 were cocultivated with Agrobacterium tumefaciens LBA4404, containing pCa2Att, pCa2P13 or pCa2T4. These vectors carry the genes for the antibacterial peptides attacin, P13 and T4 lysozymes, respectively. They also contain the selectable marker gene NPTII, which encodes kanamycin resistance, and the reporter gene GusA, which encodes ß-glucuronidase. Plantlets were regenerated in the presence of kanamycin 6 to 12 months following cocultivation of etiolated internodes. De novo callus cultures from leaves of kanamycin-resistant plantlets were established on kanamycin-containing medium. PCR analysis of DNA from callus and leaves confirmed the presence of antibacterial genes and NPTII and GusA genes. Expression of the attacin peptide was detected in the de novo callus by Western blot analysis. Several dozen transgenic anthuriums have been transplanted to potting mix for the test of antibacterial activity against Xanthomonas campestris pv. dieffenbachia, a severe bacterial pathogen of anthurium.

Acknowledgements: Thanks to Dr. J. Jaynes, Louisiana State University, Baton Rouge, for the gift of the vectors. This research was supported by the State of Hawaii Governor's Agriculture Coordinating Committee GACC Contract No. 89-22.

Y 107 A COMPARATIVE STUDY OF DIFFERENT PROMOTERS ON THE EXPRESSION OF *gus* IN CULTURED BARLEY CELLS. R.N.Chibbar, K.K.Kartha, R.S.S.Datla, N.Leung, J.Lim, K.Caswell, C.S.Mallard, M.Baga, W.L.Crosby, N.S.Nehra and L.Steinhauer. Plant Biotechnology Institute, National Research Council Canada, 110 Gymnasium Place, Saskatoon, Saskatchewan S7N 3S6, CANADA.

We have studied the efficiency of the Cauliflower mosaic virus 35S (CaMV35S), enhanced CaMV35S, maize alcoholdehydrogenase1 (*Adh1*) and rice actin (*Act1*) promoters to express *gus* marker gene in cultured barley cells. In the case of CaMV35S and enhanced CaMV35S, the maize *Adh1* intron 1 and shrunken locus (*sh*1) intron 1, were inserted between the promoter and the 5' end of the coding region of *gus*. In the constructs where *gus* was controlled by *Adh*1 or *Act*1 promoter, a DNA fragment carrying the promoter region and the first intron of the corresponding gene was inserted in front of *gus*. The plasmids were introduced into cultured barley cells using the particle gun. GUS activity was monitored 48 hours post-bombardment by histochemical and fluorimetric assays. Results showed that *Act*1 promoter with its first intron gave several-fold higher expression than any other promoter / intron combination. The level of expression from *Act1* promoter construct was followed by the enhanced CaMV35S promoter and no significant difference could be observed between the CaMV35S and *Adh*1 promoters. All the latter constructs carried the *Adh*1 intron 1.

Acknowledgements: Gene constructions with rice actin promoter and maize *Adh 1* intron 1 were kindly provided by Drs McElroy and Wu, Cornell University and Dr M.Fromm (Monsanto), respectively. Maize shrunken locus1 intron 1 was a gift from Dr L.C.Hannah, Univ. of Florida, Gainesville.

Y 108 MUTATIONS IN THE CRE/LOX RECOMBINATION SITE ENHANCE THE STABILITY OF RECOMBINATION PRODUCTS; APPLICATIONS FOR GENE TARGETING IN PLANTS. Emily C. Dale and David W. Ow. Plant Gene Expression Center, USDA/U.C. Berkeley, Albany, CA 94710.

We are investigating the use of a site-specific recombination system to promote DNA rearrangements in plants. The Cre/lox site-specific recombination system derived from bacteriophage P1 has been shown to mediate both intra- and intermolecular recombination events in plant cells[1,2]. Cre-mediated intramolecular recombination between two *lox* sites can result in either inversion or excision of the intervening sequence. We have previously reported the use of site-specific excision to remove DNA segments from the tobacco genome, including the removal of the selectable marker used to detect transgenic plants [3, 4].

Our current efforts are directed toward the development of Cre/lox intermolecular recombination to yield positional targeting (integration) of DNAs into the plant genome. Recombination between a *lox* site present in the plant genome and a second *lox*-containing plasmid would lead to the integration of this circular DNA construct into a specific site within the genome. This intermolecular event results in the physical linkage of two *lox* sites, thus the product of the integration event can be readily resolved by the highly favored intramolecular excision event. To circumvent this problem, we have introduced nucleotide changes into the *lox* sequence. We show that appropriate pairs of *lox* sites are able to recombine, yet the efficiency of recombination between the resulting product *lox* sites is greatly reduced. In this manner we have been able to introduce a directionality into the Cre/lox system. Constructs containing the altered *lox* sites have been introduced into plants and the results of our efforts towards gene targeting in plants will be presented.

1. Dale, E.C. and Ow, D.W. (1990) *Gene* 91:79-85.
2. Odell, J.T., Caimi, P.G., Sauer, B. and Russell, S.H. (1990) *Mol. Gen. Genet.* 223:369-378.
3. Dale, E.C. and Ow, D.W. (1991) *PNAS* 88: 10558-10562.
4. Bayley, C.C., Morgan, M., Dale, E.C. and Ow, D.W. (1992) *Plant Mol. Biol.* 18: 353-362.

Y 109 SOMATIC HYBRIDIZATION IN NICOTIANA: INTERSPECIFIC GENE TRANSFER BETWEEN N. TABACUM L. AND WILD NICOTIANA SPP. USING DUAL-ANTIBIOTIC SELECTION

Pauline A. Donaldson[1], Eric E. Bevis[1], Radhey S. Pandeya[2], and Stephen C. Gleddie[2]
[1]Imperial Tobacco Ltd. PO Box 6500, Montreal, Quebec H3C 3L6 Canada [2]Plant Research Centre, Agriculture Canada, Ottawa, Ontario K1A OC6 Canada

Somatic hybridizations between Nicotiana tabacum L. cultivar Delgold and several wild Nicotiana species were conducted using dual-antibiotic resistance for selection of hybrid calli. The objective was genetic improvement of tobacco by introduction of important traits such as resistance to nematodes and black root rot and blue mold pathogens (to reduce dependency on pesticides) and altered biochemistry (flavor components and alkaloid content) which are found in wild relatives of tobacco. The wild species are reproductively isolated from tobacco and the traits are not genetically charaterized. Thus somatic hybridization was used to produce hybrids which can be used as genetic bridges to transfer these traits to tobacco.

We recently described a successful heterokaryon selection system based on dual antibiotic resistance which led to the recovery of somatic hybrids between methotrexate resistant N. tabacum and kanamycin resistant N. debneyi (Sproule et al. TAG(1991)82:450-456). This approach was used for hybridization of tobacco with each of N. rustica var. NRT, N. glutinosa var. yellow, and N. sylvestris. Fertile plants, regenerated from double-resistant calli, were readily obtained from protoplast fusions with each of these wild species (and fusions with N. megalosiphon are in progress). Isoenzyme, RFLP, and morphological data demonstrate the presence of a hybrid nuclear genome in all of the somatic hybrid plants. Probing with sequences specific to mt- and cp- DNA indicated variation in the inheritance of cytoplasmic organelles among individual hybrids. Thus, the selected hybrid plants carry, in addition to the parental antibiotic markers, other nuclear encoded genes and a variety of cytoplasmic genomes which can be transferred to the tobacco parent by backcrosses.

Y 110 EFFICIENT PRODUCTION OF TRANSGENIC FLAX PLANTS FROM HYPOCOTYLS USING *AGROBACTERIUM TUMEFACIENS*, Jin-Zhuo Dong and Alan McHughen, Crop Development Centre, University of Saskatchewan, Saskatoon, S7N OWO, Canada. Flax (*Linum usitatissimum*) is an important oilseed crop as well as a source of natural fibres (linen). Flax is also suitable for molecular genetic studies as it has one of the smallest genomes of any crop species. In order to increase the efficiency of recovery of transgenic plants, we investigated the *Agrobacterium*-mediated transformation of flax hypocotyl tissue using a binary plasmid with an intron-containing β-glucuronidase (GUS) gene (designated p35SGUSINT) as a reporter, along with neomycin phosphotransferase-II (NPT-II) gene for kanamycin resistance selection, in the disarmed octopine type *Agrobacterium* strain GV2260. Regenerant shoots were scored transgenic based on a histochemical GUS assay and/or Southern blotting. Approximately 9% of inoculated hypocotyls gave rise to transgenic shoots using the best procedure. The combination of hypocotyl preculture (prior to *Agrobacterium* inoculation), removal of epidermal strips from the hypocotyl, and prolonged cocultivation duration greatly improved the intensity of cellular transformation in the hypocotyls. This, in turn, resulted in a greater recovery of transgenic shoots when regeneration was induced. Chimeric shoots (composed of transgenic and non-transgenic cell lines) accounted for about 40% of regenerants according to GUS assays. Our observations show that shoots can and do have a multicellular origin, and that transformed cells can protect nearby non-transformed cells from the selection agent (in this case, kanamycin). Also, chimeras could arise from two or more transgenic cell lines; such chimeras could explain the unusual expression patterns or progeny segregation ratios sometimes recorded from transgenic regenerants. Solid transgenic plants can be recovered from chimeras by: internode propagation, shoot induction in vitro from leaf or stem segments composed of the cell line of interest, or by careful selection from a large progeny pool.

Y 111 TRACKING THE FATE OF MICROINJECTED DNA IN EUKARYOTIC CELLS: SIZE LIMITATIONS TO NUCLEAR ACCUMULATION, Fleming G.F., Volrath S.L., Jen G.C., CIBA-GEIGY Agricultural Biotechnology Research Unit, P.O.Box 12257, Research Triangle Park, NC 27709-2257. One requirement for stable transformation of eukaryotic cells is that the transforming DNA enter the cell nucleus. To investigate this poorly understood process, we have developed an in vivo assay in which we are able to track the fate of microinjected DNA in mouse 3T3 cells. To follow the progress of the microinjected DNA through the cell, we end-label the DNA with biotinylated nucleotides. The injected DNA is visualized in the 3T3 cells by in situ staining using FITC-labeled anti-biotin IgG. Results indicate that double-stranded DNA greater in size than 2.9 kb injected into the cell cytoplasm remains in the cytoplasm and is eventually degraded. However, smaller double-stranded DNA injected into the cell cytoplasm accumulates in the cell nucleus and is not degraded. We are currently working to determine the exact upper size limit for nuclear accumulation of cytoplasmically injected DNA, as well as other parameters effecting this uptake process in animal and plant cells.

Y 112 TRANSFORMATION OF GRAMINAE USING *Agrobacterium tumefaciens*, J. H. Gould, M. E. Devey, T. S. Ko, G. Peterson, O. Hasegawa, R. H. Smith, Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843 & U.S. Institute of Forest Genetics, 2480 Carson Rd., Placerville, CA 95667.

A series of experiments were initiated to determine if *Agrobacterium tumefaciens* EHA 101 harboring a hypervirulent Ti plasmid could transform cells of graminaceous monocots when the cells were in a dividing, meristematic state. The meristematic tissue of the shoot apex of aseptically germinated seedlings was used. The group of plants generated in that year, wheat (*Triticum aestivum* L., Chinese Spring) and corn (*Zea mays* L., Funk's G90), did not show appreciable GUS activity and were considered untransformed. Later PCR and restriction analysis of genomic DNA indicated the presence of both GUS and NPT coding sequences in high molecular weight DNA in the progeny. These experiments have been extended to sorghum (*Sorghum bicolor* L. 'RTx 430') and rice (*Oryza sativa* L., 'Lemont' and 'Rexmont'). With all species used, rooted plants could be regenerated from 70-80% of the inoculated shoots within 3-6 weeks. The frequency of transformation, estimated from PCR amplification of GUS and NPT sequences was 25% in corn, 10% in wheat and 20% in sorghum. An antibiotic selection was not used with these species but was used with rice, which had a lower frequency (2-1%). Southern analysis of genomic DNA, indicated the presence of GUS and NPT II sequences in high molecular weight DNA not associated with the transforming plasmid in the regenerated plants, the F1 of all species studied and the F2 analyzed to date.

Y 113 TRANSGENIC TALL FESCUE (Festuca arundinacea Schreb.) PLANTS REGENERATED FROM PROTOPLASTS. Sam B. Ha, Fang-Sheng Wu* and Tracy K Thorne. Department of Crop and Soil Environmental Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 and *Virginia Commonwealth University, Richmond, VA 23284.

Tall fescue is one of the most important turf and forage grass species. We have established embryogenic cell cultures of two turf-type tall fescue varieties after inducing callus from seed embryos. Plants were regenerated from protoplasts of these cultured cells. In order to develop transgenic tall fescue plants the hygromycin resistance gene and the beta-glucuronidase (GUS) gene were introduced into tall fescue protoplasts through electroporation. Hygomycin-resistant colonies expressed GUS acitivity and plants were regenerated from these transformed cells. The integration of introduced genes was confirmed by Southern blot hybridization of PCR amplified DNA from transgenic plants.

Y 114 β-GLUCURONIDASE EXPRESSION IN TRANSGENIC TOBACCO INCREASES AND DECREASES WITH INCREASED COPY NUMBER, Shaun L.A. Hobbs and Catherine Delong, National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, S7N 0W9, Canada

Tobacco was transformed using pBI121 and individual transformants fell into one of two groups, high or low, according to the GUS activity of material selected to be homozygous for the inserted T-DNA in the R_2 generation of each transformant (Hobbs et al. Plant Mol. Biol. 1990. 15:851-864). Transformants in the high activity group had single T-DNA insertions, while those in the low group had multiple T-DNA insertions at the same or different loci.

In order to test whether the presence of two or more copies of the T-DNA in a genome inevitably led to reduced GUS expression, crosses were made between homozygous material of two different tranformants with high GUS expression. Each had a single T-DNA insert but at different loci. The GUS expression in the F_1 was the same as that of the parents, while the F_2 material segregated to produce some plants with very much higher levels of GUS activity than either parent. Heritability was apparently additive, there being a direct positive correlation between increased copy number and increased GUS activity.

Crosses were also made between homozygous material from transformants with high and those with low GUS activity. In all cases the GUS activity of the F_1 was low, being lower than that expected from a single copy of the T-DNA from the high parent. The F_2 material was also skewed towards low expression and Southern blots confirmed that whenever inserts from both high and low parents were present together in a plant GUS activity was low. Activity approaching that of the high parent only appeared in those F_2 plants where the T-DNA insert from the high parent was in the homozygous state and had segregated away from the T-DNA from the low parent. In this case, therefore, an increase in copy number of T-DNA insertions could produce decreased GUS activity as the T-DNA insert of the low parent apparently suppresses GUS activity from the T-DNA insert of the high parent. The reasons for this suppression are being explored.

Y 115 TRANSCRIPTIONAL ACTIVATOR CAN/ REPRESSOR AG-1 COMPETITION MODEL FOR DEVELOPMENTAL CONTROL OF THE BEAN β-PHASEOLIN GENE. Yasushi Kawagoe, Bruce R. Campell, Mark D. Burow, Partha Sen, Caryl A. Chlan and Norimoto Murai. Dept. Plant Pathology & Crop Physiol., Louisiana State University, Baton Rouge, LA 70803

Based on our *cis*- and *trans*-acting element analyses, we concluded that the CACGTG motif may be a major *cis*-acting regulatory element responsible for the seed-specific late expression of the bean seed storage protein β-phaseolin gene. Our 5'-deletion analysis revealed a 15-fold reduction in the steady-state concentration of phaseolin mRNA when the fragment containing the CACGTG motif was removed. Results from Bustos et al. (1991) indicated that the fragment containing both CACGTG and CACCTG motifs activated seed-specific GUS expression when fused to a CaMV 35S promoter. CACGTG motif-binding protein CAN was present in both bean and tobacco seeds, and was not detectable in bean leaf. We propose a transcriptional activator/repressor competition model for phaseolin gene regulation. In this model, CAN is a transcriptional activator and is a member of the basic-helix-loop-helix protein family. AAAGA motif-binding protein AG-1 is a transcriptional repressor and is a member of a high-mobility-group or the histone H1 protein family. In the repressed state of the phaseolin gene, AG-1 promotes stable nucleosome formation by binding to two AT-rich regions (-191/ -182 and -356/ -347). In the active state of the phaseolin gene, CAN protein acquires the accessibility to CACGTG (-248/ -243) and CACCTG (-163/ -158) by an unknown mechanism, and activates transcription by cooperative interaction of CAN protein. We will test the validity of our model by determining the biological functions of CACGTG and other CANNTG and AAAGA motifs in phaseolin gene regulation. We will substitute these motifs singly or in combination from the phaseolin proximal promoter (-391 or -295/+80), introduce these motifs to the CaMV35S promoter (-391/+6), and test the mutation effects by transient and stable expression assays. We will also isolate cDNA for CAN and AG-1 proteins from bean cotyledons.

Y 116 PROMOTER ANALYSIS OF AUXIN-REGULATED GENES FROM SOYBEAN AND ARABIDOPSIS. Joe L. Key, Robert E. Wyatt, Virginia H. Goekjian, Joscelyn W. Hill, Jong Chan Hong, W. Michael Ainley and Ron T. Nagao. Botany Department, University of Georgia, Athens, GA 30602.

Auxin-responsive genes from soybean (Aux22 and Aux28) and their corresponding homologs from Arabidopsis (AtAux2-11 and AtAux2-27) have been isolated. While these genes encode polypeptides with highly conserved amino acid sequence domains, the basal expression and the level of induction by auxin treatment is different between the two genes in both soybean and Arabidopsis. To understand the regulation of expression of these genes, parallel studies involving DNase I and gel retardation analyses to identify protein-binding DNA domains within the 5' flanking regions of these genes and promoter analysis in transgenic Arabidopsis plants have been initiated. Within the Aux28 gene at least eight binding domains were identified including two AT-rich sequences, a TGACGACA sequence (as-1-like element), a CCACGTGT (G-box motif) and two related sequences with a motif TAGTxxCTGT and TAGTxCTGT (D1 and D4 motifs). Multiple cDNA clones which encode binding proteins for the as-1-like and G-box motif have been isolated from a soybean expression library. The homologous promoter analysis uses the AtAux2-11 promoter fused to β-galactosidase (LacZ) reporter gene. The similar expression pattern of large scale promoter deletion constructs (-3.0, -1.5, -0.6, -0.5 and -0.4 kb) analyzed in transformed Arabidopsis suggests that relevant regulatory sequences of the AtAux2-11 gene are located within 0.6 kb of 5' flanking sequence. In another set of experiments 70 base pair linker scan deletions and fragment replacement mutations from approximately -600 to -46 were constructed and transformed into Arabidopsis. The promoter deletions tested confer tissue-specific expression patterns that are correlated with processes associated with auxin action.

Y 117 PHENYLALANINE AMMONIA-LYASE REGULATORY MUTANTS OF *ARABIDOPSIS THALIANA*. B. Kraft, S. Ohl, J. Chory, R.A. Dixon*, and C.J. Lamb. Plant Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Rd, La Jolla, CA 92037; *Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Phenylalanine ammonia-lyase (PAL) is a key regulatory enzyme in the biosynthesis of a wide variety of phenylpropanoid compounds, including lignin, flavonoids, and phytoalexins. To facilitate the identification of PAL regulatory mutants in *Arabidopsis thaliana* the transgenic line PHG1 was constructed. The PHG1 line contains a chimeric T-DNA insert at a single locus in a homozygous state and exhibits a high degree of hygromycin resistance. This T-DNA insert carries two 1.8 kb copies of the previously cloned PAL1 promoter of *A. thaliana*. One copy of the promoter directs the expression of the *hph* gene (hygromycin phosphotransferase) and the second copy directs the expression of the β-glucuronidase gene (GUS). Therefore, this strain enables the identification of mutants with decreased PAL1 promoter activity through decreased hygromycin resistance and allows testing for *trans*-acting mutations by the analysis of β-glucuronidase activity. 50,000 seeds of the PHG1 line were mutagenized with diepoxybutane (DEB), sown to soil, and M2 seed collected. 50,000 M2 seedlings were screened for decreased hygromycin resistance. 260 putative mutants were identified and 127 of these were rescued. Leaves were removed from these plants and rescreened on callus-inducing medium containing hygromycin. Leaves from 41 plants did not readily form calli. These plants were transferred to soil and 26 yielded M3 seed. Seedlings from individual M3 families exhibited a high degree of phenotypic variation and widely varying GUS activities. Due to the extreme variation within families, the M3 plants were transferred to soil and the M4 seed collected. Further characterization of the putative mutants is in progress and will be presented.

Y 118 EFFICIENT PLASTID TRANSFORMATION IN TOBACCO BY SELECTION FOR A CHIMERIC *aadA* GENE, Zora Svab and Pal Maliga, Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ, 08855-0759

The limitation of the application of genetic engineering to the plastid genome of crop plants is the low frequency of transformation. We report here that, on average, one transplastomic line is obtained per bombardment when selecting for spectinomycin resistance in tobacco by a chimeric *aadA* gene encoding aminoglycoside-3"-adenyl-transferase. This frequency represents a 100 fold increase above previously reported values (1,2). Transcription of the *aadA* gene is under control of the plastid ribosomal RNA operon promoter. Efficient translation of the *aadA* mRNA is secured by incorporating a synthetic ribosome binding site upstream of the *aadA* coding sequence. The chimeric construct has the 3' region of the plastid *psbA* gene. The *aadA* gene was cloned into plastid DNA flanks to target its insertion downstream of the *rbcL* gene. DNA gel blots indicate homoplasmy for the transformed genome copies in regenerated plants. The requirements of expressing the *aadA* gene in tobacco, as compared to *Chlamydomonas* plastids (3), will be discussed. High frequency plastid transformation makes realistic addressing questions of basic plastid biology by transformation, and applications of plastid engineering to crop improvement.

References:

1). Svab, Z., Hajdukiewicz, P. and Maliga, P. (1990) PNAS 87:8526-8530

2). Staub, J.M. and Maliga, P. (1992) Plant Cell, in press)

3). Goldschmidt-Clermont, M. (1991) NAR 19:4083-4089

Y 119 **IMPROVED EFFICIENCY FOR *AGROBACTERIUM TUMEFACIENS* MEDIATED GENE TRANSFER IN *ARABIDOPSIS THALIANA* BY USING DNA METHYLATION INHIBITORS AND CELL WALL DEGRADING ENZYMES,** **Abul Mandal, Viola Lång, Waclaw Orczyk and Tapio E. Palva, Department of Molecular Genetics, Uppsala Genetic Center, Swedish University of Agricultural Sciences, Box 7003, S-750 07 Uppsala, Sweden**

We have established a modified method for fast and very high frequency transformation of *Arabidop-sis thaliana* based on *Agrobacterium tumefaciens* T-DNA mediated gene transfer system. The modi-fications concerned mainly, methylation of T-DNA in transformed calli, and partly treatment of plant explants with cell wall degrading en-zymes prior to infection with *A. tumefacies*. The use of cell wall degrading enzymes raised the frequency of transformed calli from 71.1% to 99.1%. We have evidence that the cytosine residues of *npt-II* gene in the T-DNA were methylated and the expression of this gene was suppressed. Above 96% of calli, when treated with cytosine methylation inhibi-tor 5-azacy-tidine, resumed the expression of *npt II* gene, and regenerated transformed shoots producing fertile seeds. The copy number of T-DNA insert was determined by segregation pattern of T2 seedlings on selective medium and verified by Southern blot hybridization. From some of the transformed plants, harboring only a single copy of insertion, the T-DNA together with flanking plant DNA sequences was cloned into two *E. coli* strains (DH5α mcrAB and DH5α) by plasmid rescue. Recombination or deletion events occurred during plasmid rescue will be presented.

Y 120 PRODUCTION OF INDUSTRIAL ENZYMES IN ALFALFA,

Dennis Mathews[1], Richard Amasino[2], Sandra Austin[1], Edwin T. Bingham[3], Richard Burgess[1], Richard Koegel[4,5], Mark Shahan[1], Richard Straub[4], University of Wisconsin Biotechnology Center[1], Departments of Biochemistry[2], Agronomy[3], Agricultural Engineering[4], USDA Dairy Forage Research Center[5], University of Wisconsin, Madison, WI 53706

We have undertaken a multidisciplinary effort to assess the feasibility of producing industrial enzymes in transgenic alfalfa. Alfalfa has several advantages for "plant molecular farming": it is a hardy, perennial crop capable of multiple harvests per year, it is a legume requiring little added fertilizer and it is amenable to *Agrobacterium* transformation and regeneration. As part of an ongoing effort at the University of Wisconsin, technology has already been developed for large scale extraction of alfalfa juice and bulk precipitation of protein. This technology is being reevaluated and modified for the purpose of recovering active industrial enzymes. We are also engaged in an effort to optimize the efficiency of alfalfa transformation using *Agrobacterium* and to minimize the time required for regeneration of transgenic plants. In our feasibility study, we are focusing on industrial enzymes required in bulk quantity that do not require extensive purification. Results of our attempts to express such an enzyme in tobacco and alfalfa will be presented.

Y 121 UTILIZATION OF THE RICE ACTIN 1 GENE FOR FOREIGN GENE EXPRESSION IN TRANSGENIC CEREALS,

David McElroy[1], Deping Xu[1], Wanggen Zhang[1], Alan D. Blowers[2], and Ray Wu[1,3], [1]Field of Botany, [2]USDA-NSF-DOE Plant Science Center, and [3]Section of Biochemistry, Cornell University, Ithaca, New York 14853, USA.

One of the major limitations in the application of gene transfer technology to rice and other cereal crops has been the relative lack of efficient promoters for both constitutive and developmentally regulated expression of foreign genes in transgenic monocots. With a view towards overcoming these limitations, we have begun to evaluate the utility of the rice actin 1 gene (*Act1*), whose transcript level is relatively abundant in all rice tissues and stages examined, as a regulatory element in cereal grain transformation. *Act1-Gus* gene expression is relatively high in transformed rice, maize, sorghum, barley, and wheat suspension culture cells, and is constitutively expressed in the tissues of transgenic rice plants. We have determined that sequence elements in both the *Act1* 5'-flanking (positive regulatory elements) and transcribed regions (translation initiation region), as well as efficient splicing of the first intron, contribute to the overall high levels of *Act1-Gus* expression. Taken together, these results suggested that the rice *Act1* 5'-region might be useful for the high level constitutive expression of agronomically important genes in transgenic cereal plants. With this in mind, a series of *Act1*-based expression vectors have been constructed for directing foreign gene expression in transgenic cereals. An *Act1*-based expression vector has been used for the recovery of transgenic rice plants expressing the *bar* gene at levels which allowed the selection of phosphinothricin-resistant tissue. Finally, we observed an enhancement of gene expression in transformed cultured rice and maize cells when the *Act1* first intron was added to the transcription unit of a CaMV 35S-*Gus* chimeric gene. With this knowledge, the *Act1* intron 1 has been included in a potato wound-inducible expression cassette for use in the transformation of rice and other cereals.

Y 122 NORMAL GROWTH OF TRANSGENIC TOBACCO IN THE ABSENCE OF CYTOSOLIC PYRUVATE KINASE.

S. G. McHugh, B. L. Miki, Plant Research Centre, Agriculture Canada, Ottawa, Ont., Canada, K1A OC6.
R. S. Sangwan, S. D. Blakeley, G. C. Vanlerberghe, K. Ko, D. H. Turpin, W. C. Plaxton, D. T. Dennis, Dept. of Biology, Queen's University, Kingston, Ont., Canada, K7L 3N6.

Pyruvate kinase is a key glycolytic enzyme that catalyses the formation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP. To investigate the effect of overexpressing cytosolic pyruvate kinase (PK_c) in transgenic plants, tobacco tissue was transformed with a potato PK_c construct under the control of the cauliflower mosaic virus 35S promoter. Unexpectedly, several primary transformants were recovered which lacked PK_c in the leaves. Enzyme assays of leaf tissue failed to detect PK_c activity and no PK_c was detected by western blots. Metabolite analyses indicated that the leaves of these plants had levels of pyruvate similar to wild type, but that the levels of PEP were substantially higher than wild type, consistent with a block at the PK catalyzed step in glycolysis. Northern blot analysis demonstrated that potato PK_c transgene transcripts were expressed at high levels in transgenic plants with normal PK_c activity but were expressed only at low levels within the plants lacking PK_c. However, the levels of resident tobacco PK_c mRNA were similar in the leaves of plants with or without PK_c activity. PK_c deficiency in the leaves did not appear to adversely affect the growth of these plants. The leaves had normal rates of photosynthetic O_2 evolution and respiratory O_2 consumption. Analysis of progeny indicated that the lack of PK_c could be transmitted to offspring. Surprisingly, wild type levels of PK_c activity and protein were detected in the roots of these plants suggesting that the suppression of PK_c may be tissue specific.

Y 123 **THE PROLAMIN ELEMENT IS IMPORTANT FOR BARLEY C-HORDEIN PROMOTER ACTIVITY,**

Martin Müller and Søren Knudsen, Department of Physiology,Carlsberg Laboratory and Carlsberg Research Laboratory,Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark.

The prolamin element, also termed '-300 element', is a highly conserved circa 30 bp promoter sequence found in many prolamin genes, coding for the alcohol soluble fraction of cereal seed storage proteins. It typically incorporates two smaller motifs, a 5' sequence sharing homology with the viral SV 40 enhancer core sequence and a 3' sequence similar to the yeast GCN4- as well as the maize opaque-2 binding site. In the promoters of the barley C-hordein, the wheat α- and ω-gliadin and the rye ω-secalin genes the 3'- motif is identical to the optimal binding site ATGAG/CTCAT for the GCN4 regulatory protein. So far it has not been established, whether the prolamin element contains any information, conferring promoter activity and/or tissue specificity in cereals. This may be due to the use of heterologous transformation systems. We have carried out a C-hordein (λ-hor 1-17) promoter analysis employing a homologous expression system. Chimaeric gene constructs containing various deleted C-hordein promoters fused translationally to the β-glucuronidase gene (GUS) were transferred into intact endosperm tissue by microprojectile bombardment. Promoter strength is deduced from the number of blue dots appearing after histochemical staining (1). It is shown that sequences of the prolamin element enhance promoter activity. We are investigating, whether the sequence ATGAG/CTCAT is a nitrogen responsive element in barley endosperm as found in yeast.

(1) Entwistle, J., S. Knudsen, M. Müller and V. Cameron Mills 1991. Amber codon suppression: the in vivo and in vitro analysis of two C-hordein genes from barley. Plant Mol. Biol 17:1217

Y 124 GENE EXPRESSION IN TRANSGENIC MUSTARD PLANTS: FROM PROTOPLASTS TO PLANTS VIA SOMATIC EMBRYOGENESIS, Pua E. C. and D.G. Barfield, Institute of Molecular and Cell Biology, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Republic of Singapore.
We have previously produced transgenic mustard (*Brassica juncea* (L.) Czern & Coss) plants carrying the chimeric GUS and NPTII genes driven by the common CaMV 35S promoter. In this study, expression of these transgenes in protoplasts of *B. juncea*, isolated from shoot culture originating from transgenic seedlings of F1 progeny, and protoplast-derived plants via somatic embryogenesis was investigated. GUS activity was not visibly detected in freshly isolated protoplasts, although the mature leaves, but not the young leaves and stems, of the donor shoot culture showed GUS activity. However, GUS activity was observed at low frequencies (1-5%) in both protoplasts after 10 days and somatic embryos after 4-5 weeks of culture. Somatic embryos were developed into plants which were subsequently transferred to soil. Of all 12 protoplast-derived plants, most showed low GUS activity at the vascular tissues and tips of the mature leaves, whereas no GUS activity was observed in young leaves of all plants. At present, these plants are being characterized for the presence of transgenes and its seed progeny are also being analysed.

Y 125 CRE-*LOX* MEDIATED SITE-SPECIFIC RECOMBINATION BETWEEN PLANT CHROMOSOMES: A NOVEL APPROACH FOR GENERATING CHROMOSOME TRANSLOCATIONS
Minmin Qin, Christopher Bayley and David W. Ow, Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan St., Albany, CA 94710.
We have explored the use of site-specific recombination to generate chromosome translocations. The recombination system tested was the Cre-*lox* system of bacteriophage P1, which consists of the Cre enzyme and 34 bp *lox* site substrates. Two constructs were introduced independently into tobacco: one containing a *lox* site followed by a promoterless *hpt* coding region (*lox-hpt*) and the other containing a functional *cre* gene with a *lox* site inserted between the CaMV 35S promoter and the *cre* coding region (35S-*lox-cre*). The two constructs were then brought into the same genome by crossing plants harboring each construct. If the two *lox* sequences are in the same relative orientation with reference to the centromeres, then recombination between the *lox* sites would be expected to generate a reciprocal translocation event. Recombination between the two *lox* sites would also create a functional *hpt* gene and split the *cre* coding region from its promoter. Seedlings or shoots regenerated from these crosses were cultured in the presence of hygromycin. Progeny from three crosses between one *lox-hpt* parent and three 35S-*lox-cre* parents showed resistance to hygromycin. Polymerase chain reaction and Southern hybridization analyses of these putative recombinants showed that new junctions were created from recombination of the two *lox* sites. These and additional experiments examining recombination between two chromosomes will be presented and the feasibility in generating reciprocal translocation libraries will be discussed.

Y 126 TRANSFORMATION OF RICE WITH THE RTBV COAT PROTEIN GENE BY THE BIOLISTIC METHOD.
Rongda QU, Liangcai LI, Alexandre de KOCHKO[1],Claude FAUQUET[1] & Roger N. BEACHY. ILTAB, 1: ORSTOM, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA 92037.
It is our goal to produce transgenic plants containing the gene encoding the coat protein of the Rice Tungro Bacilliform Virus (RTBV). We have performed transformation on immature embryos, mature embryos, embryogenic calli and suspension cell cultures of indica and japonica varieties of rice via the microbombardment procedure. Tungsten particles coated with two different gene constructs, one containing the coat protein gene driven by the enhanced 35S promoter and the second containing the *hph* gene encoding for resistance to hygromycin were used. Some experiments were performed with a third plasmid containing the *gus A* gene. After shooting the embryos or calli tissueswere permitted to grow on a non-selective medium for the production of calli. After 1-2 weeks, calli were transferred to a selective medium containning 30-50 µg/ml of hygromycin. After two months on this medium, the surviving calli were transferred to a regeneration medium. The shoots that developed were further screened on selective medium. The plantlets obtained were transferred to soil and placed in the greenhouse. The presumed transformed plants were confirmed by PCR, using specific primers from the RTBV coat protein gene. Fourteen out of nineteen plantlets that grew on medium with hygromicin contained the coat protein gene. The results of western and Southern blot analysis, as well as inheritance of the CP gene, will be reported.

Y 127 MOLECULAR ANALYSIS OF TRANSGENIC MAIZE CALLUS, PLANTS, AND PROGENY. James C. Register III, Jeff M. Sillick, David J. Peterson, Paul Bullock, and Martin Wilson. ICI Seeds, Inc., Slater, IA 50244.
Now that maize transformation has become routine in many laboratories, the next issues to be dealt with are fertility of the regenerated plants and the stability of transgenes and their expression in transgenic plants obtained via direct DNA delivery. Here we have begun to address the second of these issues. Maize suspension culture cells (A188xB73) were transformed via particle bombardment with constructs containing chimeric nptII or bar genes, permitting selection on kanamycin or bialaphos, respectively. These constructs also contained a chimeric gus gene (under the control of the 35S or maize adh1 promoters). Greater than 100 of these calli have been examined for DNA insertion patterns, approximate transgene copy number, and transgene expression. Plants regenerated from many of these transformants have been similarly analyzed and the results indicate that although transgene stability through mitosis/regeneration can be expected, these plants display a range of scorable phenotypes. Most, but not all, regenerated plants show coexpression of the physically linked selectable and nonselectable genes. Both predicted and unexpected segregation ratios have been observed following outcrosses and self-pollinations of these plants. These results will be presented in detail.

Y 128 USE OF TOBACCO PLANT CELL SUSPENSIONS TO INVESTIGATE THE INTERACTION BETWEEN PLANT CELLS AND *Agrobacterium tumefaciens* DURING TRANSFORMATION, Hans Rempel, Connie Knapp and Louise Nelson, Plant Biotechnology Institute, National Research Council, Saskatoon, Canada S7N 0W9

Introduction of novel genes into plant cells is commonly achieved via Agrobacterium mediated DNA transfer. Efforts to extend the usefulness of the vector system to intractable plants, such as pea (*Pisum sativum*), usually focus on increasing T-DNA transfer by elevating *vir* gene induction. Using a tobacco (*Nicotiana tobacum*) plant cell suspension, we have devised a system for direct comparison of *vir* gene induction and efficiency of transformation. In addition, culture conditions could be identified that affected plant cell transformation rates. *Agrobacterium tumefaciens*, strain EHA101, possessing a GUS-intron binary vector and a transcriptional *virB1*-CAT fusion on a second plasmid, was cocultivated with suspension cells. The cultures were sampled at various times to determine the level of *vir* induction by measuring CAT activity. From 24 through 96 hours after cocultivation, aliquots were removed for GUS evaluations. Although GUS activity was detected within 24 hours, peak activity was found to occur between 72 and 96 hours. Also, susceptibility of the tobacco cell suspensions to transformation was greatest in the middle of log-growth phase and comparatively low in both the lag and stationary phases. Additional results will be presented to demonstrate the effectiveness of tobacco plant cell suspensions as a model system for investigating the complex interaction of bacteria and plant, from induction through integration.

Y 129 CHARACTERIZATION OF A *C-MYB* HOMOLOG FROM *ARABIDOPSIS THALIANA*. Diane M. Ruezinsky, Susan A. Hedrick, Richard A. Dixon*, Christopher J. Lamb, and Peter W. Doerner. Plant Biology Laboratory, The Salk Institute for Biological Studies, P.O. Box 85800 San Diego, CA 92138,*Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Members of the *c-myb* proto-oncogene family have been shown to be required for cell differentiation and proliferation in several species. The protein product of the first *c-myb* homolog identified in plants, the maize C1 gene, activates transcription of genes involved in anthocyanin biosynthesis. The product of another plant myb gene, GL1, is required for trichome formation in *A. thaliana*. Additional *c-myb* homologs have been isolated from barley, *Antirrhinum* and *Arabidopsis* cDNA libraries. We have cloned seven *c-myb* homologs from an *Arabidopsis thaliana* genomic library. The amino terminal end of the protein encoded by one of these genes, Atmyb4, shares 56-73% amino acid identity with previously cloned plant *c-myb* homologs. In addition, the intron/exon boundaries of C1, GL1 and Atmyb4 are positioned at the same amino acid residues. RNA slot blot analysis has shown that Atmyb4 is expressed at high levels in floral tissues; *in situ* hybridization experiments have detected expression in the xylem of floral organs. To more precisely define the expression pattern, the Atmyb4 promoter was linked to the β-glucuronidase (GUS) gene and introduced into *Arabidopsis*. To determine the function of the Atmyb4 gene product in plant development, a series of antisense, dominant negative and overexpression constructs have been introduced into *Arabidopsis*. Data on these transgenic plants will be presented.

Y 130 MICRO-TARGETING: A HIGHLY EFFICIENT AND VERY FLEXIBLE BALLISTIC GENE TRANSFER SYSTEM FOR MERISTEMS AND IMMATURE EMBRYOS

Christof Sautter, Victor A. Iglesias, Douglas M. Stein and Ingo Potrykus
Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland

Meristems and immature embryos regenerate excellently and, therefore, represent promising target tissues for gene transfer aiming at the regeneration of stably transformed plants. The macro-projectile-mediated ballistic approach ("biolistics") is not efficient for these tissues due to the unpredictable particle distribution in a relatively large target area. We constructed a novel particle delivery system by which micro-projectiles are accelerated according to the law of Bernoulli ("micro-targeting"; Sautter et al., Biotechnology 9:1080 [1991]). This new system is highly flexible allowing the adjustment of physical parameters such as the impulse of the particles, the DNA load, and the distribution of particles in a predictable target area of as little as 0.15 mm in diameter. Micro-targeting yielded about one stably transformed plant per 10^3 tobacco cells that were exposed to the particle jet. The basic function of our accelerator will be explained and some examples of its application will be presented.

Y 131 AGROBACTERIUM-MEDIATED TRANSFORMATION OF PLUM (PRUNUS DOMESTICA L.) WITH THE COAT PROTEIN GENE OF PAPAYA RINGSPOT VIRUS,

R. Scorza[1], J.M. Cordts[1], S. Mante[2], D. Gonsalves[3], V.D. Damsteegt[4], L.M. Yepes[3] and J.L. Slighton[5]
[1]USDA-ARS, Kearneysville, WV, [2]SUNY Health Science Center, Dept. of Medicine, Syracuse, NY, [3]Dept. of Plant Pathology, Cornell Univ., Geneva, NY, [4]FDWSRU, Fort Detrick, Frederick, MD, [5]The Upjohn Company, Kalamazoo, MI

The potential for papaya ringspot virus (PRV) coat protein-mediated cross protection of plum against plumpox virus (PPV), a major plum pathogen, is being tested. Both PRV and PPV are potyviruses. The transformation system utilized hypocotyl slices from ungerminated plum seeds (Mante et al., 1991. Bio/Technology 9:853-857). These were co-cultivated with A. tumefaciens EHA101 (Hood et al., 1986. J. Bact. 168:1291-1301) containing the plasmid pGA482GG/cpPRV-4. This plasmid carries the papaya ringspot virus (PRV) coat protein gene construct and chimeric NPTII and GUS genes (Fitch et al., 1990, Plant Cell Repts. 9:189-194). Shoots were regenerated from hypocotyl slices on shoot regeneration medium with appropriate antibiotics for selection. Regenerated plants tested positive for both NPTII and GUS activity. Polymerase chain reaction (PCR) amplification identified the presence of the PRV coat protein and NPTII genes in DNA isolated from young leaves. Southern blot analysis is being used to obtain information concerning the organization of the PRV cp gene in transgenic clones. The reaction of transgenic plants to plum pox virus is under test.

Y 132 EXPRESSION OF DNA MICROINJECTED INTO APICAL MERISTEM CELLS OF WHEAT.

John Simmonds, Leslie Cass and Daina Simmonds,
Agriculture Canada, Plant Research Centre, Ottawa,
Ontario, K1A 0C6 Canada

Genetic transformation of many major crop species cannot be accomplished because of limited 'in vitro' responses such as protoplast technology for direct DNA delivery and selection protocols. Even when protoplast technology or regenerative tissue cultures are adequate, these are often limited to a narrow range of genotypes, necessitating lengthy backcrossing programs to recover the desired gene in an elite cultivar. Transformation of apical meristems has the potential of providing transgenic seeds by normal zygotic embryogenesis thus eliminating the genotype dependency on cell and protoplast culture.

We describe a wheat apex culture system which routinely produces phenotypically normal, fertile plants. Non-disruptive microinjection protocols have been developed to deliver DNA into apical hypodermal cells which generate cell lineages for germ-line tissues. Histological evidence of GUS expression in the wheat apex is presented as proof that microinjection can be used to deliver functional genes into apical meristematic cells.

Y 133 *Abstract Withdrawn*

Y 134 BIOTECHNOLOGICAL APPROACHES TO FORAGE GRASS IMPROVEMENT: FESTUCA AND LOLIUM. T. Takamizo[1,2], Z. Wang[1], K. Suginobu[2], R. Perez-Vicente[1], I. Potrykus[1] and G. Spangenberg[1], 1) Institute for Plant Sciences, Swiss Federal Institute of Technology, CH 8092 Zürich, Switzerland; 2) Lab. Biotechnology, National Grassland Research Institute, Nishinasuno, Tochigi, 329-27 Japan

Somatic hybridization and genetic transformation were carried out in order to improve the forage grasses Festuca and Lolium. These graminaceous plant species are important forage grasses in temperate regions. Festuca arundinacea (tall fescue) protoplasts inactivated by iodo-acetamide and non-morphogenic protoplasts of Lolium multiflorum (Italian ryegrass) were fused electrically in order to combine agronomically desirable traits of both species, and somatic hybrids were recovered. Southern hybridization analysis using a rice ribosomal DNA probe revealed that they had both Festuca and Lolium DNA specific fragments. A cloned Lolium specific interspersed DNA probe hybridized to total genomic DNA from Lolium and somatic hybrids. Chromosome counts and zymograms of leaf esterases suggested nuclear genome instability of the somatic hybrid plants analyzed. Southern hybridization experiments using four mitochondrial and one chloroplast probe revealed that the somatic hybrids had either Festuca, additive or novel mtDNA patterns and Festuca chloroplast DNA pattern. Presently, independent regenerated somatic hybrids are being planted under field conditions for the evaluation of agronomical traits. In addition, asymmetric somatic hybrids in the same fusion combination using Italian ryegrass as donor and tall fescue as recipient are being produced in order to achieve nuclear genome transfer.
For the analysis of donor genome elimination, species-specific repetitive sequences of Festuca and Lolium have been isolated and characterized (sequence analysis, dot blot and in situ hybridizations).
Genetic transformation of F. arundinacea protoplasts and regeneration of transgenic plants grown until maturity are described. Chimeric hygromycin phosphotransferase (hph) and phosphinotricin (PPT) acetyltransferase (bar) genes were introduced into protoplasts isolated from embryogenic cell suspension cultures with polyethylene glycol treatment. Cell colonies resistant to either 200 mg/l hygromycin or 100 mg/l PPT were recovered, respectively upon selection with bead type culture systems. Stable integration of the transgenes in the genomes of plants regenerated from hygromycin resistant and PPT resistant callus clones was shown by Southern hybridization analysis. Expression of the transgenes in mature plants was proven by the corresponding HPH and PAT enzyme assays.

Y 135 BIOTECHNOLOGICAL APPROACHES TO FORAGE GRASS IMPROVEMENT: FESTUCA and LOLIUM. Takamizo[1,2], T., Wang[1], Z., Suginobu[2], K., Perez-Vicente[1], R., Potrykus[1], I. and Spangenberg[1], G. 1) Institute for Plant Sciences, Swiss Federal Institute of Technology, CH-8092, Zürich, Switzerland 2) Lab. Biotechnology, National Grassland Research Institute, Tochigi, 329-27 Japan

Somatic hybridization and genetic transformation were carried out in order to improve forage grasses Festuca and Lolium. These graminaceous plant species are important forage grasses in temperate regions. Festuca arundinacea (tall fescue) protoplasts inactivated by iodo-acetamide and non-morphogenic protoplasts of Lolium multiflorum (Italian ryegrass) were fused electrically in order to combine agronomically desirable traits of both species, and somatic hybrids were recovered. Southern hybridization analysis using a rice ribosomal DNA probe revealed that they had both Festuca and Lolium DNA specific fragments. A cloned Lolium specific interspersed DNA probe hybridized to total genomic DNA from Lolium and somatic hybrids. Chromosome counts and zymograms of leaf esterase suggested nuclear genome instability of the somatic hybrid plants analyzed. Southern hybridization experiments using four mitochondrial and one chloroplast probe revealed that the somatic hybrids had either Festuca, additive or novel mt DNA patterns and a Festuca chloroplast DNA pattern. Presently, independent regenerated somatic hybrids are being planted under field conditions for the evaluation of agronomical traits. In addition, asymmetric somatic hybrids in the same fusion combination using Italian ryegrass as donor and tall fescue as recipient are being produced in order to achieve partial nuclear genome transfer. For the analysis of donor genome elimination, species-specific repetitive sequences of Festuca and Lolium have been isolated and characterized. Genetic transformation of Festuca arundinacea protoplasts and regeneration of transgenic plants grown until maturity are described. Chimeric hygromycin phospho-transferase (hpt) and phosphinotricin (PPT) acetyl-transferase (bar) genes were introduced into protoplasts isolated from embryogenic cell suspension cultures with polyethylene glycol treatment. Cell colonies resistant to either 200 mg/l hygromycin or 100 mg/l PPT were recovered, respectively upon selection with bead type culture systems. Stable integration of the transgenes in the genomes of plants regenerated from hygromycin resistant and PPT resistant callus clones was shown by Southern hybridization analysis. Expression of the transgenes in mature plants was proven by the corresponding hpt and pat enzyme assays.

Y 136 TRANSGENIC TOBACCO PLANTS EXPRESSING A MODIFIED BACILLUS THURINGIENSIS cryIC GENE, Bert Visser, Ellie Munsterman, Guy Honée and Theo van der Salm, DLO-Centre for Plant Breeding and Reproduction Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands.
Transfer of the wild-type and modified cryIA(b) gene of Bacillus thuringiensis has resulted in insect resistant plants. Plants expressing a fully resynthesized gene showed resistance to a considerable number of lepidopteran insects including less sensitive species (Perlak et al. (1991) PNAS 88: 3324). A limited number of nucleotide residues in the cryIC gene was substituted to remove potential poly-adenylation sites and instability motifs and to interrupt long A/T stretches. To investigate whether the substitutions increased expression levels of the cryIC gene sufficiently to render transgenic plants resistant to Spodoptera species, we transferred a cryIC/NPTII fusion and a cryIC/cryIA(b) under the control of a duplicated CaMV 35S promoter to tobacco. The results of bio-assays on the transgenic plants will be presented and the potential of a strategy involving only a limited number of nucleotide substitutions based on the criteria mentioned above will be discussed.

Y 137 SUPEROXIDE DISMUTASE GENE EXPRESSION IN TRANSGENIC PLANTS, Robert P. Webb, Luis Wong-Vega, and Randy D. Allen, Department of Biological Sciences, and Department of Agronomy Horticulture and Entomology, Texas Tech University, Lubbock, TX 79409
Transgenic tobacco plants that contain a chimeric gene construct that encodes the chloroplast localized, copper and zinc containing superoxide dismutase isoform (Cu/Zn SOD) from pea have been developed. These plants constitutively express high levels of the pea SOD isoform that, in leaf tissues, is correctly localized in chloroplasts. Analysis of the SOD isoform profiles of these transgenic plants indicates that endogenous iron SOD and manganese SOD isoforms (Fe SOD and Mn SOD) are present in normal amounts; however, the endogenous Cu/Zn SOD isoforms are missing. Therefore, it appears that expression of the introduced pea Cu/Zn SOD gene somehow interferes with the expression of endogenous Cu/Zn SODs. We suspect that this type of interference may be one factor in the failure of plants that overexpress chloroplastic Cu/Zn SODs to exhibit improved resistance to oxidative stress. Preliminary analyses of the basis for the interference effect suggest that it occurs post-transcriptionally and is not due to limiting supplies of copper. We have also developed chimeric constructs that encode modified forms of Mn SOD and we are currently analyzing the effects of these genes on endogenous SOD expression in transgenic plants.

Y 138 AUXIN-INDUCIBLE RESPONSE PROMOTER ELEMENTS
Derek W.R. White, Anya Lambert, Roy Meeking, Ruth Kerr, and Paul Ealing. Plant Molecular Genetics Laboratory, Grasslands Division, Department of Scientific and Industrial Research, Private Bag, Palmerston North, New Zealand.
Auxins have been shown to regulate the transcription of an extensive set of genes in plants. However, comparison of the 5′ flanking regions of these genes does not reveal any significantly similar sequence motifs. In order to identify auxin-inducible *cis* regulatory DNA elements we have conducted a detailed analysis of the mannopine synthase (*MAS*) promoter of the divergent 1′ and 2′ genes of *Agrobacterium* T-DNA. This promoter controls an intricate pattern of tissue and cell specific expression in transgenic plants and is auxin-responsive. We have cloned a series of defined promoter fragments upstream of a promoterless β-glucuronidase (GUS) gene and tested these constructs for GUS expression in transgenic tobacco plants. We have defined a 12 base pair auxin-inducible response (AIR) element which acts in combination with other elements to control transcriptional activation. This AIR element appears to be a motif common to a variety of auxin-responsive genes. We will present the results of experiments, using site directed mutagenesis and element exchange, to further define the function of this *cis* regulatory element.

Y 139 ANALYSIS OF RICE *Act1* 5' REGION ACTIVITY IN TRANSGENIC RICE PLANTS AND THE STUDY OF REGULATORY ELEMENTS IN THIS REGION, Wanggen Zhang[1], Yixin Wang[2], David McElroy[1], and Ray Wu[1,2], [1]Field of Botany, and [2]Department of Biochemistry, Cornell University, Ithaca, New York 14853, USA.
The rice actin 1 gene (*Act1*) 5' region, containing a 447-bp 5' intron, has been developed as an efficient regulator of foreign gene expression in our laboratory. To determine the pattern and level of rice *Act1* 5' region activity, 44 transgenic rice plants containing the *Act1-Gus* were generated by PEG-mediated DNA uptake system and the direct selection technique. Two independent clonal lines of transgenic rice plants were analyzed in detail. Southern gel blot showed that the foreign gene has been integrated into rice genome at different sites. Quantitative analysis showed that tissues from these transgenic rice plants have a level of GUS protein that represents as much as 3% of total soluble protein. By a handcut thin section, We demonstrated that *Act1-Gus* gene expression is constitutive throughout all plant developmental stages. The inheritance studies of *Act1-Gus* expression in pollen of one transgenic line and in subsequently derived progeny plants revealed that there are three independent *Gus* genes in this line. By an *in vitro* gel retardation, methidiumpropyl-EDTA footprinting and transient expression assays of *Act1* 5' region deletion constructs, we identified a poly(dA·dT) positive regulatory element between -146 and -186. Deletion of this region decreased GUS activity more than 3-fold. We also detected a CCCAA pentamer repeat between -295 and -260 representing a negative regulatory element. Without this sequence, GUS activity increased about 2-fold in transformed rice protoplasts.

Y 140 TRANSGENIC RICE ANALYSES UNCOVERED PROXIMAL AND UPSTREAM *CIS*-ACTING REGULATORY ELEMENTS RESPONSIBLE FOR DEVELOPMENTAL CONTROL OF THE RICE SEED STORAGE PROTEIN GENE Gt-1. **Zhenwei Zheng, Yasushi Kawagoe, and Norimoto Murai, Dep. Plant Pathology & Crop Physiology, Louisiana State University, Baton Rouge, LA 70803.**

To identify *cis*-acting regulatory elements responsible for developmental control of the rice seed storage protein glutelin gene Gt-1, we constructed both 5'-deletion mutations of the 5.1 kbp upstream region and substitution mutations of the -508 proximal promoter region. Gt1 promoter mutation/GUS fusion genes were used to transform homologous rice protoplast. Spatial and temporal control of the Gt1 expression were determined by use of the GUS activity in developing seeds and vegetative organs of mature transgenic plants. Results of 5'-deletion analysis indicated the existence of two quantitative regulatory elements, one in the -1.8k/-508 region and another in the -5.1k/-1.8k region. These two elements together increased by 100 fold GUS expression of the -508 proximal promoter in developing seeds. Both spatial and temporal control were maintained in -154 constructs, indicating spatial and temporal regulatory elements resides 3'-downstream of -154. However, one of -154 plants lost spatial regulation and the GUS activity was found in phloem of leaf, stem and roots. Thus, there appear to be a silencer element 5'-upstream of -154 that repress the GUS expression in vegetative organs. Kim and Wu (1990) have identified in the proximal promoter region five nuclear protein-binding sites, box I (-103/-86), II (-122/-108), III (-164/-146), IV (-206/-189) and V (-595/-575). Substitution mutation of a single box did not disrupt either spatial nor temporal control of the 1.8 kbp upstream promoter. Thus, these protein-binding sites seems dispensable in the presence of the upstream quantitative element. However, combinations of substitution mutations of the protein-binding sites reduced significantly glutelin gene expression in the context of the 1.8 kbp promoter. Based on these findings, we will present a model for developmental control of the glutelin gene.

Y 141 GENETIC TRANSFORMATION OF PEANUT AND BEAN CALLUS VIA AGROBACTERIUM-MEDIATED DNA TRANSFER. C.I. Franklin, T. N. Trieu, K.M. Shorrosh, B.G. Cassidy, R.A. Dixon and R.S. Nelson, Plant Biology Division, Noble Foundation, P. O. Box 2180, Ardmore, Oklahoma 73402, USA.

Transformed peanut (Arachis hypogea c.v. Okrun) and green bean (Phaseolus vulgaris L.) callus was produced from hypocotyl or leaf explants after co-cultivation with Agrobacterium tumefaciens on a defined medium followed by selection with 200 mg/l or 50 mg/l kanamycin, respectively. For peanut, three different Agrobacterium strains (EHA101, LBA4404 and ASE-1) carrying the binary vector pKYLX71-GUS were capable of transforming callus tissue. For bean transformed with EHA101, Southern blot analysis confirmed the insertion of the GUS gene in callus cells and suggests that each transformed callus line resulted from a unique single copy insertion of the T-DNA. GUS expression in the bean cells from these callus clumps was confirmed by histochemical assay. Preliminary results based on fluorometric assay indicated a very high level of GUS gene expression in the callus. This result suggests that the high expression of GUS in this callus may be due to the absence of developmental control of gene expression in the callus which is normally present in an intact plant. Attempts to use these callus transformation methods to study coat-protein mediated protection against virus infection in peanut and basic aspects of gene expression in bean will be discussed.

Progress on Identifying New Multi-Genic Traits for Crop Improvement; Genes and Gene Expression for New Traits - Pest Control

Y 200 *Abstract Withdrawn*

Y 201 SCREENING AND CHARACTERIZATION OF MUTANTS IN AN *ARABIDOPSIS THALIANA* TRANSPOSON TAGGED SEED COLLECTION, Andreas Betzner[1], Pascual Perez[2], Denise Gérentes[2], Marie Oakes[1], William Tucker[1], Eric Huttner[1], Abdul M. Chaudhury[3] and Elizabeth S. Dennis[3]; (1) Biocem Pacific, Research School of Biological Sciences, GPO Box 475, Canberra ACT 2601, Australia; (2) Biocem, 24 Ave Des Landais, 63170 Aubière, France; (3) Division of Plant Industry, CSIRO, GPO Box 1600, Canberra ACT 2601, Australia.

RFLP mapping and chromosome walking are powerful methods for isolating genes from a mutant gene phenotype. Gene tagging provides a potentially more rapid alternative to clone mutated genes, but is dependent on the development of a suitable system. The major limitation to the use of transposable elements in heterologous species so far has been the low frequency of movement of introduced transposable elements.We have developed a tagging system in *Arabidopsis thaliana* based on modified Ac elements and have generated a population derived from 279 independent transgenic lines (T_0 plants) containing modified maize Ac transposable elements (Perez *et al.*, unpublished). Three hundred progeny of each line (T_1 plants) were allowed to self and the progeny obtained (T_2 plants) should contain in the homozygous condition mutants generated by germinal transposition in the gametes of T_0 plants, or by early somatic transposition in T_1 plants. From this population, T_2 plants derived from 150 lines were screened for mutants affected in floral morphology, plant architecture and plant development. Plants with mutant phenotypes of each class were detected and selfed or crossed.

Examples of mutants obtained and their analysis will be presented.

Y 202 ANALYSIS OF PEANUT STRIPE VIRUS CAPSID PROTEIN EXPRESSION IN TOBACCO. Brandt Cassidy[1], John Sherwood[2] and Richard Nelson[1] [1]Plant Biology Division, Noble Foundation, P. O. Box 2180, Ardmore, Oklahoma 73402, USA. [2]Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma 74078-9947, USA.

Nicotiana tabacum cv. Xanthi was transformed using Agrobacterium (strain LBA 4404) containing the binary vector pGA482 into which portions of the peanut stripe virus (PStV) capsid protein (CP) gene had been cloned. The number of gene inserts in primary transformants (R_0) from PStV CP gene constructs encoding amino acids 16-287 and 106-287 were determined by Southern blot border analysis. Transgenic tobacco plants were analyzed for NPTII and CP expression by ELISA and Western blot, respectively. Tobacco plants expressing CP were self-fertilized and the R1 generation tested for segregation. Homozygous lines expressing the PStV CP of these deletion constructs as well as lines expressing the full length CP will be tested to determine the level of resistance to tobacco etch and potato virus Y.

Y 203 **ISOLATION OF THE GENE ENCODING AN INSECT α-AMYLASE INHIBITOR / ENDO-CHITINASE FROM SEEDS OF JOB'S TEARS (*Coix lachryma jobi*)**. Donna Fairweather, Charles. H. Shaw & Martin D. Watson. Dept. of Biological Sciences, University of Durham, Durham DH1 3LE, UK

A protein inhibitor of locust α-amylase has been isolated from the seeds of *Coix lachryma*, a tropical cereal.The partial amino acid sequence shows strong homology with previously reported sequences of endochitinases from bean,potato, tobacco and barley. Endochitinase activity of the protein inhibitor has also been demonstrated [1].The potential use of this gene for crop improvement is forseable, the novel combination of functions may be relevant to protection of grains and whole plants from insect feeding and fungal infection.

Several approaches have been utilised to isolate both the cDNA and genomic sequences. These include use of the polymerase chain reaction on both genomic and λgt11 cDNA libraries. Additionally, using an antibody raised against the pure protein a λgt11 expression library has been screened.

Change in the level of the inhibitor and temporal expression of the gene during development of the seed is also being investigated.

[1]. Ary,M.B.,Richardson,M. and Shrewry,P.R. (1989) Biochimica et Biophysica Acta. 993,260-270.

Y 204 **DETERMINATION OF THE QUANTITATIVE GENETIC CONTROL OF *Rhizobium* NODULATION INTENSITY IN COMMON BEAN (*Phaseolus vulgaris*)**, Paul Gepts, Rubens O. Nodari, and Siu-Mui Tsai, Department of Agronomy and Range Science, University of California, Davis, CA 95616-8515

Leguminous plants fix atmospheric N through a symbiosis with various rhizobial symbionts, thus decreasing the need for N fertilizer. Increased nodulation has been correlated previously with higher symbiotic nitrogen fixation levels. In the work reported here, we have mapped genetic factors controlling increased nodulation by *Rhizobium tropici* in common bean. Nodulation levels were determined in F_2-derived F_3 families of a cross between BAT93 (low nodule numbers) and Jalo EEP558 (high nodule number). Bean plants grown in Leonard jars in N-free nutrient solution were inoculated after germination with *R. tropici* strain UMR-1899; nodule numbers were determined after one month of culture. A *P. vulgaris* RFLP map consisting of 150 molecular markers was established in the F_2 population of the same cross using MAPMAKER and LINKAGE-1. Factors for nodule number were located by analysis of variance. Significant differences ($P<0.001$) were observed among F_3 families for nodule number. Nodule number was influenced by factors on six unlinked chromosome regions. Three of these overlapped with regions influencing seed size. In three of the six regions, the allele of Jalo EEP558 was associated with higher nodule number, whereas in two regions the BAT93 allele was associated with higher nodule number. A sixth region showed heterosis for nodule number. If our results are confirmed, breeding for enhanced nitrogen fixation could be accelerated through marker-assisted selection.

Y 205 TRANSGENIC TOBACCO RESISTANT TO *PHYTOPHTHORA PARASITICA* : ANALYSIS OF EXPRESSION AND ACTIVITY OF A NOVEL PATHOGENESIS-RELATED (PR) PROTEIN, SAR 8.2. Christopher B. Glascock #, Sofien Kamoun *, Brett Tyler *, John Ryals §, and Danny Alexander #. # Calgene, Inc., 1920 Fifth St., Davis, CA 95616. * Department of Plant Pathology, University of California, Davis, CA 95617, § CIBA-GEIGY, Inc, 3054 Cornwallis Rd., Research Triangle Park, NC 27709.

Necrotizing pathogens are known to induce systemic resistance to a challenging pathogen in certain plant species. A multitude of genes, including PR protein genes, are induced prior to this systemic acquired resistance (SAR). SAR 8.2 is a previously uncharacterized PR protein gene family. The SAR 8.2 gene family has at least five active members, and possibly more; the family is inducible by TMV inoculation, as well as by salicylic acid treatment, of *Nicotiana tabacum* cv. Xanthi nc. Computer analysis predicts that the SAR 8.2 proteins are small (7.5 to 9.6 kd), and highly basic (pI 10.0-10.8). The C-terminal domain of the 8.2 protein has homology to metallothioneins and other metal-binding proteins. We have recently reported evidence for protection against *Phytophthora parasitica pv. nicotiana* observed during analysis of plants derived from one transgenic event which expresses constituitively the SAR 8.2 cDNA. We will report on resistance testing of additional SAR8.2 transgenic lines. We are also examining SAR 8.2 transgenic events for susceptibility to other isolates of *Phytophthora parasitica* .

In order to understand the function of the SAR 8.2 protein we are investigating its induction by metal cations, sub-cellular localization, post-translational processing during protein maturation, and proteinase inhibitory activity. Furthermore, we are examining how pre-treatment with Elicitins (proteinaceous elicitors produced by various *Phytophthora parasitica* isolates) affects the resistance or susceptibility of sense and antisense transgenic lines. The transgenic SAR8.2 sense line discussed above is highly resistant to the fungus in the absence or presence of Elicitins.

Y 206 GENETIC ANALYSES OF DOUBLED HAPLOID LINES IN MAIZE: ITS POTENTIAL USE IN GENE MAPPING.

GUITTON C., BENTOLILA S., HARDY T. and FREYSSINET G.

Rhône-Poulenc Agro, BP 9163, 69263 LYON Cédex, France.

The segregation and recombination of a common set of 94 RFLP markers was compared in an F_2 population and an anther culture (AC) derived population. One hundred F_2 individuals and 98 doubled haploid (DH) lines were obtained from the cross R6 (a proprietary line, not responsive to anther culture) x DH.89.1 (a line very responsive to anther culture). Significant deviations from the expected Mendelian segregation ratios for pooled data were observed only for the DH population. The differential transmission of alleles strongly favored the responsive parent (66 of the 69 markers which showed disturbed segregation). There was considerable heterogeneity among the markers showing a skewed segregation towards DH.89.1. This set of markers could be divided into 2 homogeneous subgroups, one comprising 59 markers (% DH.89.1: 70), the other comprising 7 markers (% DH.89.1: 84). Despite these single factor disturbed segregations, MAPMAKER program could be used to construct a linkage map with the DH population. The comparison with the F_2 linkage map demonstrated the consistency of the 2 maps for 97% of the length covered by the DH linkage map. These results are discussed in relation to the use of DH lines in maize breeding and RFLP mapping.

Y 207 ISOLATION OF A FUNGAL SESQUITERPENE CYCLASE GENE INVOLVED IN THE BIOSYNTHESIS OF ARISTOLOCHENE-DERIVED SESQUITERPENOIDS,

Thomas M. Hohn, and Robert H. Proctor, Mycotoxin Research Unit, USDA/ARS, National Center for Agricultural Utilization Research, Peoria, IL, 61604 USA

Sesquiterpene cyclase type enzymes catalyze the first step in the biosynthesis of most sesquiterpenoids. The expression of foreign sesquiterpene cyclase genes in plants may provide a means for altering plant sesquiterpenoid metabolism. Aristolochene and Aristolochene-derived sesquiterpenoids are produced by fungi, plants, and insects. In addition, aristolochene is structurally similar to 5-EPI-aristolochene, the parent compound of the tobacco phytoalexin capsidiol. We have isolated a gene coding for aristolochene synthase (AS) from *Penicillium roqueforti*. The AS gene contains two introns and codes for a polypeptide of molecular weight 39,191. Southern and Northern analyses indicate that the AS gene is present in a single copy and transcribed into an mRNA of about 1350 nt. An AS/Protein A fusion protein has been expressed in *E. coli* and shown to have sesquiterpene cyclase activity. Comparison of the AS sequence with the sequences of related enzymes did not reveal significant homologies.

Y 208 Moleculair characterization of transposition phenomena in *Petunia hybrida*, Henk S.M. Huits [1], Coen C. van der Weijden [1], Alexander G. Admiraal [1], Anton G.M. Gerats [2] and Jacques Hille[1]. [1] Department of Genetics, Free University Amsterdam, De Boelelaan 1087, 1081 HV Amsterdam, Netherlands; [2] Laboratorium voor genetica, rijksuniversiteit Gent, K.L. Ledeganckstraat 35, B9000 Gent, Belgium.

A non-autonomous transposable element, dTph1 has been isolated from petunia line. This non-autonomous transposable element of 284 bp, with 12 bp terminal inverted repeats is flanked by 8 bp target site duplications. Following crossing different petunia lines to a responsive line several lines have been identified that do or do not contain an activator for dTph1 elements. Southern-blot analysis of these petunia lines showed that "non-activator" lines contain a relatively low number of dTph1 related sequences (~ 20), whereas "activator" lines can contain both a low (~ 20) or a high number of dTph1 related sequences (>50). A genomic library was made of a petunia "activator" line harbouring a low copy number. Lambda clones of this library are characterized by using PCR techniques and purifying some of the lambda clones. Six clones have been sequenced. Two clones have a high homology with dTph1. The other clones have only small regions of homology or no homology to dTph1. By analysing more dTph1 related clones dTph families can be identified. The analysis of these dTph1 related sequences will result in information relevant for the isolation of the activating component of the dTph1 system.

Y 209 CHARACTERIZATION OF A BEET CYST NEMATODE RESISTANCE LOCUS IN BETA PATELLARIS AND BETA PROCUMBENS, Rene M. Klein-Lankhorst, Elma M.J. Salentijn, Marion Arens, Wouter Lange and Willem J. Stiekema, DLO Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, The Netherlands.

Resistance to the beet cyst nematode (bcn) Heterodera schachtii Schm. is present in wild beets of the section Procumbentes. This resistance is absent in sugar beet (B. vulgaris) making the transfer of the bcn gene to sugar beet highly desirable. As a first step in cloning the bcn-gene, an analysis of this locus in B. patellaris and B. procumbens was made using nematode resistant B. vulgaris fragment addition lines containing parts of chromosome-1 of B. patellaris or B. procumbens. 5 single copy RFLP markers were identified which are linked to the bcn locus. In addition, RAPD markers and a number of B. patellaris and B. procumbens specific repetitive RFLP markers were obtained. The genomic organisation of the bcn locus was studied by Pulsed Field gel electrophoresis and hybridisation with the molecular markers.

The construction of an addition-line specific YAC-library was initiated by cloning high molecular weight Mlu-1 fragments in the vector pYAC-RC.

Y 210 A COMPARISON OF GENOME ORGANIZATION BETWEEN BARLEY AND WHEAT, Nora L.V. Lapitan and Deana M. Namuth, Department of Agronomy, Colorado State University, Fort Collins, CO 80523.

Barley and wheat are related species that belong to the Triticea tribe of the Gramineae family. Although barley is a diploid, and wheat a hexaploid, the genomes of these two species have common features. Both have a basic chromosome number of 7. Furthermore, it has been determined based on compensation studies that each chromosome in barley is genetically similar (homoelogous) to a chromosome in each of the three genomes of wheat. The objective of this study was to determine the extent of homology between the genomes of barley and a diploid progenitor of wheat, Triticum tauschii, by means of RFLP mapping and other molecular analysis. Preliminary results show that 97% of barley cDNA and genomic clones hybridized to Southern blots containing wheat DNA detected homologous sequences, while 100% of wheat genomic clones detected homologous sequences in barley. These results indicate that the sequence composition of these two genomes are very similar. However, a difference in copy number of the clones in barley and wheat was observed based on the hybridization pattern of the clones on genomic blots. Sixty-seven percent were single copy and 31% were multiple copy in barley, whereas in wheat, 54% were single copy, 28% were multiple copy, and 18% were highly repeated. The chromosome locations and gene order of a common set of RFLP markers were also compared between the two genomes. Preliminary results show that 55% of markers map to the same homoelogous chromosomes, while 45% map to unrelated chromosomes. A comparison of the extent of rearrangements between the linkage groups of the two species is underway.

Y 211 EXPRESSION OF A RIBOSOME INHIBITING PROTEIN (RIP) OR A BACTERIAL CHITINASE LEADS TO FUNGAL RESISTANCE IN TRANSGENIC PLANTS, J. Logemann, G. Jach, S. Logemann, R. Leah[1], G. Wolf[2], J. Mundy[1], A. Oppenheim[3], I. Chet[4] and J. Schell, Max-Planck-Institut f. Züchtungsforschung, Carl von Linné Weg 10, D-5000 Cologne 30, Germany; [1]Copenhagen; [2]Göttingen; [3]Jerusalem; [4]Rehovot.

In order to protect plants against fungal attack, two genes encoding proteins exhibiting antifungal activity in vitro were expressed in transgenic tobacco plants.

1. A barley derived cDNA clone (RIP) encoding a ribosome inhibiting protein. RIP inhibits protein synthesis in fungi by specific RNA N-glycosidase modification of the 28S RNA.

2. A chitinase gene (ChiA) derived from the bacterium Serratia marcescens with the ability to destroy hyphal tips of growing fungi.

Plants expressing RIP-protein or ChiA-protein were infected with the plant pathogenic fungus Rhizocotonia solani. Whereas the growth of control plants was drastically reduced because of root and stem disease, RIP- and ChiA-transgenic plants were growing nearly as fast as uninfected tobacco plants. The fungal resistance of these transgenic tobacco plants was stably inherited. Synergistic expression of both RIP-protein and ChiA-protein in transgenic plants as well as the potential protection of other plant species against fungal infection will be discussed.

Y 212 Cloning of a barley gene, α-hordothionin, and expression in transgenic tobacco. Joyce Maddox, Jon Duvick, Craig Hastings, Helene Rouch, Margit Ross, Sandi Lane and Mike Daywalt, Department of Biotechnology Research, Pioneer Hi-Bred International, Inc., Johnston, IA 50131

Alpha-hordothionin (αHT) is a basic, 45 amino acid peptide belonging to a family of proteins known as thionins. These naturally occurring antimicrobial peptides are found in leaves and/or seeds of several monocotyledenous plants including barley, wheat, oats and rye. In its pure form, αHT inhibits bacteria, yeasts and filamentous fungi at micromolar concentrations. An αHT gene was isolated from barley (*H. vulgare* c v Morex) seed DNA using PCR to produce a genomic clone. Two introns (415 and 91 bp) were spliced out using site-directed mutagenesis to produce a cDNA clone. A dicot expression vector (pPHI1253) was also constructed containing a double cauliflower mosaic virus (CaMV) promoter, a TMV-derived transcriptional leader sequence, and a potato proteinase inhibitor II (PinII) terminator. *In vitro* -grown tobacco cotyledons were co-transformed with pPHI1253 and a plasmid coding for Basta® resistance, pPHI1285, via particle gun bombardment. Three of eleven PCR-positive regenerated plants expressed αHT at levels detectable by western blot (3+ ppm protein) using rabbit anti-αH T IgG. Further characterization of the transgenic protein and its biological activity is in progress.

Y 213 EXPRESSION OF THE TOBACCO VEIN MOTTLING VIRUS NUCLEAR INCLUSION PROTEIN (NIa) GENE IN TOBACCO, Indu B. Maiti, and Arthur G. Hunt, Plant Physiology/Biochemistry/Molecular Biology Program, Department of Agronomy, University of Kentucky, Lexington, KY 40546-0091.

The tobacco vein mottling virus (TVMV) 53 kDa nuclear inclusion protein (NIa) gene was expressed in tobacco (*Nicotiana tabacum* cv burley 21) as a single gene or as a protein fusion with the *E. coli glnH* gene. The NIa-glnH construct had a consensus NIa cleavage site at its C-terminus to yield separate NIa and *glnH* gene products; this was done to provide a convenient assay for the protease activity of NIa in transgenic plants. Five independent NIa and NIa-*glnH* transgenic lines were generated. Western blot analyses for the *glnH* gene product in three NIa-*glnH* lines showed expression levels of about 0.2% of total soluble leaf protein. In addition, the *glnH* gene product was of a size consistent with that expected if the fusion protein was cleaved at the NIa cleavage site, indicating that the NIa protease was active. In a preliminary study, seedlings from one NIa-*glnH*-containing line were inoculated with tobacco etch virus (TEV), potato virus Y (PVY), and TVMV and observed for the development of disease symptoms. TEV- and PVY-infected plants developed normal symptoms whereas TVMV-infected plants showed no symptoms after 21 days. These preliminary studies indicate that expression of the protease gene can effect the response of plants to the homologous virus.

Y 214 GENOME MAPPING, PHYLOGENETIC ANALYSIS AND GENE TAGGING IN THE TROPICAL PASTURE LEGUMES STYLOSANTHES USING RAPD GENETIC MARKERS, John M. Manners, Kemal Kazan and Don F. Cameron, CSIRO, Division of Tropical Crops and Pastures, The Cunningham Laboratories, Carmody Road, St. Lucia, Brisbane, 4067 Australia.

The genus Stylosanthes is of high economic importance as a pasture legume in tropical regions around the world. Although it is amenable to improvement via breeding and molecular genetic transformation, little is known of its genome organisation and no linkage groups and few genetic markers have been described. We report here the development of Random Amplified Polymorphic DNA markers (RAPDs, Williams et al. 1990 Nucl. Acids Res. 18: 6531) for genetic analysis of this genus. A survey of twenty genotypes representing four species of Stylosanthes has revealed high levels of inter-specific DNA polymorphisms between species using 20 PCR primers. Phenetic analysis of the data indicated the following order of decreasing genetic similarity: S. humilis, S. hamata, S. scabra and S. guianensis. The RAPD analysis supports the contention that S. humilis is one diploid progenitor of the allotetraploid S. hamata and also is able to distinguish individuals within a species which have been suspected of being divergent on the basis of morphological analysis and sexual compatibility. RAPDs have also been shown to be useful for distinguishing proprieted cultivars. Genetic mapping has been undertaken using the F2 population of an inter-specific cross between S. scabra and S. hamata. Most RAPD markers were inherited in a Mendelian manner but some exceptions were observed. So far, 46 loci have been mapped in 10 linkage groups proving the value of RAPDs for mapping in this genus. The ability to use RAPDs for linkage analysis provides a means to tag genes of economic importance in this genus. Currently near-isogenic lines and bulked segregants are being prepared for the RAPD tagging genes for resistance to anthracnose disease which is a major agricultural problem in this legume.

This work was funded by the Australian Meat Research Council.

Y 215 SUBCELLULAR LOCALIZATION OF ACIDIC AND BASIC CHITINASES IN CERCOSPORA INFECTED SUGAR BEETS

J.D. Mikkelsen, K.K. Nielsen, J. Nielsen, L. Janniche, and K. Bojsen.

MARIBO Seed, Biotechnology, 1, Langebrogade, DK-1001 Copenhagen K, Denmark.

Thirteen different chitinase isozymes have been purified to homogeneity from sugar beet leaves infected with Cercospora beticola. When analyzed by Immuno-blotting polyclonal antibodies could classify the 13 chitinase isozymes into four serologically different classes, SP, SE, CH2, and CH4. SP and SE are acidic proteins whereas CH2 and CH4 are basic proteins with pI of 4.2, 3.0, 8.9, and 9.1, respectively.

By immunocytochemical analysis on both light and electron-microscopy level we have shown that both the acidic and basic chitinases accumulate around the necrotic spots of Cercospora induced lesions. Both the basic and acidic proteins were deposited extracellularly and not in the central vacuole of the plant cell. In accordance with this, analysis of the gene structure of the basic sugar beet chitinase, CH4, revealed that no signal peptide for vacuolar targeting was present.

Y 216 A STRATEGY FOR GENE TARGETING IN BARLEY USING MEIOTIC RECOMBINATION. Peter Mouritzen and Preben B. Holm, Carlsberg Research Laboratory, 10, Gl. Carlsberg Vej, DK-2500 Copenhagen, Valby, Denmark.

In higher plants a number of transformation techniques are available to introduce foreign DNA into the genome of the plant cell. It is however a major limitation of these techniques that the introduced DNA integrates virtually at random in the plant genome. This implies that gene targeting, i.e., the modification of a chromosomal locus by homologous recombination between the endogenous gene and a modified counterpart is very rarely achieved. As an alternative to the conventional approaches of developing techniques for gene targeting in somatic cells we propose to explore the natural process for facilitated homologous recombination, i.e., meiosis. As a working hypothesis we consider that gene targeting in higher organisms during meiosis may be achieved in either of two ways: 1) By short conversions where DNA segments of partial homology to an endogenous gene are introduced when synapsis of the homologues are initiated, i.e., at the beginning of zygotene. 2) By double crossovers at pachytene between an introduced large segment, such as a yeast artificial chromosome (YAC), and a segment of partial homology in the plant genome. In order to test this hypothesis we have developed a technique, which allows the culture of barley cv. Igri anthers in isolated spikes and spikelets through meiosis until the mid-late uninucleate microspore stage whereafter plants are regenerated by microspore embryogenesis. In the isolated spikelets the top half of the anthers is exposed and thus accessible for particle bombardment and microinjection. Light microscopical analyses and measurements of the duration of the individual substages of meiosis and microsporogenesis revealed a developmental sequence indistinguishable from that observed in spikes on plants. Embryogenesis was induced in microspores at the mid - late uninucleate stage and green plants were regenerated from the embryoids. On a modified Murashige-Skoog medium with 100 g/l maltose and 1 mg/l 6-benzyl amino purine 62 % of the anthers survived, while 51 % of these anthers responded with microspore embryogenesis and a mean of 2.4 green plants were regenerated per responding anther. The albino to green plant ratio was 0.5.

Y 217 EFFECT OF FUNGAL SUPPRESSOR OF HYPERSENSITIVITY ON CHITINASE IN PLANT, Naotaka Furuichi and Satomi Hirai, Faculty of Agriculture, Niigata University, 950-21and Bio Science Laboratory, Yamagata 990, Japan

We reported that treatment of potato leaf disks with suppressor oligosaccharide isolated from *Phytophthora infestans* inhibits the accumulation of phytoalexin, rishitin, and hypersensitive cell death of potato cells. In contrast, Mr 4700 glucan isolated from the fungus enhance the rishitin accumulation in potato cells when unsaturated fatty acids elicitor of the fungus are treated with these glucans in the host cells. To investigate the effect of suppressor on defense genes of potato, we observed the mRNA level of basic chitinase after the pretreatment of the Mr 4700 glucan (200 ppm) for 2 hr following the hyphal cell wall components (HCW, 500 ppm) treatment by using vacuum infiltration.

The results showed that the glucan enhance the activation of chitinase mRNA caused by the HCW at 120 min and 240 min after the treatment.

Our data indicate that the glucan of *P. infestans* has ability of both suppressor of hypersensitivity and enhancer of defense gene in potato.

Y 218 PROGRESS TOWARDS MAPPING QTLs FOR WOOD SPECIFIC GRAVITY IN LOBLOLLY PINE, David B. Neale, Andrew T. Groover, Claire G. Williams[1], Robert A. Megraw [2], Troy A. Fiddler, Curtis L., Tucker, and Michael E. Devey, Institute of Forest Genetics, USDA Forest Service, Placerville, CA 95667, [1]Weyerhaeuser Company, PO Box 1361, New Bern, NC 28563-1391, [2]Weyerhaeuser Company, 32901 Weyerhaeuser Way South, Federal Way, WA 98003

We are attempting to map quantitative trait loci (QTL) which determine wood specific gravity (wood density) in loblolly pine (Pinus taeda L.). A three-generation outbred pedigree was chosen for the mapping population. Pairs of grandparent trees were chosen which had extreme values for wood specific gravity (Range .45-.58). We measured specific gravity for 280 F_2 progeny resulting from the mating of the two F_1s. We intend to employ selective genotyping. Thus, we will select 100+ progeny from the extremes of distribution of specific gravity values.

Previously, we constructed a low-density RFLP map for loblolly pine using a second three-generation pedigree. This map currently includes 100+ mapped cDNA and genomic probes. We expect that a large number of these probes will segregate and can thus be mapped in the specific gravity cross. RFLP-QTL cosegregation analysis will be conducted using a set of 200 marker loci.

Currently, statistical methods do not exist for mapping QTLs in outbred tree pedigrees. We are working to extend the interval mapping approach of Lander and Botstein (1990) for mapping QTLs in trees.

Y 219 MODIFICATION OF LIGNIN BIOSYNTHESIS BY GENETIC MANIPULATION OF CAFFEIC ACID O-METHYLTRANSFERASE, Weiting Ni, Nancy L. Paiva and Richard A. Dixon, Plant Biology Division, The Samuel Roberts Noble Foundation, Ardmore, OK 73402

Lignin is a major cell wall component which lends mechanical rigidity to plant tissues. Furthermore, lignin deposition is a common response of plants to infection or wounding. Our laboratory has cloned caffeic acid O-methyltransferase (COMT), a key enzyme of lignin monomer biosynthesis, from alfalfa. COMT methylates caffeic acid to form ferulic acid. Blocking COMT activity in vivo might reduce overall lignin content, and increase the relative proportion of p-coumaryl units, compared to guiacyl and syringyl units, in the polymer. We have made gene constructs for the overexpression and underexpression (by antisense) of COMT in transgenic alfalfa and tobacco, and in electroporated protoplasts. Preliminary experiments indicate that the transient expression of these constructs in alfalfa protoplasts does not lead to inhibition of the expression of COMT activity, even though COMT transcript levels are very low. We will analyze stably transformed plants for lignin content and composition, and relate this to fungal pathogen resistance. A second goal of this project is to improve forage digestibility in alfalfa by reducing lignin content.

Y 220 PLANT-PARASITIC NEMATODES: GENE STRUCTURE AND A NEW HOST, *Arabidopsis thaliana*, Charles E. Novitski, Department of Biology, Central Michigan University, Mt. Pleasant, MI 48859.

Plant-parasitic nematodes and their interactions with plant hosts are still poorly understood at a molecular level. We have explored the structure of the plant parasitic-nematode gene structure, by determining the sequence of members of the gene family of the major sperm proteins of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. The promoters have family-member-specific sequences as well as typical eukaryotic features; introns at evolutionarily conserved sites are observed. Using fluorescently tagged monoclonal antibodies we have also explored nematode gene products, some of which are secreted by soybean cyst nematode, *Heterodera glycines*, esophageal glands into plant root tissue during the establishment of nematode-induced plant syncytial cells.

Plant-parasitic nematodes lie at the intersection of two valuable model systems, *Arabidopsis thaliana* and *Caenorhabditis elegans*. *Arabidopsis* can be infected by the sugar beet cyst nematode, *Heterodera schachtii*, and by other cyst, root-knot and lesion nematodes. This opens the door to an accelerated genetic analysis of these host-parasite interactions, and should lead to a better understanding of nematode resistance promoters and genes, which can be transferred to, or identified in, other plant species.

Y 221 ACTIVATION OF DEFENSE-RELATED GENES IN COTYLEDONS OF MEDICAGO SATIVA BY SPECIES AND RACES OF COLLETOTRICHUM. Nichole R. O'Neill, USDA, ARS, Beltsville, MD 20705.

The phenomenon of induced disease resistance to fungal infection in plants has been described many times in the plant pathological literature. The transcriptional activation of genes coding for enzymes which lead to the accumulation of isoflavanoid phytoalexins has been implicated in this resistance. In alfalfa, the accumulation of the phytoalexin medicarpin is one of the responses specifically associated with cultivars with genes for resistance to anthracnose during attempted infection by Colletotrichum trifolii. Although there is evidence that medicarpin may be an important determinant of resistance, the specific role of medicarpin in limiting fungal ingress or growth, and the chronology of its accumulation during induced resistant interactions suggest the presense of a number of defense mechanisms. The induced defense responses in alfalfa cotyledons with the An_1 or An_2 genes for anthracnose resistance will be presented.

Y 222 ANALYSIS OF A GENOMIC CLONE FOR ISOFLAVONE REDUCTASE - A DEFENSE RESPONSE GENE FROM ALFALFA, Abraham Oommen, Nancy L. Paiva and Richard A. Dixon, The Noble Foundation, P.O. Box 2180, Ardmore, Oklahoma 73402.

The penultimate step in the biosynthesis of medicarpin, a major isoflavonoid phytoalexin in alfalfa, requires the enzyme isoflavone reductase (IFR). Using a cDNA clone for IFR (1) as probe, we have isolated a clone from an alfalfa genomic library. This clone contains the entire coding region and 435 bp of the putative promoter in a 2.4 kb EcoRI fragment. An additional 2.0 kb upstream of the 435 bp promoter region is within a PstI/HindIII fragment. Sequencing of the 2.4 kb EcoRI fragment revealed 4 introns in the coding region. Except for the TATA box and a potential CAAT box, no known cis-element consensus sequences were found within the 435 bp promoter region. The start of transcription was identified by primer extension and agrees with the site predicted by computer analysis of the sequence. IFR is constitutively expressed only in roots and root nodules. In cell cultures of alfalfa, IFR transcription can be induced more than 35-fold using fungal elicitors (1). Promoter-GUS fusions are being made with the clones we have to study (a) the inducibility of the promoter and (b) the tissue and organ specific expression of IFR, and to dissect the cis-elements that control (a) and (b).
Potential applications of infection-inducible promoters for crop improvement are being explored.

1. Paiva et. al. Plant Mol. Biol. 17, 653-667 (1991).

Y 223 MOLECULAR MARKERS FOR GENETIC ANALYSIS OF THEOBROMA cacao, J.K. Osei and P.J. Fritz
ACRI-Cocoa Molecular Biology Lab., Food Science Dept., Penn State University, University Park, PA 16802

The commercially valuable cocoa tree (Theobroma cacao), 2n=20, genome size=200Mbp, family Sterculiaceae, originates near the Amazon river headwaters but much of the crop is produced in West Africa, Brazil and Malaysia within 20° N/S of the equator. Fat expressed from the fermented, dried and roasted seeds is used to manufacture chocolate and cosmetics. Cocoa powder from the seeds is the source of chocolate flavor.

High yielding, early maturing, disease resistant genotypes are selected from three main populations, namely Upper Amazon forasteros, Lower Amazon forasteros and Trinitarios to breed F1 commercial varieties. Progress in breeding has been slow because of the long generation time (3-5 years), the complex polygenic inheritance of desirable characters, and the absence of a linkage map.

In order to accelerate breeding, a linkage map for cocoa is greatly needed. To this end, probes including a 21 kDA cocoa cDNA encoding a trypsin inhibitor, a cocoa seed vicilin gene, and a cocoa stearoyl acyl desaturase gene are being used to detect RFLP markers that can be used to construct a linkage map for cocoa. RAPD markers are also being used in PCR reactions to detect more polymorphisms.

Cocoa cultivars Catongo and Pound 12 and their backcross progeny are being used in the study. Catongo is a white seeded, self compatible Lower Amazon forastero discovered in Bahia, Brazil. It is moderately resistant to Phytophthora palmivora. Pound 12 is a purple seeded, self incompatible, Upper Amazon forastero, susceptible to P. palmivora.

The Catongo X (Catongo X Pound 12) backcross segregated into 1:1 purple:white seeds. The height at first jorquette (first plagiotropic branching) of 112 greenhouse grown seedlings varied from 60cm to 100cm nine months after planting. One plant flowered nine months after planting and three plants flowered 11 months after planting. These highly desirable indications of early maturity are much prized in cocoa breeding which will be greatly accelerated when molecular markers that correlate with such desirable characters are found.

Support from the American Cocoa Research Institute (ACRI) and from the African-American Institute is gratefully acknowledged.

Y 224 *Abstract Withdrawn*

Y 225 GENETIC ANALYSIS OF RESISTANCE TO *Gibberella zeae* IN MAIZE BASED ON RFLP MARKERS, M. Enrico Pe, Paolo Angelini*, Giorgio Bineili, Maria Dani*, Luca Gianfranceschi and Graziana Taramino, Department of Genetics and Microbiology, University of Milan, Milan, Italy, *Enichem Agricolture Research Center, Massa, Italy

Gibberella stalk and ear rot is a widespread disease of corn, whose phenotypical expression is modulated by environmental factors, mainly by weather. Thus, data obtained from analysis of cultivated hybrids and/or inbred lines do not allow direct estimation of genetic differences as distinct from non-heritable effects. Our experimental approach to obtain informations about the genetic basis of susceptibility to Gibberella infection was to estimate the genetic correlation between RFLPs and the character, measured as the amount of diseased tissue 40 days after an inoculum of a conidial suspension of *Gibberella zeae* in the first stalk internode. A sensitive and a resistant parental inbred were crossed to obtain F_1 and F_2 populations: the analysis of the segregation of 106 RFLP clones was performed on a population of 150 F_2 individuals. The analysis of the degree of infection was made on the F_3 families obtained by selfing the F_2 plants used for RFLP analysis. The experimental plan was a Randomized Blocks Design. QTL detection was based on the regression of family mean value on the value attributed to the RFLP polymorphic loci in the F_2 population, using the GLM procedure of SAS.

Y 226 *Abstract Withdrawn*

Y 227 *Ac* INDUCED INSTABILITY AT THE TOMATO *XANTHOPHYLLIC* LOCUS.

Peter W. Peterson and John I. Yoder, Department of Vegetable Crops, University of California, Davis, CA 95616.

We are using a whole plant genetic marker to detect genomic instability caused by the maize *Ac* transposable elements in tomato. The incompletely dominant *Xanthophyllic* (*Xa*) gene causes leaves to be yellow. Somatic instability at the *Xa* locus appears as sectors which have lost the yellow phenotype. Transgenic *Ac* containing tomato plants which differ in the location and number of their *Ac* elements were crossed to *Xa* tester lines. Of 800 test and control F1 progeny screened, only 4 plants showed significant somatic sectoring. All 4 of the plants were derived from multiple copy *Ac* parents. Three plants had green/white twin sectors and the other had green/pale green twin sectors.

The sectoring phenotypes were inherited and were mapped relative to the *Xa* locus. The green/pale green locus maps less than 10cM from the *Xa* locus. The green/white sectoring loci are also linked to *Xa* but on the other homeolog. We believe that the two phenotypes arise from the same mechanism; the only difference being that the sectoring loci are on the homologous chromosomes. The green/pale green phenotype cosegregates with one particular *Ac*. The high rate of *Ac* transposition in the green/white sectoring plants has made indentifing a cosegregating *Ac* difficult.

In other systems twin sectors can result from either mitotic recombination or chromosome breakage followed by breakage-fusion-bridge cycle. We will determine if either mechanism causes our twin sectors by probing DNA from twin sectors with RFLP markers which flank the *Xa* locus. Since altered *Ac* elements can cause chromosome breaks we are cloning and determining the integrity of the cosegregating *Ac* element(s) .

Y 228 PLUM POX VIRUS COAT PROTEIN MEDIATED RESISTANCE IN TRANSGENIC TOBACCOS.

Michel Ravelonandro, Pierre Yves Teycheney, Marie.Monsion, René Delbos and Jean Dunez. Station de Pathologie Végétale, INRA, BP 81, 33 883 Villenave d'Ornon, France.

Plum pox virus (PPV) coat protein gene was cloned into plant expression vector and was then expressed in Nicotianae plants in attempt to produce resistant plants. Two species of tobacco were used, Nicotiana tabacum cv xanthi against potato virus Y (PVY) and Nicotiana benthamiana against PPV. Transgenic and non-transgenic Nicotianae were respectively challenged with PVY or with PPV. The plants were later evaluated by symptomatology and ELISA to determine infection. Systemic infection corresponding to green island and mottling were present in non-transformed Nicotiana benthamiana and non-protected transgenic plants when infected by PPV. Susceptibility to infection by PVY of inoculated plants was discriminated from 10 days after inoculation. We have identified a few plant lines that are resistant when compared to the control plants. Other lines have intermediate level of resistance and the rest that express PPV capsid have no protection. ELISA tests were correlated with infectivity. Analysis of ribonucleic acid extracts from each sample of Nicotiana benthamiana by dot-blot hybridization with molecular probes corresponding to a cDNA of PPV helper component gene indicated that PPV RNA is not present in immune plants. Our results confirm again the data obtained with transgenic plants that express a potyviral capsid conferring protection against infections by homologous and heterologous potyviruses.

Y 229 MOLECULAR MARKER ANALYSES OF MAIZE POPULATIONS INVOLVED IN LONG TERM SELECTION EXPERIMENTS,

Torbert R. Rocheford, Jay R. Sughroue, and Irwin L. Goldman, Department of Agronomy, University of Illinois, Urbana, IL 61801

We are performing molecular marker analyses on the Illinois long term selection chemical strains and Iowa Stiff Stalk Synthetic recurrent selection materials. We are characterizing molecular marker allelic frequency differences among generations 65 and 90 of the Illinois high oil, low oil, reverse high oil, reverse low oil as well as the Illinois high protein, low protein, reverse high protein, and reverse low protein selection strains. We have detected considerable molecular marker polymorphism within and among the various long term selection strains. We have detected molecular marker allelic frequency changes on chromosome arms 1L, 2S, 3L, 4S, 5L & 6L in the oil selection strains and on chromosome arm 5L in the protein selection strains. We are currently mapping quantitative trait loci controlling % protein, % starch, and % oil in F3 families developed from crosses of the high and low protein strains and oil strains. These investigations represent a transition from classical quantitative genetic analyses to molecular genetic analyses of the Illinois Long Term Selection materials. We have detected significant change in the ribosomal spacer variant composition between the lines that comprised cycle 0 of Iowa Stiff Stalk Synthetic (BSSS) versus cycle 7 of BSSS and cycle 11 of BSSS(R) that have undergone 7 and 11 cycles of selection for agronomic performance, respectively. Molecular marker analyses with single copy probes close to the NOR region have revealed no clear linkage of these markers with the ribosomal spacer variants that have increased in frequency with selection. This suggests the ribosomal variants may be under direct selection in these two populations.

Y 230 GENETIC ANALYSIS OF TOMATO YELLOW LEAF CURL VIRUS (THAILAND), Dean E. Rochester, Claude M. Fauquet, and Roger N. Beachy, Department of Biology. Washington University, St. Louis, MO 63130

The genome of the geminivirus, Tomato Yellow Leaf Curl Virus (Thailand), has been cloned and the entire nucleotide sequence determined. Analysis of the cloned DNAs indicated that the genome was comprised of two DNAs, namely A- and B-DNA. Location and sequence similarity of the open reading frames on the DNAs indicated relatedness to the bipartite geminiviruses (Subgroup II). Five open reading frames (ORFs) capable of encoding proteins greater than 10 kilodaltons in molecular weight were found on the A-DNA and two such ORFs were found on the B-DNA. Generally, the highest sequence similarity was found in comparisons with African Cassava Mosaic Virus. Systemic infection was possible following agroinoculation with only the A-DNA. At the same time, infection with both A- and B-DNAs resulted in more severe symptoms than with A-DNA alone. Mutation of the coat protein ORF greatly decreased the efficient systemic infection properties of the geminivirus. Properties of Tomato Yellow Leaf Curl Virus (Thailand) will be presented with special emphasis on the relationships to other geminiviruses of all three established subgroups.

Y 231 REGULATORY ELEMENTS FOR TRANSCRIPTIONAL ACTIVATION DURING LATE BLIGHT DISEASE IN POTATO, Günter Strittmatter, Norbert Martini and Irmgard Rüntz, Department of Biochemistry, Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30, FRG.

Development of late blight disease in potato is accompanied by transcriptional activation of a pathogenesis-related gene (*prp1-1*) which shows structural similarity with the gene encoding heat-shock protein HSP26 in soybean. Among different stresses tested, only infection of plants with fungal spores and treatment with fungal elicitor or salicylic acid can induce the accumulation of PRP1 mRNA. The regulatory function of *prp1-1* upstream fragments was determined by measuring the expression of chimeric promoter/GUS-gene constructs during compatible interactions of potato cultivar Désirée (R0) with *Phytophthora infestans* race 4. A 273 bp long promoter segment turned out to be sufficient for rapid transcriptional activation at infection sites. Basal activity of this element before infection was only detected in tips of stolons as analyzed by histochemical GUS-staining of tissue sections from transgenic potato plants. Gel-retardation assays proved the interaction of the 273 bp long promoter fragment with DNA binding proteins *in vitro*. Because of its unique characteristics the hereby identified regulatory region is ideal to drive the synthesis of products that interfere with the development of the disease at early stages.

Y 232 TRANSGENIC SQUASH PLANTS EXHIBIT COAT PROTEIN MEDIATED PROTECTION UNDER FIELD CONDITIONS, David M. Tricoli*[1], Kim J. Carney[1], Hector Quemada[1], Paul F. Russell[1] J. Russell McMaster[1], Maury L. Boeshore[1], David W. Groff[2], Keisha Hadden[2], Jon P. Hubbard[3], [1]Experimental Plant Genetics, The Upjohn Company, Kalamazoo, Michigan 49001, [2]Southeast Breeding Station, Asgrow Seed Company, Tifton, Georgia 31794, [3]Plant Pathology Research Center, Asgrow Seed Company, San Juan Bautista, California 95045 Using *Agrobacterium tumefaciens* transformation procedure we have produced squash plants that express one or more coat protein (CP) genes from cucumber mosaic virus (CMV), watermelon mosaic virus-2 (WMV-2) or zucchini yellow mosaic virus (ZYMV). Segregating R_1 or R_2 progeny from selected transgenic lines were germinated in the greenhouse and evaluated for neomycin phosphotransferase (NPTII) enzyme activity in order to identify those seedlings which contain the linked coat protein gene(s). Both the CP+ and CP- segregants were mechanically inoculated in the greenhouse with virus. Approximately one week after inoculation the plants were transplanted into the field where they were subjected to natural virus inoculation by aphids. Both mechanically inoculated and non-inoculated control plants were included in the trial in order to evaluate the efficiency of our mechanical inoculations, as well as to monitor for natural virus spread by aphids. In these trials, we have identified squash lines that exhibit a significant level of protection against more than one virus. The foliage and fruits of these plant lines remained non symptomatic even though 100% of both the mechanically and nonmechanically inoculated control plants become symptomatic. These plant lines have been incorporated in our breeding program and will be further tested in 1992.

Y 233 CLONING AND CHARACTERIZATION OF POKEWEED ANTIVIRAL PROTEIN, Nilgun Tumer, Jennifer Lodge, Wojciech Kaniewski and Keith O'Connell, Monsanto Agricultural Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

Pokeweed antiviral protein (PAP) is a ribosome inhibiting protein found in the cell walls of *Phytolacca americana*. Three forms of the protein with limited amino acid similarities have been identified, including PAP from spring leaves, PAP-II from summer leaves and PAP-S from seeds. We demonstrated that exogenously applied PAP-S is very effective against viruses from different groups, including DNA viruses. We isolated overlapping cDNA clones encoding the 5' and 3' halves of PAP and constructed a clone containing the entire coding sequence. The derived amino acid sequence matches the known sequence of N-terminus of PAP and has homology to tryptic fragments of PAP-S. This clone hybridizes to at least ten DNA fragments on a genomic Southern blot suggesting that PAP is a multigene family. The clone also hybridizes well to RNA from leaves but poorly to RNA from seeds, suggesting that although the PAP and PAP-S proteins are related at the amino acid level, their genes are not highly conserved at the nucleotide level.

Y 234 EXTRACELLULAR TARGETED VACUOLAR PR-PROTEINS EXHIBIT ANTIFUNGAL ACTIVITY IN THE EXTRA CELLULAR FLUID OF TRANSGENIC PLANTS, Marianne B. Sela-Buurlage, Leo S. Melchers, Sandra A. Vloemans, Charles P. Woloshuk, Jeroen S.C. van Roekel, Peter J.M. van den Elzen and Ben J.C. Cornelissen, MOGEN International nv, Einsteinweg 97, 2333 CB Leiden, The Netherlands.

Induced resistance in tobacco is accompanied by the increased expression of at least 5 groups of proteins, PR-1 to PR-5. Within each group one set of proteins accumulates in the extracellular space and has a low pI. A second set of basic highly homologous proteins (high pI) accumulates in the vacuole.

The PR-2 group contains β-1,3-glucanases while the PR-3 group consists of proteins with chitinase activity. Recently, we demonstrated that one of the basic PR-5 proteins, AP24, is able to lyse *sporangia* of *Phytophthora infestans in vitro*.

In this study we demonstrated that purified intracellular chitinases have an antifungal effect on *Fusarium solani in vitro* by lysis of germ tubes. Addition of intracellular glucanase synergistically enchances this effect. Extracellular chitinases and glucanases, however, do not show any antifungal nor synergistic effect against this fungus.

In order to obtain fungal resistance in plants by introducing an extra defense barrier, we targeted AP24, intracellular chitinase and intracellular glucanase to the extracellular space of tobacco leaves. We demonstrated that the C-terminal propeptide of tobacco AP24, intracellular chitinase and intracellular glucanase is necessary for correct sorting of these proteins to the vacuole. In addition, we showed that these proteins have retained their antifungal activity: Intercellular fluids, isolated from these transgenic plants have a significant *in vitro* effect on *Phytophthora infestans* and *Fusarium solani* respectively.

Y 235 DEVELOPMENT OF A WOUND-INDUCIBLE PROMOTER SYSTEM FOR GENE EXPRESSION IN TRANSGENIC CEREALS, Deping Xu[1], David McElroy[1], Jun Cao[2], Wanggen Zhang[1], and Ray Wu[1,3], [1]Field of Botany, [2]USDA-NSF-DOE Plant Science Center, and [3]Department of Biochemistry, Cornell University, Ithaca, New York 14853, USA.

Recent progress in gene transfer technology has made it possible to express natural insecticidal proteins in transgenic cereal plants. Before evaluating the utility of such transgenic cereals we need to anticipate the evolution of insect resistance and the need limit the production of such proteins to specific plant parts to conserve plant metabolic energy. As a first step towards producing cereals expressing insecticidal proteins in response to specific damage thresholds we have optimized the wound-inducible promoter of the potato proteinase inhibitor II gene (*PinII*) for use in rice transformation. In transgenic rice plants a *PinII-Gus* fusion construct showed a weak wound response. However, inclusion of the first intron of the rice actin 1 gene (*Act1* 5'-intron) in the 5'-transcribed region of the *PinII-Gus* fusion resulted in a high level systemic wound response. The pattern, magnitude and kinetics of *PinII - Act1* 5' intron - *Gus* fusion gene expression in wounded and non-wounded rice plants was determined by *in situ* histochemical localization and quantitative analysis of both *Gus* mRNA levels and GUS specific activities. In wounded transgenic rice plants the activity of the optimized *PinII* promoter appeared highest in leaf and root vascular tissue, leaf trichome and epidermal cells, root tip and root zone of elongation. The activity of the *PinII* promoter in transgenic rice was increased over 10 fold by wounding and this wound-induction was increased a further 15 fold by the presence of the *Act1* 5'-intron. The kinetics of induction of the optimized *PinII* promoter in transgenic rice was similar to what is found in potato, with maximal mRNA and protein levels being found 24 hours after wounding. The *PinII* fusion constructs have also been found to be active in transformed maize, barley and sorghum tissue. A series of optimized *PinII*-based expression vectors for use in cereal transformation have been developed and these vectors are being used to express natural insecticidal proteins in transgenic rice plants.

Y 236 SOMATIC TAGGING IN TOMATO, John I. Yoder, Francois Belzile and Peter Peterson, Department of Vegetable Crops, University of California, Davis, CA 95616. The maize transposable element *Ac* is active during the somatic development of transformed tomatoes. High levels of activity can lead to the rapid proliferation of *Ac* elements throughout the tomato genome in a single generation. For example, we isolated 24 different *Ac* insertions from a single plant whose parent had only one copy of *Ac*. We localized 20 of these insertions to nine of the twelve tomato chromosomes by RFLP mapping; only one of the transposed *Ac*'s was linked to the donor T-DNA. Therefore, somatic transposition can lead to the rapid distribution of elements to genetically unlinked sites.

Somatic transposition of *Ac* elements to multiple chromosomal sites suggests that a number of different insertional events are likely represented on a single plant. For species like tomato which are indeterminate and amenable to tissue culture, the tagged gene can potentially be cloned from tissue derived vegetatively from the somatic sector. To test the efficacy of somatic tagging, we targeted three endogenous tomato loci whose mutant phenotypes are readily apparent in vegetative sectors: 1) *Xanthophyllic* (*Xa*), an incompletely dominant gene on chromosome 10 which causes leaves to be bright yellow; 2) *White anthers* (*Wa*), a dominant gene which causes anthers, normally yellow, to be white; and 3) *Ms48*, a dominant male sterility gene which conditions the complete absence of pollen. Tester lines bearing these markers were crossed to 32 independently derived tomato lines containing active *Ac* elements. Transgenic lines in which *Ac* had been lost through genetic segregation were used as controls. Because each of the marker genes were dominant, their expression could be scored directly in the F1 hybrids.

In one F1 individual carrying the *Xa* marker, we localized an active *Ac* to within 10 cM of the locus (see abstract by Peterson and Yoder). From over 20,000 progeny derived from this individual, we identified plants which had yellow, *Xa*-like sectors consistent with an unstable insertion into the *Xa* gene. In a related transgenic field experiment, 2500 tomato plants bearing *Ac* were scored for the loss of the accompanying *Ms48* marker by screening for fruit set. We estimate that well over 25 million flowers were potentially scored for loss of *Ms48* in this targeted tagging experiment. The molecular analyses presently underway should be illuminating as to the mechanism of instability at the *Xa*, *Ms48* and *Wa* loci.

Y 237 GENE TRANSFER IN CORN AND ALFALFA BY POLLEN ELECTROTRANSFORMATION, Camelia R. Smith and James A. Saunders, SARL, USDA/ARS, Beltsville, MD 20705

Pollen is nature's vector used to transfer genetic material during pollination of plants. We are developing a pollen electrotransformation system to create transgenic plants by electroporating germination pollen in the presence of foreign DNA. Reversibly permeabilized membranes allow the influx of genetic material into the pollen which may be subsequently incorporated into the putative transformed seed. This technique eliminates the cost, time and involvement that tissue culture and protoplast regeneration entails. We have successfully used both square wave and exponential discharge pluses (BTX models 200 and 600), with a 2mm gap electrode chamber to transfer pCAT in corn and alfalfa pollen. Species specific modifications in the electroporation media maintain the viability of germinating pollen while optimizing the DNA uptake. The optimal amplitude of the electroporation pulse is characteristic of the pulse shape. For example, typical results show that a positive ^{14}C chloramphenicol acetyltransferase assay is obtained with a 4.5 kV/cm, 80μsec square wave pulse. In alfalfa, using an exponential pulse, transient CAT expression was obtained with a 4.83 msec pulse of 7.5 kV/cm. Optimizing these parameters with a transient assay provides positive operational guidelines for the development of transgenic seed by this technology. The ability to maneuver genetic material by pollen electrotransformation could have tremendous impact in the improvement of economic crops which are recalcitrant to other methods of gene transfer.

Genes and Gene Expression for New Traits-Food Quality; Technology Transfer to Developing Countries

Y 300 ISOLATION AND CHARACTERIZATION OF cDNAS AND GENOMIC CLONES OF POTATO INVERTASE INHIBITOR, Christine Blomendahl, Heike Glaczinski, Joel Vandekerkhove, Meike Köster-Töpfer, Francesco Salamini, Christiane Gebhardt, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne Weg 10, D-5000 Köln 30

Potato tubers, when stored at cold temperatures, accumulate the reducing sugars glucose and fructose which causes browning of the tubers during the frying process (so called Maillard reaction). The level of reducing sugars is correlated in a temperature and genotype dependent manner to the activities of invertase and its proteinaceous inhibitor.

Sequencing of isolated invertase inhibitor protein revealed an N-terminal sequence of 15 amino acids. Using a degenerated oligonucleotide for the 5' end and a specific oligonucleotide for the 3' end of 1st strand cDNAs we were able to amplify in a Polymerase Chain Reaction a 0.75 kb fragment which was cloned and used for screening a cDNA library. Two of the resulting clones were sequenced and found to show high homology between each other. The clones encode proteins of the expected size of about 21 kd for the mature protein of 193 amino acids and show 28 amino acids for a presumed signal peptide. Northern blot analysis revealed temperature dependet RNA levels as a function of time. Antibodies raised against the purified inhibitor protein recognized the cDNA encoded proteins when expressed as fusionproteins in E. coli. Using one of the cDNA clones a genomic library was screened resulting in 8 clones. Restriction analysis showed two different types of clones which a currently under investigation.

Y 301 PURIFICATION, CHARACTERISATION AND CLONING OF POTATO INVERTASE GENES, Howard Davies, Robbie Waugh, Gordon Machray, Lindsay Burch and Peter Hedley, Scottish Crop Research Institute, Invergowrie, Dundee DD25DA, United Kingdom. We are isolating potato invertase genes with the objective of manipulating hexose accumulation in cold-stored tubers. So far, two invertase genes have been cloned, one by exploiting homology with carrot cell wall invertase (Inv 1), the other by screening libraries with antibodies raised to a 58kDa invertase purified from tubers (Inv 2). Inv 1 is expressed in source and sink leaves and stems but not, apparently, in roots or developing tubers. Inv 2 is expressed in all tissues. A soluble 58kDa acid invertase has been purified from leaves which, in V8 digests, shows no apparent homology with either Inv 1 or Inv 2. Other invertases exist in certain tissues (e.g. an insoluble [tenaciously bound wall?] invertase in leaves, roots and stems and an alkaline invertase in leaves and stems). A full genomic sequence has been established for Inv 1. Constructs have been prepared for transformation with both Invs 1 and 2. Further characterisation of other invertases is underway.

Y 302 STUDIES ON LIPID DEGRADATION AND LIPOXYGENASE IN GERMINATING BARLEY, F. Heidekamp, R. Bakhuizen, R. Derksen, A. Doderer, G. van Duijn, I. Kokkelink, J.R. van Mechelen, K. van Rijn, M. Smits, B.E. Valk and A.C. Douma, Center for Phytotechnology RUL/TNO, Department of Molecular Plant Biotechnology TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands

Barley grain contains up to 4.4 % of lipid which is mainly stored in the embryo and the aleurone in the form of lipid bodies. We studied the degradation of lipids in barley upon germination, and focussed on the role of lipoxygenase.

The lipid content of barley embryo and aleurone was analyzed using two different methods, namely gas chromatographical analysis of the fatty acid content and morphometrical analysis of lipid bodies. These studies revealed that approximately one quarter of the amount of lipid originally present was degraded after four days of germination in both grain parts.

Lipoxygenase activity is restricted to the embryo. Activity patterns of this enzyme in the different parts of the embryo in the course of germination have been established. The highest activity was measured in the scutellum. Two lipoxygenase isoenzymes have been purified, which differ in the broadness of their pH optimum, their isoelectric point and the product they form when linoleic acid is used as a substrate. One of the isoenzymes, lipoxygenase 1, solely forms the 9-hydroperoxide as a product, whereas lipoxygenase 2 exclusively produces the 13-hydroperoxide. Monoclonal antibodies specific for each of the isoenzymes have been prepared and were used to screen an expression library of embryos from germinating grain. A nearly full length cDNA sequence, most probably encoding lipoxygenase 1, has been obtained, which is highly homologous to other plant lipoxygenases. Recently 2 different cDNA clones, encoding 2 additional lipoxygenases have been isolated. In future studies the expression of the lipoxygenase isoenzymes during germination will be investigated.

Y 303 BIOCHEMICAL STUDIES OF TRANSGENIC POTATO PLANTS IN WHICH CARBOHYDRATE METABOLISM HAS BEEN ALTERED
H.W. Heldt, D. Heineke, D. Büssis, G. Günter, K. Leidreiter
Institut für Biochemie der Pflanze, Universität Göttingen, Germany
U. Sonnewald, B. Müller-Röber, L. Willmitzer
Institut für Gentechnologische Forschung, Berlin, Germany

By expression of yeast acid invertase in the apoplast the export of sucrose from the leaf mesophyll cells via the sieve tubes was decreased.

By expression of the "anti sense" DNA of ADP glucose pyrophosphorylase, starch biosynthesis was decreased.

The effects of these transformations on the metabolism of the leaves and the tubers have been investigated by measurements of photosynthesis and tubers growth, and several other parameters including metabolites of carbon and nitrogen metabolism in leaves, in the phloem sap obtained from these leaves and in the tubers. The results will be discussed.

Y 304 A TRANSGENIC CORN LINE WITH ALTERED LEVELS OF A HIGH-METHIONINE STORAGE PROTEIN, Julie Anderson Kirihara, Gary Sandahl, Bruce Odegaard, Diane Potts and James Woodman, Plant Science Research, Inc., 10320 Bren Road East, Minnetonka, MN 55343

Corn seed with elevated methionine levels would be valuable in formulating poultry feed, where synthetic methionine is routinely added as a nutritional supplement. One approach to elevate methionine levels involves overproduction of methionine-rich proteins in the seed. To this end, a gene encoding the high-methionine 10 kD zein protein from maize was modified for enhanced expression in the seed by constructing a gene fusion with the promoter from a highly-expressed seed-specific gene. The resulting chimeric 10 kD-zein gene was introduced into corn cells by microprojectile bombardment, along with plasmids carrying a CaMV 35S::HPTII gene as a selectable marker and a CaMV 35S::GUS construct as a screenable marker. A hygromycin-resistant callus line was obtained and fertile plants were regenerated. Southern blot and PCR analysis confirmed that the callus line and the regenerated plants were transgenic, and carried the HPTII, GUS and chimeric 10 kD-zein gene sequences. Analysis of R1, R2 and R3 progeny showed that introduced DNA sequences are inherited in a pattern consistent with Mendelian segregation of a single locus. Comparative DNA and protein analyses indicated that the chimeric Z10 gene is expressed, resulting in elevated levels of 10 kD zein protein in the seeds of transgenic plants. Analyses of seed generated in 1991 field trials are in progress.

Y 305 PURIFICATION AND MOLECULAR CLONING OF ACYL-ACP THIOESTERASE FROM IMMATURE SAFFLOWER EMBRYOS, Jean C. Kridl, Deborah S. Knutzon, Janice L. Bleibaum, Janet Nelsen, William E. Schreckengost, and Gregory A. Thompson, Calgene Inc., 1920 Fifth Street, Davis, CA 95616

Acyl-ACP thioesterases from various plant species display characteristic specificities for fatty acyl chain length and are at least partly responsible for differences in seed oil composition. We have partially purified an 18:1-ACP thioesterase from developing embryos of safflower, *Carthamus tinctorius*, using a modified version of the protocol published by McKeon and Stumpf (*J. Biol. Chem.* **257**:12141-12147, 1982). Two protein species identifiable by SDS-PAGE at 40 kd and 34 kd co-purified with thioesterase activity. The N-terminal amino acid sequence of the two proteins was identical suggesting that the 34 kd species was a processed or partially degraded form of the longer polypeptide. Additional amino acid sequence derived from CNBr peptides was used to design degenerate oligonucleotide primers for use in PCR. A 600 bp PCR product was used to identify two classes of cDNA clones in developing embryo tissue. DNA sequence analysis indicated that they contain open reading frames of 389 and 385 amino acids, including plastid transit peptides of 60 amino acids. The mature protein sequences share 81% amino acid identity. Results of expression of the cloned safflower acyl-ACP thioesterase in *E. coli* will be presented.

Y 306 ANTISENSE RNA MEDIATED SUPPRESSION OF GBSS GENE EXPRESSION IN POTATO USING DIFFERENT CONSTRUCTS. Anja G.J. Kuipers, Evert Jacobsen and Richard G.F. Visser. Dept. of Plant Breeding, Agricultural University of Wageningen, PO.box 386, NL-6700 AJ Wageningen, The Netherlands.

The synthesis of amylose in amyloplasts is catalysed by granule-bound starch synthase (GBSS). In a model system introduction of an antisense GBSS gene into the potato genome was shown to result in stable and total suppression of GBSS activity in tuber starch. To study the effect of different constructs and to find the best suited conditions for the application of this technique in culture varieties a set of constructs was made. In these constructs the full length GBSS cDNA or genomic DNA sequences (the complete gene or parts thereof) were cloned in antisense orientation behind the 35S CaMV promoter or the tuber specific GBSS promoter. Using Agrobacterium rhizogenes mediated transformation the antisense effect of these constructs was evaluated in hairy roots and in tubers of regenerated plants. Transgenic plants of several potato genotypes (including cultural varieties), transformed via A.tumefaciens with all constructs, were grown for tuber production. The analysis and comparison of the effect of the suppression of GBSS gene expression on enzyme activity, starch composition and RNA level in greenhouse grown and in vitro grown tubers of A.tumefaciens and A.rhizogenes transformed plants will be presented.

Y 307 EFFECT OF S-ADENOSYLMETHIONINE HYDROLASE EXPRESSION ON ETHYLENE BIOSYNTHESIS IN TRANSGENIC TOMATOES, Dan P. Langhoff, Wendy Wagoner, Jill A. Kellogg, James A. Stamp and Richard K. Bestwick, Agritope, Inc., 8505 S.W. Creekside Place, Beaverton, OR 97005

We have previously utilized the bacteriophage T3-encoded enzyme S-adenosylmethionine hydrolase (AdoMetase or SAMase) to generate transgenic tobacco plants with reduced capacity to synthesize ethylene. SAM is the metabolic precursor of 1-aminocyclopropane-1-carboxylic acid (ACC), the proximal precursor to ethylene. SAMase catalyzes the conversion of SAM to methylthioadenosine (MTA) and homoserine. The native SAMase gene was engineered to contain a consensus Kozak initiation sequence (SAM-K) and was placed under the influence of the CaMV 35S promoter. Constitutive, high-level expression of SAM-K in tobacco plants resulted in plants that grew slowly and were dwarfed. To restrict the expression of SAMase to tissues that are producing ethylene, the E8 promoter from tomato was used to control expression. The native E8 gene is expressed only in ripening tomatoes beginning at the mature green stage (Lincoln, etal, PNAS 84:2793-2797, 1987). By placing the SAM-K gene under the influence of the E8 promoter, SAMase expression is restricted to ripening fruit. The *Agrobacterium* vector pGA482 was used to construct pGA-ESKN which contains the E8:SAM-K:NOS-terminator expression cassette. Transgenic tomato plants (cv. Large Red Cherry) containing pGA-ESKN have been isolated and Southern blot analyses indicate they contain the SAMase gene. Analysis of SAMase expression and its impact on ethylene biosynthesis in ripening tomato fruit will be reported.

Y 308 GENERATING A DOUBLE MUTANT OF *ARABIDOPSIS THALIANA* DEFICIENT IN POLYUNSATURATED FATTY ACIDS Michele M. McConn, Martine Miquel, and John A. Browse. Institute of Biological Chemistry, Washington State University, Pullman WA 99164-6340 There are two pathways, eukaryotic and procaryotic, for lipid synthesis in higher plants. Single mutants alone are not able to block desaturation in both pathways, thus a double mutant must be constructed. Here we describe the generation of a double mutant which is severely deficient in the desaturation of 16:1 and 18:1 fatty acids. Seeds with a double mutant genotype were generated in a mendialian ratio from a *fadC-|-:fad2+|-*, however less than 40% of these seeds gave rise to viable seedlings. The availability of double mutant plants will allow us to extend our investigations on the importance of polyunsaturated membrane lipids to photosynthesis and to the growth and development of plants.

Y 309 MAIZE MUTANTS OF STORAGE PROTEIN DEPOSITION Mario Motto, Massimo Maddaloni, Hans Hartings, Carlotta Balconi, Eduardo Rizzi, Adriano Marocco, Enzo Martegani[1], Isabella Mauri[1], Francesco Salamini[2], Stephan Lohmer[2] and Richard Thompson[2], Istituto Sperimentale Cerealicoltura, Bergamo, Italy; 1) Dip. Fisiologia e Biochimica Generali, Milano; 2) Max-Planck-Institut, Köln, Germany
We have investigated the effect of three dominant mutations fl2, De-B30, and Mc which reduce zein level in the endosperm. It is shown that both b-70s resemble heat shock proteins in that they bind ATP, cross react with HSP antibodies and has N-terminal sequence homology to chaperon-like HSP70. The physical similarity of b-70 to known molecular chaperone proteins and its association with abnormal accumulation in endosperm mutants may reflect a biological function to mediate protein folding and assemby in maize endosperm. Out of the recessive mutants o2, o6 and o7, the locus O2 play a central role in regulating the expression of certain members of the zein gene family and the expression of an abundant cytosolic albumin, b-32. The protein encoded by O2 is a member of the bZip family of eukaryotic transcription factors. By expressing the O2 gene product (O2-protein) in tobacco leaf protoplasts, one can trans-activate 22 kDa zein and b-32 promoters, demonstrating that O2 protein acts as a transcriptional activator. DNA binding analyses indicate that O2 recognizes a target site that is present in promoter of 22-kDa zein and b-32 genes. Additional experiments show that co-expression of b-32 in tobacco protoplasts potentiates the trans-activation of target promoters by O2. This indicated that b-32 appears to increase in a non-specific manner the translation of reporter genes expressed in this assay and therefore presumably interacts with some component of the translation machinery. It was shown that the presence of uORFs of the major coding sequence of the O2 locus reduces trans-activation by O2 probably by interferring in the translation of O2 mRNA. A functional homology between the GCN4 protein and the O2 product has been obtained through expression of O2 cDNA in the yeast S.cerevisiae. The regulation of zein synthesis in endosperm cells by amino acid supply will be also presented and discussed.

Y 310 BEAN SEED STORAGE PROTEIN ß-PHASEOLIN WAS EXPRESSED IN THE ENDOSPERMS OF TRANSGENIC RICE PLANTS. Norimoto Murai, John M. Dyer and Zhenwei Zheng. Department of Plant Pathology and Crop Physiology, Louisiana State University, Baton Rouge, LA 70803
To improve nutritional quality of rice seed protein, the bean 7S storage protein ß-phaseolin gene was used to transform rice. A 1.8 kbp promoter fragment of the rice storage protein glutelin (Gt-1) gene or a 785bp phaseolin gene promoter was fused to either genomic or cDNA coding sequences of the bean ß-phaseolin gene. The highest quantity (3.3%) of phaseolin was detected by ELISA in the endosperm of mature rice seeds when the phaseolin genomic coding sequence was expressed under control of the 1.8 kbp glutelin gene promoter. Both phaseolin genomic and cDNA coding sequences were used to produce intact phaseolin polypeptides, corresponding to the glycosylated (48 kd) and non-glycosylated forms (45.5 kd) of ß-phaseolin. The phaseolin trait was segregated into a 3:1 ratio among 30 T1 seeds tested, indicating the gene was inserted into a single chromosomal locus or tightly linked loci. Preliminary results suggested that the phaseolin promoter may be functional in the embryo but not in the endosperm of rice. We are also studying the overall efficiency of bean protein expression in rice, i.e. intron processing, glycosylation, endoproteolitic cleavage, and accumulation of bean storage protein in the vacuolar protein body of rice endosperms. An alternative approach to improve the nutritional quality of rice seed protein is by protein engineering. We used the bean 7S seed storage protein as a model system because phaseolin is amenable to the protein engineering experiments. The α-carbon coordinates of phaseolin were established using X-ray crystallography by Lawrence *et al.* We (J. M. Dyer, J. Nelson & N. Murai) determined the complete three-dimensional structure of phaseolin by adding side chain configurations to α-carbons in a VAX computer using the CHARMM, SYBYL and Macromodel molecular analysis package. The native structure is used as a template for simulation modification (i.e. increased methionine or lysine content) in the protein structure.

Y 311 STRUCTURE AND EXPRESSION OF THE POTATO TUBER ADPGLUCOSE PYROPHOSPHORYLASE SMALL SUBUNIT. Paul A. Nakata, Joseph M. Anderson, Thomas W. Okita, and *Jack Preiss. Institute of Biological Chemistry, Washington State University, Pullman, WA. 99164-6340. *Department of Biochemistry, Michigan State University, East Lansing, MI 48824.
The biosynthesis of α1,4,-glucans in both bacteria and plants is controlled primarily by the expression and allosteric regulation of ADPglucose pyrophosphorylase. Recently, both cDNA and genomic clones of the potato tuber ADPglucose pyrophosphorylase small subunit have been isolated and their structure determined. Spatial and temporal expression of the small subunit as well as its correlation to starch accumulation has been elucidated. A comparison of the rice endosperm and potato tuber ADPglucose pyrophosphorylase cDNA's revealed a 74% identity at the nucleotide level and a 91% identity at the amino acid level. Similarly, both the rice and potato pyrophosphorylase genes exhibit a complex gene structure as do other genes involved in starch metabolism. Currently, cis acting elements and their involvement in spatial and temporal expression are under investigation. (Supported in part by a McKnight Foundation Graduate Fellowship and DoE).

Y 312 CLONING AND CHARACTERIZATION OF GENOMIC ß-KETOACYL-ACP SYNTHASE GENES FROM CASTOR.

Jeffery Q'Neal, David W. McCarter and Andree Genez.
Calgene Inc., 1920 Fifth St., Davis, CA 95616

ß-Ketoacyl-ACP synthases catalyze the condensation reactions of fatty acid synthesis in plants, required for the formation of fatty acids for membranes and storage lipids. Each form of the enzyme displays a unique range of substrate specificity: ß-ketoacyl-ACP synthase I (KAS I) prefers C4 to C14-ACP and ß-ketoacyl-ACP synthase II (KAS II) is most active on C16-ACP.

A fraction was purified from developing castor endosperm which contains synthase activities capable of elongating C10-ACP and C16-ACP. SDS-PAGE analysis of the purified preparation showed protein bands of 46 kDa and 50 kDa. cDNA clones for each protein species were isolated from a castor endosperm cDNA library and used to probe a castor genomic library for homologous sequences. Genomic sequences corresponding to each cDNA were isolated and subcloned.

We report here the sequence and molecular characterization of the castor KAS I and KAS II genomic clones, and their preparation for expression in heterologous plant systems.

Y 313 PRODUCTION OF ACTIVE ALPHA-AMYLASE IN PLANTS AND THE APPLICATION IN STARCH LIQUEFACTION, Jan Pen, Peter J.M. van den Elzen, Albert J.J. van Ooyen* and André Hoekema, MOGEN N.V., Einsteinweg 97, Leiden and *Gist-brocades N.V., P.O. Box 1, Delft, The Netherlands.

Plants may be exploited for economic production of proteins for *e.g.* food, feed, or processing industries. Industrial enzymes may be suitable candidates for production in plants, because of their applicability in many processes.

We have produced a biologically active α-amylase from *Bacillus licheniformis* in tobacco at a maximum level of 0.3% of soluble protein. The enzyme was targeted outside the cell. Complex-type carbohydrate chains were found to be attached to the protein as produced in tobacco.

Different approaches to the production and application of industrial enzymes in plants were tested:

I. Direct application of transgenic plant material.

Direct application of transgenic plant material in an industrial process has the clear advantage of reduced purification costs and the absence of formulation costs. This concept was exemplified by using milled transgenic seeds containing α-amylase in starch liquefaction. Virtually identical degradation products were obtained as with the *B. licheniformis* α-amylase.

II. Process-dependent conversion.

Conversion may become process-dependent by production of the enzyme sequestered from its substrate. The feasibility of this concept was demonstrated in this study. Staining of leaves for the presence of starch revealed no difference between transgenic and control plants, as expected from the different locations of enzyme and substrate. Homogenization of transgenic leaf-material, followed by incubation at 95°C resulted in degradation of the starch present.

These approaches will result in lower manufacturing costs and consequently increase the competitiveness of plants for production of industrial enzymes.

Y 314 EXPRESSION OF A THERMOSTABLE (1-3,1-4)-β-GLUCANASE IN BARLEY

Belinda Phillipson, Department of Physiology, Carlsberg Laboratory, DK-2500 Valby, Copenhagen, Denmark.

Barley malt is produced by initiating germination of the grain. Insufficient (1-3,1-4)-β-glucanase activity during malting and mashing may result in increased levels of high MW β-glucans in the mash, giving lowered rates of mash and beer filtration. It would therefore be desirable to have a (1-3,1-4)-β-glucanase which could survive kiln drying and be active in the subsequent mashing step when the barley (1-3,1-4)-β-glucanases are irreversibly heat inactivated. A series of thermostable hybrid bacterial (1-3,1-4)-β-glucanases that fulfill these criteria have been constructed.

A 320 bp promoter fragment derived from a barley low pI α-amylase gene expressed during germination, was isolated using the PCR technique. The expression driven by this promoter was assayed by fusing it to the coding region of the CAT gene and then using the construct to transfect barley aleurone protoplasts. This promoter fragment was shown to confer hormone responsiveness and drive high levels of expression. Therefore, it was used to make further constructs where the coding region for CAT was replaced with fragments encoding a hybrid bacterial (1-3,1-4)-β-glucanase. The performance of these constructs was tested in the barley aleurone protoplast system. However, significant expression of active, heat stable (1-3,1-4)-β-glucanase could not be detected. The low pI α-amylase promoter fragment was then exchanged with the pEMU promoter. These plasmids have been used to transfect both barley leaf and aleurone protoplasts and the levels of active, heat stable (1-3,1-4)-β-glucanase compared.

Y 315 STRATEGIES FOR THE ISOLATION OF GENES INVOLVED IN DE NOVO FATTY ACID BIOSYNTHESIS Wolfgang Schulte, Barbara Klein, Katharina Pawlowski, Jeff Schell, Reinhard Töpfer, Abteilung genetische Grundlagen der Pflanzenzüchtung, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne Weg 10, D-5000 Köln 30 FRG

The modification of the triacylglycerol composition in oil seed rape (Brassica napus) is one of the major objectives in the improvement of plant storage compounds. We are focussing on the isolation of genes involved in de novo fatty acid biosynthesis from Brassica napus and Cuphea lanceolata: acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS). Initial experiments for testing heterologous probes from rat, yeast and Eschericha coli revealed no crosshybridization with the rape seed genome. Even crosshybridization of a plant FAS component such as ß-ketoacyl-ACP reductase from Cuphea lanceolata (Klein et al. MGG in press) with rape seed could not be detected, indicating that also between plants the FAS components are evolutionary divergent. Therefore, other strategies for gene isolation were followed: For isolation of the acetyl-CoA carboxylase (ACC) gene we are useing 1. the classical line by purification of the protein in order to raise an antibody against the enzyme for screening an expression library and 2. we are screening an cDNA expression library from size fractionated large mRNAs for biotin containing enzymes using peroxidase linked streptavidin. As an alternative approach PCR was used for the synthesis of homologous hybridization probes starting from mRNA. Thus, we were able to isolate ß-ketoacyl-ACP reductase cDNAs from C. lanceolata.

Y 316 REGULATION OF ETHYLENE BIOSYNTHESIS IN TOMATO BY EXPRESSION OF A *PSEUDOMONAS* ACC DEAMINASE GENE, Raymond Sheehy, Virginia Ursin, Belinda Martineau, William Hiatt, Calgene, Inc. 1920 Fifth Street, Davis, CA 95616, USA

Ethylene, a plant hormone, regulates a variety of growth and developmental processes, in particular senescence and fruit ripening. A gene encoding 1-aminocyclopropane-1-carboxylate deaminase (ACCD) from a *Pseudomonas* species has been introduced into tomato to produce an alternate branch point in the ethylene biosynthetic pathway. (Cloning of the *Pseudomonas* ACCD gene has been described[1].) Deamination of ACC to form ammonia and α-ketobutyrate[2] in plants depletes the pool of ACC, the rate-limiting precursor of ethylene, and therefore reduces ethylene biosynthesis. Analysis of tomato plants transformed with a chimeric gene utilizing an enhanced 35S promoter and the *Pseudomonas* ACCD coding region will be described.

1. R.E. Sheehy, M. Honma, M. Yamada, T. Sasaki, B. Martineau, W.R. Hiatt. 1991. J. Bacteriology 173:5250-5265.

2. M. Honma and T. Shimomura. 1978. Agric. Biol. Chem., 42:1825-1831.

Y 317 MOLECULAR CLONING OF THE SOYBEAN GENE ENCODING DIHYDROPICOLINATE SYNTHASE (DS), Gregg W. Silk and Benjamin F. Matthews, USDA, Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD, 20705-2350

In plants, the first committed step in the pathway to lysine synthesis is the condensation of aspartic B-semialdehyde and pyruvate, catalyzed by the enzyme dihyrdopicolinate synthase (DS). DS activity undergoes feedback inhibition by lysine, suggesting that the regulation of DS activity may control lysine synthesis. Cloning of the soybean DS gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the DS gene may be a way of increasing lysine synthesis in soybeans and other crop plants. The gene which encodes DS has been cloned from monocots, but it has not been previously cloned from dicots. We have amplified a portion of the soybean (G. max var. Century) DS gene using PCR. The DS gene fragment was cloned and used to isolate copies of the gene from genomic DNA and cDNA libraries. Southern blots show that the soybean genome contains a small number of DS genes. Although the soybean gene hybridizes to the cloned maize DS gene, the level of homology is not high enough for the soybean probe to detect the maize DS gene in genomic Southern blots. Northern blots indicate that the soybean DS mRNA is present at only very low levels.

Y 318 ISOLATION AND CHARACTERIZATION OF GAMMA-ZEIN GENES FROM QUALITY PROTEIN MAIZE (QPM), Koichi Takasaki, Mauricio A. Lopes and Brian A. Larkins, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Quality Protein Maize (QPM) are genetically modified *opaque-2* mutants with hard and vitreous endosperm. The most characteristic biochemical feature of QPM endosperms is the elevated amount of the 27 kD gamma-zein, a storage protein that appears to play important roles in protein body formation and endosperm structure. Highly modified genotypes contain two- to three-times the normal level of this protein in the endosperm, and its amount is proportional to the degree of modification in partially modified endosperms. Using the Polymerase Chain Reaction (PCR) we isolated the coding and promoter regions of the gamma-zein from QPM varieties. RFLP's in the coding and promoter regions of these genes allowed us to investigate allelic variation at this locus, by analysis of PCR-amplified products. As is true of other maize genotypes, QPM contains 1 or 2 genes which are in the form of 3 types of alleles: A, B, Ra. Comparisons with other published sequences revealed no major differences in the coding region from QPM and inbred lines. Furthermore, the promoter regions (up to -1 kb) revealed a high degree of similarity among QPM and inbred lines. It remains to be established if the large amount of gamma-zein synthesized in QPM varieties is a consequence of cis effects, trans effects, or a combination of the two. Studies are in progress to determine if the 5' flanking region contains specific DNA sequences involved in promoter activity and interaction of transacting factor.

Y 319 METABOLISM OF THE *LATHYRUS SATIVUS L.* NEUROTOXIN, β-N-OXALYL-L α, β-DIAMINO PROPRIONIC ACID, BY A PURE CULTURE OF A SOIL-BORNE *ENTEROBACTER CLOACAE*. Michael P. Timko[1], Vijay K. Yadav[1,2], I.M. Santha[2], and Shanti L. Mehta[2], Dept. of Biology[1], University of Virginia, Charlottesville, VA 22901 USA and Division of Biochemistry[2], IARI, New Delhi 110 012, India

Lathyrus sativus L., commonly called the chickling pea, is an exceptionally hardy, protein-rich legume crop noted for its ease of culture and high climatic adaptability permitting growth even under such extreme conditions as drought or water logging. All of these factors make *L. sativus* potentially a valuable food crop for arid regions. However, the full potential of *L. sativus* has not been realized due to the presence of an unusual, non-protein amino acid known as β-N-oxalyl-L-α, β-diaminoproprionic acid (Ox-Dapro) in the seeds and vegetative portions of the plant. Ox-Dapro has been shown in clinical tests to be a potent neurotoxic compound that acts as a stereospecific glutamate-receptor agonist. Prolonged or excessive consumption of the seed meal or other plant parts has been implicated in the human neurological disorder known as neurolathyrism. Pure cultures of bacteria (BYA1, BYT1, and BYK1) capable of utilizing Ox-Dapro as their sole carbon and nitrogen source have been isolated from soil-sludge filtrates by repetitive growth on the neurotoxin. Of the three isolates, strain BYA1 demonstrated the highest capacity for Ox-Dapro utilization, degrading greater than 98 % of the Ox-Dapro present in the culture media within 12 hrs. Using a variety of morphological and biochemical criteria BYA1 was identified as an unusual *Enterobacter cloacae*. The bacteria harbors a single large plasmid (designated pBYA-1) approximately 40-50 kb in size that contains the genetic information for Ox-Dapro utilization and antibiotic resistance. Transformation experiments with *E. coli* recipient strains were used to further define the location of the sequences involved in Ox-Dapro metabolism. Detailed analysis of this region is now in progress.

Y 320 CHARACTERIZATION OF ß-KETOACYL-ACP REDUCTASE OF CUPHEA LANCEOLATA, Reinhard Töpfer, Barbara Klein, Christa Höricke-Grandpierre, Jeff Schell, Katharina Pawlowski, Abteilung genetische Grundlagen der Pflanzenzüchtung, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linne Weg 10, D-5000 Köln 30 FRG

A cDNA library, constructed from immature embryos of Cuphea lanceolata, was screened using a 340 bp cloned PCR fragment showing homology to ß-ketoacyl ACP reductase of avocado peptide sequences. Four of the cDNA clones obtained were larger than 1100 bp and complete sequencing revealed that three clones (clone 16, 21, 27) were identical while clone number 10 showed 95 % identity on amino acid level to clone 27. The cDNAs code for a 27 kd polypeptide consisting of 320 amino acids. A fusion protein of glutathione-S-transferase with the coding sequence of clone 27 was constructed and isolated from E. coli. When subjected to an enzyme assay for ß-ketoacyl-ACP reductase, the fusionprotein showed a substrate specificity for NADPH while NADH as reduction equivalent was used less effective. Further analysis of the recombinant protein is in progress.

Y 321 BIOCHEMICAL PROPERTIES OF DORMANT AND NON-DORMANT BARLEY GRAINS. J.M.M. van Beckum, R.C. Schuurink, F. Heidekamp and M. Wang, Center for Fytotechnology RUL/TNO, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

We are interested in the elucidation of the molecular mechanisms controlling inhibition of germination. Therefore we have grown barley plants in a phytotron and were able to select 2 growing conditions which led to the harvest of mature grains with a high and a low level of dormancy.

We studied the response of the aleurone layers from dormant and non-dormant grains upon incubation with the plant hormones abscisic acid (ABA) and gibberellic acid (GA_3). The results show that dormant aleurone layers and non-dormant aleurone layers have different expression levels of the RAB (Responsive to ABA) gene upon incubation with ABA. The basal RAB mRNA level in dormant aleurone layers (4 days imbibition in water) is also much higher than that of non-dormant one. It is known that incubation of aleurone layers from barley grains with GA_3 results in α-amylase secretion from the aleurone cells. However, the GA_3 induced levels of α-amylase in the aleurone layers from dormant grains is strongly reduced. Furthermore, there is a great difference in sensitivity of dormant and non-dormant aleurone layers in reacting to GA_3.

A possible explanation for this observation may be that the hormone signal transduction pathways are different in both dormant and non-dormant grains. To investigate this we measured the cytosolic free calcium concentration (Ca_i) and the intracellular pH (pHi) in protoplasts from dormant and non-dormant aleurone layers. Our data demonstrate that there are clear differences in both Ca_i and pH_i. A possible explanation for those differences regarding to hormone action will be discussed.

Genes and Gene Expression for New Traits - Yield Improvement; Promising New Research Areas

Y 400 **ISOLATION AND CHARACTERIZATION OF REPRODUCTIVE BUD SPECIFIC GENES FROM *POPULUS TRICHOCARPA***, Teresa K. Boes, William H. Rottmann, Lorraine S. Nyers, and Steven H. Strauss, Department of Forest Science, Oregon State University, Corvallis, OR, 97331-5705.

Populus is well suited to forest farming, and is an important species for the production of pulp, paper, and woody biomass. In addition to its commercial value, *Populus* is an excellent model system for woody plant molecular genetics since it has a small genome size, can be regenerated in culture, and is susceptible to genetic transformation by *Agrobacterium*. *P. trichocarpa* is the female parent of the highly productive hybrid *P. trichocarpa* x *deltoides*, which is intensively grown in the northwestern United States. We are involved in several projects aimed at understanding floral development and gene expression in *P. trichocarpa*. To document the stages of floral structure development in male and female trees we have prepared an anatomical study of seasonal morphology. Bud collection—from branches observed flowering the previous spring—began approximately four weeks after anthesis and continued on a weekly basis through the summer. We have cloned cDNAs synthesized from mRNAs transcribed during the earliest identifiable stages of male and female reproductive bud development. We applied three rounds of subtractive hybridization to enrich floral probes for sequences expressed uniquely in reproductive buds. We then differentially screened potential clones using the enriched floral cDNA and vegetative bud cDNA. The tissue-specificity of candidate clones was verified by probing cDNA library inserts amplified by PCR. Characterization of selected cDNAs will be discussed. Once reproductive bud-specific genes are adequately characterized, the ultimate goal of this project is to genetically engineer sterility in a woody plant species.

Y 401 INTRODUCTION OF A XYLANASE GENE FROM A RUMEN BACTERIUM INTO THE CHROMOSOME OF A COLONIC BACTERIUM FOR THE PRODUCTION OF XYLANASE, R. J. Bothast, T. R. Whitehead, and M. A. Cotta, USDA, ARS, National Center Agric. Utilzn. Res., Peoria, IL 61604

Xylanolytic enzymes are important because of their involvement in bioconversions. Targeted industries for applications include pulp and paper, textiles, food processing, brewing, biofuels and chemical feedstocks, plant agriculture, and animal nutrition. Our research has focused on improving incomplete digestion of hemicellulose (xylans) in the rumen. Our approach is to genetically modify bacteria for more efficient degradation of xylans. The xylanase gene from the ruminal bacterium Bacteroides ruminicola 23 was introduced into the chromosome of the colonic organism B. thetaiotaomicron 5482, where the gene was highly expressed. The gene was found to be stable in continuous culture in the recombinant strain BTX. Furthermore, BTX was able to effectively compete in coculture with the parent strain and against B. ruminicola strain D31d. BTX was able to degrade xylan to oligosaccharides, but could not use these products for growth. Xylanolytic ruminal organisms could utilize the xylooligosaccharides produced by BTX (>80%), and strains of the nonxylanolytic bacterium Selenomonas ruminantium could also use these hydrolysis products (approx. 40%).

Y 402 THE PEA LIPOXYGENASE GENE FAMILY. Rod Casey, Claire Domoney, Shaun Hobbs, Helen North and Paul Ealing, John Innes Institute, NORWICH UK

Lipoxygenase(s) (Lox) catalyze the formation of fatty acid hydroperoxides which are metabolized to a range of compounds with different physiological effects and organoleptic properties. Lox are implicated in the plants' response to pathogen and pest attack, wounding and stress and are commercially important through their effects in bread-making, the production of natural tastes and flavours, and the formation of 'off-flavours' in stored or frozen vegetables. This paper describes the *Pisum* (pea) Lox multigene family.

Pea seeds contain two major Lox polypeptides, equivalent in sequence to soybean Lox-2 and -3, and at least three minor species, one of which appears to be produced at very early (predominantly mitotic) stages of seed development. A range of Lox transcripts/translation products has been identified in pea flowers, stems, leaves and roots. Each of the two major seed Lox polypeptides is encoded by two or three genes that map close to *le* on linkage group 4. Other unlinked Lox loci have been identified through RFLP analysis. Variants that lack the Lox-2-like, or almost lack the Lox-3-like polypeptides are being used to introduce low-Lox phenotypes into standard backgrounds for commercial assessment; the molecular bases of the mutations are under investigation.

Lox genes have been isolated from a pea λGEM12 library and a promoter from a Lox-2-like gene has been cloned *via* inverse PCR and sequenced; deletions of the promoter fused to GUS are being introduced into transgenic plants.

Y 403 MOLECULAR ANALYSIS OF CAROTENOID BIOSYNTHESIS. Daniel Chamovitz, Iris Pecker, Gerhard Sandmann* and Joseph Hirschberg. Department of Genetics, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, and *Lehrstul für Physiologie und Biochemie der Pflanzen, Universitat Konstanz, Germany

In order to study the regulation of carotenoid biosynthesis we have cloned the genes that encode phytoene synthase (*pys*) from cyanobacteria and phytoene desaturase (*pds*) from cyanobacteria, algae and plants. *pys* encodes a protein of 307 amino acids that when expressed in cells of *E. coli* catalyzes the two step reaction from geranylgeranyl pyrophosphate to phytoene. The deduced amino acid sequence of the cyanobacterial *pys* is highly homologous to the tomato fruit ripening cDNA clone pTOM5 (59% amino acid identity), indicating that pTOM5 also encodes phytoene synthase.

The cyanobacterial *pds* encodes a protein comprising of 474 amino acids. The PDS's from *Synechococcus* PCC7942, *Dunaliella bardwill* and tomato are highly conserved (over 64% identity), except for an additional leader region in the two eukaryotic proteins. *in vitro* translated tomato PDS is imported into pea chloroplasts and processed to a size similar to the cyanobacterial protein. Expression of *pds* in *E. coli* shows that PDS catalyzes the formation of ζ-carotene from phytoene. There is a ten fold increase in accumulation of *pds* transcripts from mature green to orange tomato fruits.

Several norflurazon and fluridone-resistant strains of *Synechococcus* PCC7942 have been isolated following mutagenesis. Point mutations in *pds* that lead to amino acid substitutions in phytoene desaturase were found to confer herbicide resistance in these mutants. All of the residues which are mutated are conserved in all other species. Alteration in the catalytic kinetics of PDS in the mutants results in a reduction of carotenoid biosynthesis *in-vivo*. Furthermore, over-expression of *pds* in cyanobacteria increases carotenoid content. This demonstrates that PDS is a rate limiting step in carotenoid biosynthesis.

Y 404 *Abstract Withdrawn*

Y 405 ISOLATION AND CHARACTERIZATION OF cDNA ENCODING LIMONENE CYCLASE IN SPEARMINT. Sheila M. Colby, William R. Alonso, and Rodney Croteau. Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

The monoterpine cyclase, 4S-limonene synthase, from *Mentha spicata* is responsible for the conversion of geranyl pyrophosphate to limonene. To isolate and study the gene(s) encoding limonene synthase and to produce enough protein to study enzyme structure by NMR and X-ray methods, RNA was extracted from young leaves and a cDNA library was constructed in lambda Uni ZAP XR from spearmint leaf poly (A)+ RNA. Three degenerate oligonucleotides were designed based on internal amino acid sequences obtained from Edman degradation of purified 4S-limonene synthase peptides (Alonso et al., submitted) and 250,000 clones were screened with these probes. Six positive clones that hybridize to all three oligonucleotides have persisted after tertiary screening. Spearmint 4S-limonene synthase cDNA candidates will be confirmed by comparing nucleic acid with amino acid sequence and then further characterized. A T7 polymerase expression vector will be employed to obtain high levels of enzyme. A 4S-limonene synthase cDNA clone will also be used to probe a spearmint genomic library.

Y 406 *VSPA/VSPB* ENCODE VACUOLAR ACID PHOSPHATASES: ANALYSIS OF GENE EXPRESSION AND PROTEIN TARGETING, Daryll B. DeWald, Hugh S. Mason, and John E. Mullet, Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843.

Soybean vegetative storage protein (VSP) is a vacuolar glycoprotein which occurs as dimers of nonidentical subunits α and β, encoded by the genes *vspA* and *vspB*. We have shown that purified soybean VSP from seedling hypocotyls and young leaves has acid phosphatase activity. We determined the pH optimum for cleavage of a standard substrate and report the relative efficiency of cleavage of a variety of different phosphate containing substrates. In addition to describing an enzyme activity for VSP, we have examined whether the n-terminal leader portion of the VSPα translation product will target β-glucuronidase (GUS) to the vacuole of transgenic tobacco. We compared the efficiency of vacuolar localization of the above fusion protein with unmodified GUS and GUS with a signal peptide fused to its n-terminus.

The expression of *vsp* genes is high in growing shoot tissues, is modulated developmentally in response to sink demand, and is inducible in mature tissues by wounding and water deficit. We have investigated the roles of sugars and the putative plant hormone methyl jasmonate (Me-JA) in the regulation of *vsp* gene expression in soybean plants and suspension cultures, and in transgenic tobacco. Accumulation of *vspA* and *vspB* mRNAs was induced in excised mature soybean leaves when treated in the dark with 0.2M sucrose and 10^{-5} M Me-JA, but not by either compound alone. Glucose and fructose substitute efficiently for sucrose in this induction. The time courses of *vsp* mRNA induction in excised leaves treated with sucrose/Me-JA and in wounded leaves were similar. A portion (-787 to +46 relative to the transcription start site) of a genomic clone of the *vspB* gene was fused to the coding region of GUS. Transgenic tobacco plants harboring this construct showed greatly increased GUS activity when excised leaves were treated with 0.2M sucrose and 10^{-5} M Me-JA. These data indicate that the *vsp* genes are responsive to both metabolic and hormonal signals, which may also be important in the regulation of sink activity. In order to delineate promoter sequences which are important in these responses, we have generated a series of 5' and 3' deletions of the *vspB* promoter and transformed tobacco with the GUS fusion constructs. These data are presented and discussed.

Y 407 DIFFERENTIAL EXPRESSION OF *fbp1*: A GENE INVOLVED IN FLOWER MORPHOGENESIS OF PETUNIA.

J.J.M. Dons[1], G.C. Angenent[1], M. Busscher[1], J. Franken[1], J.N.M. Mol[2] and A.J. van Tunen[1]. [1]Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, Wageningen, The Netherlands and [2]Free University, Amsterdam.

Using the conserved sequence of the DNA binding part of homeotic genes regulating flower morphogenesis, a cDNA clone was isolated from young *Petunia hybrida* flower buds. This cDNA clone was used to screen a flower specific cDNA library. A full size clone, designated as *fbp1* (floral binding protein 1) was isolated. The cDNA codes for a 24 kd protein, which has several characteristics of a transcription factor: a DNA binding domain (the MADS box) and a domain for dimerisation. Both domains show a high degree of homology with *agamous* and *deficiens*. The corresponding *fbp1* gene was isolated and sequenced. Southern blot analysis revealed that FBP is most likely encoded by a single copy gene.The gene was expressed exclusively in whorls 2 and 3 (petals and stamen) of wild type *Petunia hybrida* flowers. In contrast, PBP1 protein was only detectable in petals and not in stamen, suggesting a differential expression at the mRNA and protein level. In the *Blind (Bl)* mutant, expression in the antheroid structures (transformed limbs) was as in normal anthers. In the homeotic flower mutant *Green petals (Gp)*, expression of *fbp1* was strongly reduced in the sepaloid structures which replace the petals in this mutant. In contrast to the proposed models describing floral morphogenesis, these data show that homeotic genes can be functional in one whorl only.

Y 408 REGULATION OF CHLOROPLAST DEVELOPMENT AND GENE EXPRESSION DURING PEA LEAF GROWTH IN DARKNESS, FAR-RED OR WHITE LIGHT. Arnold N. DuBell, Brian J. Baumgartner, and John E. Mullet. Dept. Biochemistry and Biophysics, Texas A&M University, College Station, TX. 77843.

In barley, primary leaf growth is largely light independent. Similarly, plastid transcriptional activity and DNA copy number were shown to increase early during leaf development in a light independent manner (1). We characterized the development of pea (Pisum sativum, var. Little Marvel) to determine if monocot and dicot leaf and chloroplast biogenesis followed a similar pattern.

Pea leaves do not elongate in darkness, but continual far-red (FR) light allows partial elongation. Growth in white light allowed a higher rate of cellular DNA accumulation than growth in darkness; by 14 days post imbibition (dpi), the amount of DNA in white light-grown peas was around 32 μg per leaf. This paralleled the increase in leaf length. In FR light, DNA accumulated to 32 μg/leaf by 8 dpi then declined to 20 μg/leaf by 14 days. The difference between growth in FR or white light is not the result of changes in mesophyll cell size as both increase to about 75 μm by 7 days.

Plastid number per cell remains low in dark grown peas but increases from an average of 20 at 5 dpi to 60 at 10 dpi in white light illuminated plants. Plastid number increases to 50 per cell by 7 to 8 dpi in FR light. The increase in plastid number under either light condition parallels changes in DNA content per leaf. Plastid transcription remains low in dark-grown pea plants. In contrast, overall plastid transcription activity increases to a maximum by 6 dpi in FR or white light although the maximum level in FR is approximately twice that of white light. Transcription activity in continuously illuminated plants declines to the low levels seen in dark-grown peas between 7 to 9 dpi. The abundance of RNA's hybridizing to plastid genes encoding components of the transcriptional and translational apparatus as well as the photosynthetic apparatus will be reported.

In conclusion, chloroplast development in pea leaves and barley leaves is similar except that leaf growth and chloroplast development in pea is modulated by light.

1. Baumgartner, B.J., J.C. Rapp, and J.E. Mullet. (1989) Plant Physiol. 89:1011-1018.

Y 409 TUBULIN ISOTYPE EXPRESSION IN TWO HERBICIDE RESISTANT AND SENSITIVE SPECIES

[1]J. Raymond Ellis, [2]Teresa Waldin and [2]Patrick J. Hussey. [1]ICI Seeds, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK; [2]Royal Holloway & Bedford New College, University of London, Egham, Surrey, TW20 0EX, UK.

In higher plant microtubules the constituent α- and β-tubulins are present as a family of closely related proteins, which are the expression products of multigene families. Dinitroaniline herbicides, such as trifluralin, are believed to interfere with microtubule mediated processes by binding to tubulin subunits. Plants which have evolved resistance to dinitroanilines offer an opportunity to investigate the tubulin-herbicide interaction.

We have examined the tubulin complement of susceptible and resistant biotypes of two grasses, *Eleusine indica* (goosegrass) and *Setaria viridis* (green foxtail). Seedlings were grown in darkness for 2 weeks and soluble proteins extracted and separated either on 1-D gels by PAGE or on 2-D gels by IEF and PAGE. Tubulin isotypes were visualised by immunoreaction with α-specific or β-specific antibodies.

In *Eleusine* one complex α-tubulin and four β-tubulin isotypes were detected. In *Setaria* two α-tubulin and two β-tubulin isotypes were identified. In neither species was any difference apparent between susceptible and resistant biotypes in the mobility or abundance of the α- or β-tubulin isotypes. We conclude that at the level of resolution provided by 2-D gels and immunoblotting there was no modification in the size, charge, or abundance of a tubulin isotype associated with dinitroaniline resistance. Thus we have no evidence that the resistance trait is attributable to a modified tubulin target site in the two grass species examined.

Y 410 CHARACTERISATION OF A SMALL GENE FAMILY ENCODING SAPORINS: TYPE 1 RIBOSOME INACTIVATING PROTEINS, Anthony P. Fordham-Skelton, Lesley Sinclair and Ronald R.D. Croy, Plant Molecular Biology Group, Department of Biological Sciences, University of Durham, UK, DH1 3LE.

Ribosome-inactivating proteins (RIPs) have been isolated from many diverse plant species. RIPs inactivate ribosomes by removing a specific adenine residue present in a conserved region of the large ribosomal RNA subunit, thus causing the cessation of protein synthesis. RIPs have been classified as either type 1 or type 2 depending upon their subunit composition. Type 1 RIPs possess a single active polypeptide chain whilst type 2 RIPs have an active A-chain and a B-chain with a lectin activity.

Whilst much attention has focused on the use of RIPs as the cytotoxic component of immunotoxins little is known about their function in plants. We have isolated genomic clones encoding saporins, type 1 RIPs found in the leaves and seeds of the soapwort plant (*Saponaria officinalis* L.).

The genes have leader peptide sequences but do not contain introns. The promoter sequences exhibit no significant homology. Two of the genes have extremely divergent C-terminal peptide sequences, one of which has a potential N-linked glycosylation site. Comparison with other plant proteins which have C-terminal processed propeptides suggests they may have a role in the targeting of saporin proteins.

Comparison of the saporin coding sequences with other RIPs reveals conserved residues in the region of the putative active site of the protein. PCR was used to introduce a promoter and initiation codon upstream of the coding sequence, simultaneously removing the leader sequences. *In vitro* expression of the modified saporin coding sequences showed that rabbit reticulocyte ribosomes were modified in the same manner as native saporin.

We are currently investigating the expression of the genes in leaf and seed by Northern analysis and *in situ* hybridisation. Immunolocalisation is being used to determine the final destination of saporins in leaf tissue and developing seeds.

Y 411 INTRODUCTION OF RESISTANCE TO BROMOXYNIL INTO CANOLA

FREYSSINET M., KUZIO S., PELISSIER B., OUDEYER J. C., RENARD M., BREJON M. and FREYSSINET G.

Rhône-Poulenc Agro, B.P. 9163, 69263 LYON Cédex, France.

Bromoxynil is a contact herbicide which acts by blocking photosynthesis in dicot plants. A bacterial gene coding for a bromoxynil specific nitrilase has been isolated (STALKER *et al.*, 1987). The region coding for this enzyme has been put under the control of eucaryotic regulatory sequences and introduced into canola using an *Agrobacterium*-mediated transformation/ regeneration procedure. *In vitro* selection is done either with kanamycine or bromoxynil. Regenerated plants are sprayed, under greenhouse conditions, with bromoxynil at doses up to 1,200 g a.i./ha and show no phytotoxicity. The progenies obtained after self-pollination have been analyzed at the molecular and phenotypic levels. Plants with one gene copy were analyzed under field conditions. We did not see differences in the growth and behavior of the treated transgenic canola compared to non-treated, non transgenic ones.

STALKER D.M. *et al.*, J. Bact. 169 (1987) 955-960.

Y 412 CLONING AND ANALYSIS OF A cDNA ENCODING A GLUTATHIONE-S-TRANSFERASE SUBUNIT FROM MAIZE WHICH IS INDUCED BY TREATMENT WITH HERBICIDE SAFENERS, Andrew Greenland, Ian Jepson, Philip Bell, Simon Bright, David Holt, Venetia Lay, ICI Seeds, Jealott's Hill, Bracknell, Berkshire, RG12 6EY, UK.

Glutathione-S-transferases (GSTs) in maize are a family of enzymes which detoxify several commonly used herbicides by conjugation with glutathione. Total GST activity and protection against herbicides can be enhanced by the application of chemicals known as safeners. In maize, three isoforms of GST have been identified. GST I and GST III are homodimers of 29kD and 26kD subunits respectively (1, 2, 3). We are studying the third isoform, GST II, which is induced by safener treatment. Pure GST II is a heterodimer composed of 29kD (GST II-29) and 27kD (GST II-27) subunits which are immunologically distinct. Amino acid sequence analysis of tryptic fragments of GST II-29 shows that the 29kD subunits of GST II and GST I are identical. Immunoscreening of an expression cDNA library made from maize roots treated with safener has identified a cDNA encoding GST II-27. GST II-27 and GST II-29 exhibit an identity of 56% at the protein level. Currently we are analyzing the expression of GST II-27 in plants treated with safener.

1. Wiegand *et al* (1986) Plant Mol. Biol. 7, 235-243.
2. Moore *et al* (1986) Nucl. Acids Res. 14, 7227-7235.
3. Grove *et al* (1988) Nucl. Acids Res. 16(2) 425-438.

Y 413 OSMOTIC STRESS AND REGULATION OF GENES OF PROLINE BIOSYNTHETIC PATHWAY, Chien-an A. Hu, Ashton J. Delauney, and Desh Pal S. Verma, Department of Molecular Genetics and Biotechnology Center, 1060 Carmack Road, The Ohio State University, Columbus, OH 43210-1002.

Drought and high salinity are the most important environmental factors that cause osmotic stress and impact negatively on plant growth and crop productivity. To counter these stresses, many plants increase osmotic potential by synthesizing and accumulating compatible osmolytes such as proline and glycine betaine. Proline has been found to accumulate in many plants that are osmotically stressed; however, the exact metabolic route and enzymes involved in the synthesis of proline have not been unequivocally identified. Working from the assumption that the proline biosynthetic pathway is conserved in bacteria and plants and that structural genes of this pathway may have functional homologies, we have recently isolated a cDNA clone for soybean Δ^1-pyrroline-5-carboxylate (P5C) reductase (P5CR) by direct complementation of an *Escherichia coli proC* proline auxotroph with a soybean nodule cDNA expression library (Mol. Gen. Genet. [1990] 221: 299-305). While attempting to isolate *proB* and *proA* homologues of plants using similar strategy, we discovered a clone encoding a novel bifunctional enzyme, P5C synthetase (P5CS), in a mothbean nodule cDNA expression library. This enzyme has both γ-glutamyl kinase and glutamyl semi-aldehyde dehydrogenase activities. P5CS activity is feedback regulated by proline. Since P5C can also be synthesized from ornithine *via* ornithine aminotransferase, we transformed an *E. coli* CSH26 *proBA* deletion mutant with a mothbean cDNA library and selected for proline prototrophy on minimal media supplemented with ornithine. Several clones encoding ornithine aminotransferase were isolated. We have thus established the proline biosynthetic pathway in plants. Expression of P5CR and P5CS genes increases with salt treatment, suggesting the involvement of these genes in osmoregulation. The availability of plant genes encoding the enzymes in proline biosynthesis opens the way for genetically regulating this pathway in transgenic plants, with the potential for increasing tolerance to drought and salinity stresses.

Y 414 HORMONAL AND TISSUE-SPECIFIC REGULATION OF CELLULASE. Elizabeth Kemmerer, Gail Matters, Susan Koehler, Susan Baird, Panagiotis Kalaitzis and Mark Tucker. USDA/ARS, Plant Mol. Biol. Lab., BARC-West, Beltsville, MD 20705.

Cellulase (endo-1,4-β-D-glucanase) is one of several cell wall hydrolases playing a critical role in many plant developmental processes including root initiation, abscission, and fruit softening. We are studying the hormonal and tissue-specific regulation of cellulase during adventitious rooting in soybean and leaf abscission in bean. Exogenously applied auxin induces adventitious rooting in seedling cuttings. Cellulase facilitates rooting by breaking down cell wall components in the stem, allowing the new roots to emerge. Cellulase enzyme activity increases 10-fold in hypocotyls by the third day after auxin treatment (the day of maximum hypocotyl swelling and root emergence) after which the cellulase enzyme activity decreases as the roots elongate. Epicotyl and leaf tissues, which do not swell or form roots, show no distinct pattern of cellulase activity. Experiments with explants treated with anti-auxin and anti-ethylene agents reveal that it is the endogenous ethylene produced by the plant in response to the exogenous auxin application that induces cellulase activity. We have isolated two different soybean cellulase clones. Studies of cellulase regulation at the mRNA level during rooting are in progress. We have also identified cDNA and genomic clones for bean abscission cellulase. Northern blot analysis showed that the abscission cellulase mRNA accumulates in ethylene induced abscission zones. Auxin treatments inhibit the development of abscission zones as well as the accumulation of abscission cellulase mRNA. We are utilizing bean abscission cellulase promoter deletion constructs in transient assays in bean and in transgenic tomato plants to identify cis-acting regulatory elements.

Y 415 GENETIC MANIPULATION OF GLUTATHIONE METABOLISM: A STRATEGY TO IMPROVE RESISTANCE TO OXIDATIVE STRESS, Karl [illegible] Kunert[1] and Christine H. Foyer[2], AECI Ltd, PO Modderfontein 1645, South Africa[1] and Laboratoire du Métabolisme, I.N.R.A, Versailles, 78026 Versailles, France[2]

Aerobic organisms possess an antioxidative defense system which comprises of enzymatic and non-enzymatic components, such as glutathione, for scavenging oxidizing free radicals. Manipulation of the capacity for synthesis and reduction of glutathione in plant cells may therefore have considerable potential for improving the viability of plants exposed to extreme oxidative environmental conditions. This includes exposure to pollutants, prooxidative herbicides and low temperatures. It is our aim to enhance the endogenous protective capacity of plants via transformation with selected bacterial genes involved in glutathione metabolism. We have initially investigated the influence of glutathione reductase (GOR) on glutathione metabolism in both E. coli and tobacco. Using a bacterial mutant lacking GOR activity high levels of glutathione and GOR activity correlate with an increased, rather than a decreased, sensitivity to oxidation. GOR was however essential for maintaining a high glutathione content. In tobacco, glutathione metabolism was unaffected by high GOR levels. The glutathione pool was always maintained constant. Additional protection against severe oxidation was not found after significant elevation of GOR activty obtained by expression of the bacterial gene coding for GOR in the cytoplasm of transgenic plants. Results suggest that overproduction of a single enzyme seems not to be a feasable way to completely overcome the consequences of cellular oxidation. However, we are currently investigating the possible advantages of overproduction of other enzymes involved in glutathione metabolism, such as γ-glutamylcysteine synthetase and glutathione synthetase, either alone or together with GOR.

Y 416 GENE EXPRESSION ASSOCIATED WITH CHILLING TEMPERATURE ALLEVIATION OF DORMANCY IN FLOWER BUDS OF WOODY PERENNIALS, Gregory A. Lang and Jiaxun Tao, Department of Horticulture, Louisiana State University, Baton Rouge, LA 70803

The application of molecular techniques to some plant science phenomena cannot be addressed in herbaceous plant systems. One such phenomenon is bud endodormancy in woody perennials and the mechanism(s) by which exposures to winter chilling temperatures "accumulate" to alleviate the dormant state. Both bud dormancy and the heritable "chilling unit requirement" are poorly understood, yet vital to improvement of yield in temperate zone fruit crops. We were the first to report alterations in dormant floral bud gene products associated with chilling unit accumulation (1) and have demonstrated that a protein of ~61 kD decreases during the final stage of chilling unit accumulation in both low- and moderate-chilling requirement peach germplasm. The relative abundance of this protein is differentially regulated by both temperature and stage of endodormancy (2). Poly A(+) RNA has been isolated from these experiments and in vitro translations are currently being attempted to further characterize regulation at the nucleic acid level. A timecourse of changes in expression with chilling promotive or inhibitive temperatures is also being developed.

We have recently initiated additional analyses of dormancy-related changes in gene expression during the chilling accumulation period in several other fruit crops, including apple, blueberry, grape, pear, and plum. Results from these experiments and those further characterizing the regulation of the 61 kD protein will be presented.

1. Lang, G.A. and Tao, J. 1990. Analysis of fruit bud proteins associated with plant dormancy. HortSci. 25:1068.
2. Lang, G.A. and Tao, J. 1992. Changes in dormant peach (Prunus persica Batsch.) flower bud proteins associated with chilling unit accumulation temperatures. Plant Physiol. 97:in review.

Y 417 SALT STRESS INDUCED PROTEINS IN SALT TOLERANT RICE VARIETIES, S.M.S. Naqvi, V.C. Özalp, H.A.Öktem, D.Nalbant, F.Özkan, A.R.Memon and M. Yücel, Department of Biology, Middle East Technical University, 06531, Ankara, TURKEY

Plants are known to synthesize unique sets of proteins when exposed to different environmental onslaughts. In an attempt to investigate such proteins in salt tolerant rice cultivars, eight days old seedling of Nona bokra grown asceptically on Murashige and Skoog (MS) medium were subjected to 2% NaCl in MS for eight hours. Whole root protein extracts were subjected to isoelectric focusing in a pH range of 3-8 in the first dimension and to SDS PAGE gradient gel (8-18%) in second dimension. With subsequent silver staining at least four proteins unique to salt treated plants have been identified with mol. wts of 15,26, 36 and 62 kDs and approximate pI values of 6, 5.5, 6.1 and 5.5 respectively. A comparison of different stains and organelle specificity of these proteins have been established which may help in elucidating the critical members with respect to salt tolerance.

Y 418 IDENTIFICATION OF STRESS INDUCED PROTEINS IN DIFFERENT VARIETIES OF POPPY *(Papaver somniferum L.)*

H.A.Öktem, V.C.Özalp, F.Özkan, D.Nalbant, S.M.S. Naqvi, A.R.Memon, M. Yücel, Department of Biology, Middle East Technical University, 06531 Ankara, TURKEY

In this study a preliminary analysis of synthesis of heat, metal and cold stress induced proteins of 10 days old poppy seedlings *(Papaver somniferum L.)* were presented. Following stress conditions total soluble protein profiles were analyzed by one and two dimensional polyacrylamide gel electrophoresis. Upon 4 hours 40°C heat stress, at least two distinct proteins were observed at high molecular weight range (>70 kDa). When seedlings were subjected to 5 hours of cold acclimation at 4°C there were obvious synthesis of several acidic proteins at 35 kDa and 90 kDa. Three days of 3 mM $Cd(NO_3)_2$ treatment of the seedlings caused induction and synthesis of novel proteins especially at low molecular weight ranges of 8-10 kDa and 17 kDa. The results are important for the selection of the heat, cold and metal resistant species. To the best of our knowledge, the resent study is the first demonstration of stress associated proteins in different poppy varieties.

Y 419 ORGAN DEVELOPMENT MUTANTS IN MAIZE, Peter A. Peterson, Department of Agronomy, Iowa State University, Ames, IA 50011.--Mutants affecting various plant organs and tissues have been uncovered in our maize genetics nursery plots. Some have been extensively analyzed while others have received only preliminary analyses.--***Mn*** (Miniature Seed)*: This mutant is tagged by the transposable element *Uq* (identified as *Mn::Uq*). It is the only gene site with a *Uq* element. *Mn* is dominant and not male transmissible. The pollen does not germinate.--**RSS** (Reduced Seed Set): This incompatibility phenotype is expressed in the presence of three recessive genes--two on the male side (*cim1, cim2*) and one on the female side (*cif*); the definitive genetic cross is: *cif/cif, Cim1/Cim1, Cim2/Cim2* x *Cif/Cif, cim1/cim1, cim2/cim2*. This results in reduced seed set. There are some "escapes" but no more than in most incompatibility crosses. It is not allelic to *ga1*.--***ba4*** (barren ear is absent): This is a newly originated barren mutant that is not allelic to the previously described *ba* mutants. With the identification of *ba4*, four pathway interruptions are now recognized that cause the barren phenotype.--**Grass-type-*Tp 889703-1***: This grass-type mutant represents a complex of effects that are currently being examined. The major effect is a grass-type, many tillered plant with (mostly) an incomplete or absent tassel. The ear type varies in size but it is teopod. This mutant, under greenhouse growth conditions can have a relatively good tassel which facilitates outcrossing. Outgrowths of normal looking tassels from an otherwise grass-type plant suggests that the mutant is unstable.--**Tassel-less**: This is a normal looking plant whose tassel develops through the microscope stage but the tassel emerges completely dried up. It appears that the base of the tassel has an abscission layer that aborts the tassel.--These mutants will be illustrated.--***Pan, Yong-Bao** and **Peter A. Peterson**. 1989. Tagging of a maize gene involved in kernel development by an activated *Uq* transposable element. Mol. Gen. Genet. **219**:324-327.

Y 420 Soybean Inositol 1,3,4,5,6-Pentakisphosphate 2-Kinase, Brian Q. Phillippy, USDA, ARS, Southern Regional Research Center, New Orleans, LA 70124

Inositol hexakisphosphate ($InsP_6$), commonly known as phytic acid, comprises approximately 1 % of the weight of most seeds. It is considered an antinutrient because it reduces the bioavailability of trace minerals and phosphate in foods and feeds. $InsP_6$ in animal excreta is increasingly recognized as a major contributor to the water pollution caused by farm-raised animals. Biotechnological approaches to these problems will be to either increase $InsP_6$ degradation or to lower the rate of its synthesis. Inositol 1,3,4,5,6-pentakisphosphate ($InsP_5$) is a precursor of $InsP_6$ in plants and animals. $InsP_5$ 2-kinase catalyzes the transfer of the gamma phosphate of ATP to $InsP_5$ and may regulate the in vivo accumulation of $InsP_6$.

In order to evaluate its importance to $InsP_6$ synthesis, $InsP_5$ 2-kinase was purified one thousand fold from soybean seeds using anion exchange, blue dye affinity, and hydroxylapatite columns. Similar results were obtained using dried mature or green immature seeds and in the presence or absence of an initial ammonium sulfate precipitation. The pH optimum of the reaction was 7.0, and 5 mM magnesium ions was required for optimal activity. The enzyme displayed Km values for $InsP_5$ and MgATP of 3 uM and 6 uM, respectively, and a Keq of 1. Hopefully, genetic engineering using antisense technology to reduce the activity of the 2-kinase will reduce the $InsP_6$ levels of the seeds without affecting viability.

Y 421 OHP: ISOLATION AND CHARACTERIZATION OF A MAIZE bZIP PROTEIN WHICH INTERACTS WITH OPAQUE-2. Leonard D. Pysh, Milo J. Aukerman, and Robert J. Schmidt, Department of Biology and Center for Molecular Genetics, University of California at San Diego, La Jolla, CA 92093.

The Opaque-2 (O2) protein is required for the expression of a subclass of seed storage proteins (zeins) in the maize endosperm and is a member of the bZIP family of transcriptional regulatory proteins. We have isolated and characterized another bZIP protein from maize which is capable of heterodimerizing with O2 and has been called OHP (O2 heterodimerizing protein.)

A maize endosperm cDNA library was screened with a fragment of the *O2* gene which contained the basic domain and the first four leucine residues of the leucine zipper. Two clones showed hybridization at reduced stringencies but no hybridization to the 3' end of the *O2* gene. A 900 bp EcoRI fragment from one of the clones was subcloned into Bluescript and sequenced. The deduced amino acid sequence revealed the presence of a bZIP domain. The newly-discovered gene is 76% identical to *O2* at both the nucleotide and amino acid level over the bZIP domain but diverges greatly outside of this domain. Southern analysis revealed the presence of two closely-related genes, with OHP mapping to the long arm of chromosome 1. Northern analyses indicate that the OHP message is approximately 1.8 kb and is present in endosperm, root, shoot, embryo, and leaf tissue but not female flower tissue. Gel shift analysis demonstrates that *in vitro* transcribed and translated OHP protein is capable of binding to the O2 target site as both a homodimer and in a heterodimeric complex with O2. Progress on characterizing the OHP gene product will be presented.

Y 422 GENETIC MANIPULATION OF *ARABIDOPSIS*, Ethan R. Signer, Department of Biology, Massachusetts Institute of Technology, Cambridge MA 02139-4307. Genetic technology for manipulation of the *Arabidopsis* genome should be of considerable value, initially for basic research and ultimately for practical engineering of crop species in agronomy. This laboratory is developing a variety of methods for targeted gene replacement and similar aims based on fundamental studies of recombination that are also under way. The current status of these methods will be described.

Y 423 RAFFINOSE AND STACHYOSE INCREASE DURING COLD ACCLIMATION, Cecil Stushnoff, Richard L. Remmele Jr., M. McNeil, and Virgil D. Esensee, Departments of Horticulture, Biochemistry and Microbiology, Colorado State University, Fort Collins, CO 80523. Temperate zone plants produce endogenous cryoprotectants in response to induction of cold acclimation. Dormant buds of apple and other woody plants can be induced to increase hardiness from about -5° C for non-acclimated buds, to as low as -196° C in the fully acclimated, desiccated condition. We monitored seven woody plant taxa to determine quantitative and qualitative metabolic changes in search of oligosaccharides that might be associated with induction and loss of cold hardiness. Under controlled acclimation, at the first stage of hardiness the lethal freezing temperature changed from -5° C before induction, to -15 to -20° C. This increase in cold hardiness was accompanied by a ten-fold increase in raffinose and about a three-fold increase in endogenous stachyose. In the field samples, endogenous raffinose increased from < 0.02% in August to 2-11% (wt % dwb) for cortical stem tissues of all cold acclimated taxa at maximum hardiness. The tetrasaccharide, stachyose increased from <0.02% to 0.25-2.5% for similar comparisons. Although endogenous production of glucose, fructose, sorbitol and sucrose increased with exposure to low temperature for some taxa, the levels remained static or decreased for others. The lack of consistent patterns for all sugars monitored, except for the raffinose family oligosaccharides (RFO), leads us to conclude that the RFO's play a special role in the biochemistry of induced cold acclimation. Others have reported that RFO's increase following a single exposure to low temperature in cabbage, soybean, kidney bean, conifer needles and *Chlorella*. Our research shows a direct relationship to acclimation, maximum hardiness and deacclimation under controlled induction, and under field conditions. Accordingly, we hypothesize that endogenous production of RFO's may play an important role in metabolic events associated with the induction of resistance to low temperature stress and are conducting studies to determine the genetic control of this metabolic pathway.

Y 424 ANALYSIS OF COTTON CELL LINES WITH INCREASED RESISTANCE TO CHILLING TEMPERATURE, Norma L. Trolinder, Jiying Huang, and Candace H. Haigler, U.S. Department of Agriculture, ARS, Route 3, Box 215, Lubbock, TX 79401 Cotton cell lines were selected in vitro for increased resistance to chilling temperature (5°C). Shoot tips of plants regenerated from these cell lines were able to root normally at 22°C, whereas those of the control cell lines did not. After prolonged passage in the absence of the selection pressure, the selected cell lines retained the acquired resistance. Glucose uptake, cell viability, and growth were greater in the selected cell lines than the control cell lines when grown under either continuous 15°C or under a cycling regime of 30°C:15°C. PAGE and 2-D gel analysis revealed several major differences in gene expression between the selected versus the control cell lines when grown under cycling low temperature.

Y 425 ANALYSIS OF CO-SUPPRESSION OF ANTHOCYANIN BIOSYNTHETIC GENES IN PETUNIA, William T. Tucker, Alison Morgan and Neal Courtney-Gutterson, DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, CA 94608

Co-suppression of gene expression by the introduction of constitutively expressed copies of homologous genes has been reported in a number of diverse biological systems. The effect on expression of genes of the anthocyanin biosynthetic pathway in petunia (*Petunia hybrida*) has been examined following the introduction of anthocyanin biosynthetic genes from both homologous and heterologous plant species. Chalcone synthase (CHS) and Dihydroflavanol reductase (DFR) genes isolated from either petunia or chyrsanthemum, expressed from the CAMV 35S promoter were introduced into the Petunia line V26 and the phenotypes examined. Crosses were performed using Petunia lines carrying non-co-suppressing transgenes to identical lines carrying co-suppressing transgenes to determine the effect of both introduced genes on flower phenotype.

Y 426 CELL WALL PROTEINS IN SOYBEAN ISOGENIC LINES, Lila O. Vodkin, Jon T. Lindstrom, Christopher Nicholas and Joselyn Todd, Department of Agronomy, University of Illinois, Urbana, Illinois, 61801. We are investigating the composition and structure of the soybean seed coat during development. Two genes that affect seed coat pigmentation and structure are the *I* and *T* loci. We have demonstrated an unexpected and novel correlation between the *I* gene which inhibits anthocyanin accumulation and the quantitative levels of a specific developmentally regulated proline rich cell wall protein, PRP1 (*Plant Cell* 3: 561-571, 1991). We have extended these studies to more than 10 additional isogenic pairs of soybean lines. Each cultivar with recessive *i/i t/t* genotype has defective seed coats and reduced levels of PRP1 cell wall protein. We show that seed coats of *i/i T/T* genotypes also contain polyphenolic compounds, possibly proanthocyanidins, that have in vitro "tannin" activity toward protein and RNA. These compounds accumulate even at very early stages of seed development (10-25 mg total seed weight) more than 25 days before the development of visible anthocyanin accumulation. We have also found a correlation between another soybean gene that affects anthocyanin, the *Im* locus, and the size of two proline rich cell wall proteins, PRP1 and PRP2, in a number of soybean lines. We are currently examining whether genetic linkage or protein modification is the basis of the size variation.

Late Abstracts

THE USE OF THE pEMU PROMOTER IN SUCCESSFUL SELECTIONS OF TRANSGENIC RICE AND WHEAT , Douglas A. Chamberlain, Richard I.S. Brettell, Alex M. Drew, David I. Last, Barbara Witrzens, David McElroy and Elizabeth S. Dennis, CSIRO Division of Plant Industry, Canberra, Australia 2602.

The pEmu promoter, which is based on that of maize *Adh1*, and uses additional enhancer elements from various sources, has been shown in transient studies to promote far greater gene expression in cultured cereal cells than any of the 35S constructs we tested (Last *et al* 1991). We have linked this promoter with the *nptII*, *hpt* and *bar* genes to produce selectable marker genes that are also well expressed in cereal tissues. Conditions for the efficient selection of rice plants and wheat cultures from protoplasts electroporated with these genes have now been established.

Herbicide and antibiotic resistance genes linked to promoters conferring high levels of expression in cereal cells will prove useful for the transformation of species such as wheat, barley and sugarcane in which the 35S promoter shows low efficacy, (Rathus 1990, Lazzeri 1991, Vasil *et al* 1991, Bower and Birch 1992).

Bower R. and Birch R.G. The Plant Journal (In press).
Last D.I., Brettell R.I.S., Chamberlain D.A., Chaudhury A.M., Larkin P.J., Marsh E.L., Peacock W.J. and Dennis E.S. Theor Appl Genet (1991) 81:581-588.
Lazzeri P. personal communication.
Rathus C. Phd Thesis(1990) University of Queensland, Botany Dept.
Vasil V., Brown S.M., Re D., Fromm M.E. and Vasil I.K.(1991) Bio/Technology 9:743-747.

U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT INITIATIVES IN CROP BIOTECHNOLOGY FOR THE DEVELOPING WORLD, Judith A. Chambers*, Mariam B. Sticklen, R. Horsch*** and Joel I. Cohen*, *Office of Agriculture, Bureau for Research and Development, Washington D.C. 20523 and **Plant Tissue Culture and Genetic Engineering Lab, Michigan State University, East Lansing, MI 48824-1311, *** Monsanto Agricultural Company, St. Louis, MO 63198**

This century has witnessed a critical transition in agriculture, from a system historically dependent on utilization of natural resources, to one reliant on science and technology to increase agricultural yields. The lack of well-developed public and private infrastructure in developing countries presents a distinct disadvantage in the application of biotechnological innovations. Therefore, the U.S. Agency for International Development, Office of Agriculture is vigorously supporting several programs in crop biotechnology research and application addressing developing country production constraints. Programmatic support has capitalized on expertise resident within U.S. public and private sectors to increase technical capacity in biotechnology. These programs will also enhance developing country capacity in biosafety and intellectual property and in the ability to commercialize beneficial products of research. Two grants are discussed in detail: (1) production of genetically-engineered virus resistance in cassava, yams, and sweet potato for Africa via Monsanto; (2) Michigan State University, as the lead managing institution in a consortium of public and private institutions which include Cornell University, Texas A&M, and DNA Plant Technologies, to utilize recombinant DNA and tissue culture techniques directed towards the improvement of indigenous varieties of such developing country crops as cucurbits, maize, potato, banana, coffee and pineapple.

INVOLVEMENT OF MITOCHONDRIA IN COLD-INDUCED SUGAR ACCUMULATION, Yannis Gounaris, U.S. Department of Agriculture, Potato Research Laboratory, 311 5th Ave. NE, P.O. Box 113, East Grand Forks, Minnesota 56721. Mitochondria were isolated from cold-stressed tubers of four potato cultivars differing in their ability to accumulate sugars when subjected to low temperatures. Low temperatures induced changes in the buoyant density of the mitochondria. The degree of change corresponded to their ability to accumulate sugars. A 26-kDa protein appeared in the cold-stressed mitochondria of the high sugar accumulating cultivars. Low sugar accumulating cultivars lacked or showed only limited amounts of the 26-kDa protein. Examination of the most resistant to cold-induced sweetening cultivar, ND 860-2, showed that it differs from the high sugar accumulating clones in the mitochondrial DNA restriction pattern and the ribosomal RNA bands.

IN SITU FOREIGN GENE EXPRESSION IN SEEDLINGS OF SOME MONOCOTYLEDONOUS CROPS USING THE IMPROVED PARTICLE GUN DRIVEN BY COMPRESSED N_2 GAS, H. Kakuta, Y. Hashidoko, T. Seki, M. Matsui, T. Anai, K. Hasegawa and J. Mizutani. Plant Ecochemicals Project, Research development Corporation of Japan, Eniwa RPB, 1-1-3 Megumino Kita, Eniwa-shi, Hokkaido 061-13, JAPAN

Using an improved N_2-compressed particle gun, a foreign gene introduction was performed on leaves, stems and roots of intact seedlings of oat and wheat. The improved particle gun has a stainless steel cylinder (5mm, internal diameter; 300mm long as standard) equipped with a stopper and vent holes. The cylinder goes through the upper blind flange into the cylindrical vacuum chamber (267mm, internal diameter; 368mm, internal height), which is connected to a solenoid valve. The degree of vacuum in the chamber during the acceleration of macroprojectiles, was kept constant by the vacuum controller with a quartz gauge. The lower extremity of the cylinder was loaded with teflon or polyacetal macroprojectiles covered with gold particles coated with plasmid DNA, after removing it from the chamber. The bombardments were done at a pressure of 100mmHg, produced with nitrgen gas pressure (22kg/cm^2), with the plant samples distant approximately 5cm from the stopper. The advantage of the improved gun is: 1) any plant material can basically be treated; 2) treatment of the material is simple and easy; 3) the appaturus is applicable to intact plant gowing on pot; 4) chamber is exchangeable on the purpose of the experiment; 5) cylinder is also exchangeable to optional size. Thus, it is possible to set up the gun condition to cover most of plant materials as the objective. Plasmid DNA (300ngDNA/75µg particle), pBI221 and pBI121 (Clontech, Palo Alto/CA) carrying the GUS gene coated onto gold particles (0.78 µm, diameter) were introduced into the embryo cell of water-absorbed oat and wheat seeds by bombardment. The seedlings grown in sterilized pot (2-7 days) were tested GUS gene expression by X-Gluc staining. In oat, transient GUS gene (pBI221) expression was observed at the embryos, stems, leaves and roots. In wheat, GUS gene (pBI121) expression was observed at lower part of stems.

PROTEIN COMPOSITION AND BIOSYNTHETIC ACTIVITIES OF TWO CLASSES OF RUBBER PARTICLES (RP) IN *HEVEA BRASILIENSIS*.

Elisabeth Goyvaerts, Hoong-Yeet Yeang* and Nam-Hai Chua**. Institute of Molecular and Cell Biology, National University of Singapore, Singapore 0511, *Rubber Research Institute of Malaysia, Experimental Station, Sungei Buloh, Malaysia and **The Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York 10021-6396.

In the laticifers of *Hevea brasiliensis*, rubber (cis 1-4 polyisoprene) is made at the surface of RP. Two major proteins of Mr 14000 (REF) and 24000 (p24), are associated with the RP. To examine the role of the two RP surface proteins in rubber biosynthesis, we analysed the RP in greater detail. By ultracentrifugation RP can be separated in two zones. The low density zone1 contains large RP (50Å to 3µm) with a higher rubber content (ρ_{rubber} = 0.93 g/cm^3) while the heavier zone2 contains smaller RP (50-3000Å) with less rubber per particle. The rubber Mr distribution does not correlate with the RP size. REF is more abundant in zone1 than zone2 while more of the p24 is found in zone2. These findings suggest that one class of particles may originate from the other and both are part of the same RP biogenesis pathway. Alternatively, the two different classes of RP may coexist, each having its own biogenesis pathway and biosynthetic properties. The necessary involvement of REF in stereospecificity during rubber polymerisation may be questioned since it may be absent in RP2. Or, the p24 may play a similar role as REF in rubber biosynthesis. Light *et al.* (JBC 264, 18598) reported that prenyltransferases from a variety of sources elongate the rubber chain by subsequent additions of isopenthenyl pyrophosphate (IPP) whereby REF renders *cis*-stereospecificity to the rubber polymerization reaction. Rubber biosynthesis can be monitored *in vitro* by measuring the amount of ^{14}C-IPP incorporated into high Mr particles (by gel filtration) or into rubber (by floatation or solvent extraction). The smaller RP of zone2 are about 4 times more active than zone1 RP on the basis of protein or dry rubber content (DRC) and about 1.4 times more active than whole latex on basis of DRC. To study the role of prenyltransferase in rubber biosynthesis, we added recombinant rat liver prenyltransferase (PT) to the RP. Addition of PT to zone1 RP doubled ^{14}C-IPP incorporation to the level of whole latex after PT addition. Zone2 ^{14}C-IPP incorporation remained unchanged. Our results suggest that zone2 may contain a prenyltransferase activity absent in zone1. Alternatively, a prenyltransferase which is involved in synthesizing starter molecules may be absent in zone1 RP.

STUDIES ON THE MECHANISM OF MULTIPLE VIRUS RESISTANCE IN *Solanum brevidens* AND ITS TRANSFER TO POTATO VIA ASYMMETRIC HYBRIDIZATION, Pehu, E., Xu, Y-S, Valkonen, J., Pehu, T. and Lehto, K., Department of Crop Production, University of Helsinki, 00710 Helsinki, Finland.

Recently we have confirmed the extreme resistance of *Solanum brevidens* to PLRV and PVY and shown that it is also extremely resistant to PVA and moderately to PVX. Studies on 25 accessions showed very little variation in the degree of resistance. The resistance in *Solanum brevidens* is not expressed in isolated protoplasts, which may suggest it to be related to slow cell-to-cell spread. Experiments are on-going to resolve the role of the virus functions in the resistance mechanism at the molecular level. To transfer the resistance to cultivated potato we have by 'gamma-fusion' produced 30 asymmetric hybrids, which contain 15 - 55% of the donor genome as determined by species specific probes. Survey of the virus resistant and susceptible hybrids by linkage group specific RFLPs is on-going and the results will be presented in the poster.

APRT -- A POTENTIAL SELECTABLE MARKER FOR PLANT TRANSFORMATION, Dennis A. Schaff, Sunita K. Agarwal, and David J. Schultz, Department of Plant and Soil Sciences, College of Agricultural Sciences, University of Delaware, Newark, DE 19717-1303.

Currently, selectable markers for plant transformation and the production of transgenic plants confer resistance to either antibiotics or herbicides. There are public concerns which border on fear that antibiotic resistance genes might somehow be incorporated in pathogenic bacteria, and antibiotics will no longer be effective. There is also concern that herbicide resistance will spread to weed populations by cross pollination with herbicide resistant crop plants. Therefore, the use of a neutral plant gene, such as APRT (adenine phosphoribosyltransferase; EC 2.4.2.7), as a plant selectable marker will help circumvent these public concerns and fears. The APRT system has the added advantage over resistance to phytotoxic chemicals (antibiotics or herbicides) of both positive forward or backward/reverse selection, which can be used in the selection of transformed plants. APRT has a simple selection system with positive selection for both the functional APRT enzyme ($APRTase^+$) and non-functional APRT enzyme ($APRTase^-$). APRTase does not show a high degree of specificity for the exact structure of adenine and can also act on cytokinins and adenine analogues like 6-methlypurine, 2,6-diaminopurine, and 2-fluoroadenine. APRTase can utilize these adenine analogues as substrates and convert them to their nucleotide forms, which are toxic. Plants that lack APRTase activity survive in the presence of these analogous. The amount of adenine analogue used for selecting $APRTase^-$ plants is such that it kills all $APRTase^+$ (wild type) plants. The APRT system can function as a conditional auxotroph for AMP. The $APRTase^-$ phenotype can be conditioned as an auxotroph when alanosine and azaserine are added to the growth medium — these chemicals block the *de novo* pathway for AMP biosynthesis, and the $APRTase^-$ plants will not survive because they cannot produce AMP. We are selecting $APRTase^-$ plants and are cloning APRT genes from soybeans and other plants. After which we will attempt to complement the $APRTase^-$ plant with a wild-type APRT gene to select for transformation.

ISOLATION, CHARACTERIZATION AND CLONING OF TOXINS FROM BACILLUS THURINGIENSIS ACTIVE AGAINST PLANT PARASITIC NEMATODES, George E. Schwab, Kenneth E. Narva and Leo Kim, Department of Molecular Biology, Mycogen Corp., San Diego, CA 92121

The parasporal inclusion bodies of certain strains of Bacillus thuringiensis possess demonstrated toxicity to a wide range of insects including dipteran, lepidopteran and coleopteran pests. The specificity and environmentally benign nature of these toxins make them attractive alternatives to conventional chemical pesticides. We have recently cloned and characterized six novel genes encoding proteins toxic to a number of plant parasitic nematode species that include the root lesion and root knot nematodes, Pratylenchus penetrans and Meloidigyne javanica, respectively. Evidence is presented for the segregation of these six genes into two new Bacillus thuringiensis toxin gene families.

"THE ACID TEST": A PH INDICATOR-BASED TEST TO IDENTIFY TRANSFORMANTS EXPRESSING PPT ACETYL-TRANSFERASE.

Raymond D. Shillito, Joseph J. DiMaio, Trang Le, Gleta K. Carswell and Catherine M. Kramer. CIBA-GEIGY Biotechnology Research. P.O. Box 12257, RTP NC 27709-2257

Phosphinothricin (PPT; glufosinate; Hoechst AG) is a herbicide which has been used extensively for selection of transformed plant cells. We have developed a novel method for identifying maize cells transformed with the BAR (Bialaphos resistance; PAT) gene based on inclusion of a pH-indicator in the culture medium.

The method allows one to identify transformed colonies more quickly and efficiently by observing color changes in the medium. We use the identification method as part of an efficient selection system for maize transformants carrying and expressing the BAR gene. By combining PPT selection with an the assay, we are able to identify transformants only 6 weeks after transformation. Transformed plants can also be screened using the test.

TRANSFORMATION OF PLANT MITOCHONDRIA, Henri Wintz, Maureen Hanson and Kathy J. Newton *, Plant Science Center, Biotechnology Building, Cornell University, Ithaca, NY 14853, (*) Department of Biological Sciences, Tucker Hall, University of Missouri, Columbia, MO 65211

Technically the introduction of foreign genes into organelles is possible with the gene gun since it has been achieved with yeast mitochondria and plant and algea chloroplasts. However higher plant mitochondria transformation has been hindered by the lack of a selection scheme for the transformants. We are attempting to use a maize cell line that has a mutation in the mitochondrial genome as a host in the transformation experiments and use the restoration of the wild type phenotype in a selection scheme. The NCS (Non Chromosomal Stripe) series of maize mutants are the only plant mitochondrial mutants known today. The phenotype of the plants, yellow striping of the leaves, affected growth, and zone of aborted kernel development on the cob, are due to deletions in the mitochondrial genome. In the case of NCS-6 line that we are using there is a partial deletion of a mitochondrial gene coding for a component of the cytochrome oxidase (the *cox*II gene). The plants are heteroplasmic as well as cell cultures obtained from immature embryos; however we have shown, by using PCR that single cells are homoplasmic. We have also obtained homoplasmic mutant calli through protoplast regeneration. The strategy that we plan to use to select mitochondrial transformants relies on the fact that the mutant cells do not have a functional cytochrome oxidase and therefore have to use the alternative or cyanide insensitive pathway for their respiration. Selection of the transformants can be done by specifically blocking the alternative pathway allowing only cells with a restored cytochrome oxidase to survive.