IMPROVED CROP AND PLANT PRODUCTS THROUGH BIOTECHNOLOGY

Organizers: Barbara Mazur, Jan Leemans and Richard Flavell

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Product Performance Criteria and Regulatory Approval for Transgenic Crops

X1-001 CROPS CARRYING NEW HERBICIDE AND INSECT RESISTANCE TRAITS, Robert T. Fraley, Stephen R. Padgette, Roy L. Fuchs, Stephen G. Rogers, The Agricultural Group of Monsanto Company, 800 N. Lindbergh Boulevard, St. Louis, MO 63167. The development and commercialization of genetically engineered crops is underway today in many countries around the world as scientists strive to meet the challenges of increasing food production for a growing world population. Seven hundred field tests at over 1,500 sites have now been conducted with crops expressing new genes that improve pest resistance, enhance grain or fruit quality or provide farmers with better options for controlling weeds. Monsanto Company has developed a number of genetically-improved crops which have been field tested for several years and are currently in advanced stages of performance and safety assessment. Two examples--cotton lines engineered to express the Bt insect control protein for budworm and bollworm protection and soybean varieties safened for in-crop Roundup® herbicide application--will be discussed to illustrate the detailed efforts undertaken to support plant breeding, field performance evaluation, regulatory agency (USDA, EPA and FDA) approval and commercialization. The scientific framework for evaluating the food, feed and environmental safety for these advanced commercial crop candidates will be reviewed in depth. Finally, potential barriers to commercialization (legal actions, public acceptance, international trade, etc.) of these crops will be discussed.

X1-002 COMMERCIAL INTRODUCTION OF FLAVR SAVR™ TOMATOES, William R. Hiatt, Calgene Fresh, Inc., Davis, CA 95616.

FLAVR SAVR™ tomatoes have greatly reduced levels of polygalacturonase as a result of antisense technology. These fruit soften and rot more slowly, allowing for ripening on the vine and development of flavor. Requirements for commercial introduction will be discussed including variety development, regulatory considerations and product specifications.

Host Plant Resistance to Pathogens and Insects

X1-003 TRANSGENIC AND NATURAL RESISTANCE MECHANISMS TO POTATO VIRUS X INFECTION, David C. Baulcombe, The Sainsbury Laboratory, Norwich Research Park, Colney Lane, Norwich NR4 7UH, UK.

Replicase mediated resistance to potato virus X. Transgenic plants were produced that expressed various forms of the replicase gene of potato virus X in the expectation that the plants would be resistant to the virus. It was anticipated that the resistance would result from an imbalance in the levels of host and virus encoded components of the replicase complex or, if the viral transgene encoded a dysfunctional protein, interference with the function of the wild type protein of the inoculated virus. The plants were resistant, as predicted, but the properties of the resistance indicated that the mechanism may not depend on expression of the virus encoded protein. There was also a non linear relationship of accumulation of the RNA or protein products of the transgene and the degree of resistance: some plants accumulated the products of the transgene at a low level but showed extreme resistance whereas other plants were fully susceptible and accumulated the transgene products at a high level. In the hybrid progeny of these two types of plant the viral transgene giving high level accumulation was co-suppressed by the transgene associated with low level accumulation of the transgenic RNA and the plants were resistant to PVX. These data indicate that the resistance mechanism in these transgenic plants may be related to a mechanism of co-suppression operating outside the nucleus.

Natural resistance to potato virus in potato. The coat protein of PVX is the elicitor of the extreme resistance in potato conferred by the *Rx* locus. This resistance is expressed in protoplasts by inhibition of accumulation of PVX RNA, including the negative strand RNA which is a replication intermediate. In resistant (*Rx*) protoplasts co-inoculated with PVX and an unrelated virus, either cucumber mosaic virus or tobacco mosaic virus, the accumulation of the second virus was also inhibited. We interpret this result to indicate that the *Rx*-mediated resistance is a two phase process in which recognition is virus specific and distinct from the induced resistance mechanism that is not virus specific. Identification and manipulation of the genes for components of the *Rx*-mediated resistance mechanism may allow genetic engineering of broad spectrum virus resistance.

X1-004 NOVEL GENES FOR THE PRODUCTION OF INSECT RESISTANT TRANSGENIC PLANTS
John A. Gatehouse, Angharad M.R. Gatehouse, Kevin S. Powell, Ying Shi, Mingbo Wang, Claire Brough, Vaughan A. Hilder*, and Donald Boulter. Department of Biological Sciences, University of Durham, South Road, Durham DH1 3LE, U.K.; *Agricultural Genetics Company. Cambridge, U.K.

Increasing awareness of the deleterious effects of indiscriminate usage of pesticides on the environment, and on human producers and consumers, has led to a search for alternatives to control insect pests. Transgenic plant technology is one method of producing crops with inherent resistance to insect attack, and is able to introduce entirely novel resistance genes into a plant species. Although most work in this area has been applied to the expression of insecticidal *Bacillus thuringiensis* δ-endotoxins in transgenic plants, an alternative approach is to use plant genes which encode proteins with insecticidal properties.

Protease inhibitors are involved in endogenous plant defence against insects, and their accumulation can either be constitutive in certain tissues (usually with a storage role) or induced by insect attack. Over-expression of several inhibitors from constitutive promoters has been shown to afford protection in transgenic tobacco plants against attack by Lepidopteran larvae. However, the degree of protection obtained has not been sufficient for commercial requirements, and while certain inhibitors are effective against a number of insect species, others seem to be ineffective, or only effective against a limited number of species. If these genes are to be made effective as crop protection agents, development of the technology will be necessary. By assaying the interactions of protease inhibitors with insect gut proteases *in vitro*, the most effective inhibitor (i.e. the inhibitor giving the greatest inhibition of proteolysis at the lowest concentration) can be selected for a particular insect species. Data from bioassays of insects using artificial diets, and with transgenic plants, suggest that the *in vitro* assay of relative inhibitor effectiveness is consistent with the effects of different inhibitors on insect development and survival *in vivo*. Alternatives to screening naturally occurring inhibitors for effectiveness against insect pests will be considered.

Sucking insect pests do not rely on proteolysis to obtain necessary amino acids and nitrogen, instead taking in free amino acids in the phloem sap. A different approach is therefore necessary with pests from this insect order, particularly as Bt toxins effective against Homopterans have not been reported to date. Bioassay in artificial diet was used to identify plant proteins with insecticidal effects on the rice brown planthopper (a model Homopteran). The lectin from snowdrop (GNA) was found to be the most effective of the proteins tested, giving approximately 80% corrected mortality in bioassay. Gene constructs containing the GNA coding sequence fused to either a constitutive (CaMV 35S), or a phloem-specific (rice sucrose synthase *RSs1*) promoter, were produced and tested for expression pattern in transgenic plants. GNA was shown to be present in the phloem sap of a *RSs1*-GNA transgenic tobacco plant by immunoassay of honeydew produced by aphids feeding on it. GNA is also insecticidal to the aphid *Myzus persicae*, which will feed on tobacco, and thus a bioassay of transgenic tobacco, to "prove" the technology, can be carried out.

A possible improvement to the use of a single introduced resistance gene would be to combine different resistance genes in the same transgenic plant to improve the effectiveness of protection. This procedure has been exemplified by the use of genes encoding cowpea trypsin inhibitor and pea lectin to protect transgenic tobacco against larvae of the Lepidopteran pest *Heliothis virescens*, and further examples will be considered.

X1-005 THE MOLECULAR BIOLOGY OF SYSTEMIC ACQUIRED RESISTANCE, John Ryals, Danny Alexander, Danielle Chandler, Terrance Delaney, Leslie Friedrich, Manuela Gut-Rella[1], Helmut Kessmann[2], Kay Lawton, Sharon Potter, Scott Uknes, Bernard Vernooij, Eric Ward, and Kris Weymann, Ciba-Geigy Agricultural Biotechnology Research Unit, Research Triangle Park, NC 27709-2257, [1]Ciba Limited, Seeds Division, CH-4002 Basel, Switzerland, [2]Ciba Limited, Plant Protection Division, CH-4002 Basel, Switzerland.

Systemic acquired resistance (SAR) is a particularly interesting response of plants to pathogens since a broad spectrum resistance can be induced that may last from several weeks to months. We have studied SAR at a biochemical and molecular biology level with the hope that a better understanding of SAR will lead to the development of both improved crop varieties and low-usage-rate, novel mode-of-action fungicides. Toward this end, we have isolated and characterized various SAR-related cDNA's. Transgenic plants have been engineered that express these cDNA's and they have been evaluated for fungal viral, and bacterial resistance. Several of these genes impart significant, though not total, tolerance to particular pathogens.

Salicylic acid (SA) is a probable signal in the pathway leading to the onset of SAR. Transgenic plants that express a bacterial enzyme that degrades SA can not be induced to resistance (Science 261:754-756). To determine if SA is the mobile SAR signal, we carried out grafting experiments using the transgenic salicylate hydroxylase plants. Transgenic rootstocks, although unable to accumulate SA, are fully capable of delivering a signal that renders non-transgenic scions resistant to further infection. This result indicates that a translocated signal other than SA is responsible for triggering SAR, and further, that SA is a local signal in the response. The salicylate hydroxylase expressing transgenic plants are also dramatically more susceptible to a number of pathogens. This increased susceptibility is manifested as a decrease of both quantitative and qualitative resistance reactions to fungi (ie *Peronospora parasitica*), bacteria (ie *Pseudomonas syringae*) and viruses (ie tobacco mosaic virus). This result suggests that the SA-dependent signalling pathway is critical even in local responses to pathogens. In other studies on signal transduction, we have demonstrated that the role for ethylene in SAR is as an enhancer or modifier of SA-dependent responses. Gaseous ethylene cannot induce resistance in arabidopsis but it can enhance the response to SA. We have also found that the action of ethephon as it relates to acquired resistance is not due to ethylene, but to the synergistic action of the breakdown products of ethephon, phosphonic acid, hydrochloric acid and ethylene,.

Because the SAR signal transduction pathway is likely to be complex we have established a working model of acquired resistance in arabidopsis (The Plant Cell. 5: 159-169). We have used our knowledge of this system to establish selection schemes for the isolation of mutants involved in acquired resistance. One of these mutants, cim1, had spontaneously forming lesions. We have demonstrated that this class of disease-lesion mimics constitutively express SAR.

X1-006 GENETIC DISSECTION OF BACTERIAL DISEASE RESISTANCE, Brian Staskawicz, Andrew Bent, Kit Brown, Karen Century, Douglas Dahlbeck, Matthew Hinsch, Barbara Kunkel, Department of Plant Biology, University of California, Berkeley, CA 94720.

We are currently developing *Arabidopsis thaliana* as a model host plant to study the genetic basis of plant disease resistance towards strains of *Pseudomonas syringae* . Our work has focused on isolating disease susceptible plant mutants that longer recognize strains of *Pseudomonas syringae* containing the *avrRpt2* avirulence gene. Several allelic *rps2* mutants have been identified and localized to chromosome 4 near the RFLP marker M600. These mutants are specific for this avirulence gene as these mutants are still capable of recognizing the avirulence genes *avrB* and *avrRpm1*. A YAC contig has been constructed that spans this locus and has been employed to isolate a set of overlapping *Agrobacterium* binary cosmids clones. Currently, we are in the process of introducing these clones into disease susceptible mutants to determine whether we can identify a clone that complements disease resistance. In addition to the *rps2* mutants, we have isolated two other classes of disease susceptible plant mutants. One class is unable to recognize either the avirulence genes *avrB* or *avrRpm1*, but still retains the ability to recognize *avrRpt2*. These data suggest that a single plant disease resistance gene is able to recognize two different avirulence genes. Finally, a third class of mutants has been identified that is unable to recognize all three avirulence genes. Preliminary data suggest that this mutant is controlled by a single recessive gene. The genetic characterization of this mutant is in progress.

Dissection of Signal Transduction Pathways

X1-007 CYCLIC GMP AND CALCIUM MEDIATE PHYTOCHROME PHOTOTRANSDUCTION, Chris Bowler, Gunther Neuhaus[1], Hiroshi Yamagata, and Nam-Hai Chua, Laboratory of Plant Molecular Biology, The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, 1Institut fur Pflanzenwissenschaften, ETH-Zentrum, Universitatstrasse 2, CH-8092 Zurich, Switzerland.

Phytochrome is a well-characterized plant photoreceptor, able to modulate many morphological, physiological, and biochemical events through as yet undefined mechanisms. By developing single-cell assays to visualize phytochrome responses, we have studied the effects of microinjecting putative signaling intermediates into phytochrome-deficient tomato cells. We demonstrate that phytochrome phototransduction initially involves the activation of one or more G proteins that are coupled to at least two different pathways. One pathway requires calcium and activated calmodulin and can stimulate expression of a photoregulated *cab*-GUS reporter gene together with the synthesis and assembly of PSII, LHCI and II, ATP synthase, and RUBISCO. The other pathway is mediated by cGMP and leads to the activation of CHS-GUS and anthocyanin biosynthesis. Synthesis of PSI and *cyt* b_6-f complexes, however, requires the cooperation of both pathways. Furthermore, our results reveal that phytochrome signalling is cell-autonomous and is not likely to require any light-activated steps downstream of the G protein.

This work was supported by a National Institutes of Health Grant GM44640.

X1-008 CELL SIGNALLING THROUGH CYTOSOLIC CALCIUM. Anthony Trewavas[1], Marc Knight[1], Steve Smith[1], Tony Campbell[2] and Nick Read[1]. [1]Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, EH9 3JH Scotland, [2]Medical Biochemistry, University College of Wales, Cardiff, S. Wales.

Plant cells receive a constant stream of environmental information to which they respond. Many of these signals impinge upon a cellular reaction network of considerable complexity and regulation which is constructed around calcium. Calcium enters the cytoplasm through channels clustered to varying degrees in the plasma membrane and some organelle membranes; channels may also direct calcium into intraorganelle spaces such as the nucleus. There is fine-grain differentiation of free calcium concentrations particularly in membrane microdomains and these change in ways unknown during signalling. Understanding aspects of this complex control system requires knowledge of the cellular distribution and movement of calcium-sensitive enzymes and proteins and the local distributions of intracellular calcium itself. The molecular signalling group at Edinburgh has developed a novel method with which the complexity of distributions of calcium can be approached. Plants have been transformed with the cDNA for the calcium sensitive protein aequorin; luminous plants have been generated whose luminosity measures intracellular calcium. This technology will be described and its future indicated. The information so far gained concerning mechanical signalling and oxidative stress will be outlined.

X1-009 SIGNAL TRANSDUCTION IN OXIDATIVE STRESS, Marc Van Montagu, Elena Babiychuk, Didier Herouart, Sergei Kushnir, Wim Van Camp, Hilde Willekens and Dirk Inzé, University Gent, Laboratory of Genetics, B-9000 Gent (Belgium).

Numerous environmental stress conditions are know to mediate, at least in part, cellular damage by the enhanced production of reduced oxygen species such as superoxide radials, hydrogen peroxide, and hydroxyl radials. Plants have evolved a wide range of enzymatic and non-enzymatic mechanisms to content with this so called oxidative stress. Key enzymes in the protection are superoxide dismutases (SODs), which converts superoxide radials into hydrogen peroxide, catalases (CAT1) and peroxidases (ascorbate peroxidase; APx and glutathione peroxidase, GPx), which scavenge hydrogen peroxide in water and oxygen.

In recent years genes encoding the various SOD isoforms, APx, catalases, GPx, have been isolated from *Nicotiana plumbaginofolia* and *Arabidopsis thaliana*. We showed that the expression of these genes is strongly affected by oxidative stress, albeit not co-regulated. Our data suggest that the expression of each gene is governed by the extent of generated oxidative stress within the particular subcellular location of each enzyme.

To unravel signal transduction cascades involved in sensing and transmitting the oxidative stress signal, three approaches are followed. First, we developed a protoplast based assay to identify molecules able to induce or influence the expression of a reporter gene, consisting out of the GUS coding sequence fused to a promoter of an oxidative stress responsive gene. As such we showed that thiolmolecules induce the expression of both the cytosolic Cu/ZnSOD and APx genes. This finding let to the identification of a protein factor with a putative role in sensing oxidative stress.

Second, a detailed DMS and DNASE-I in vivo footprinting analysis has been carried out on the Arabidopsis APx promoter. Several cis-acting elements were identified with a putative role in oxidative stress activated gene expression. A progress report will be given on our attempts to clone interacting transcription factors.

The third approach is based on the isolation and characterization of *Arabidopsis* mutants resistant to lethal concentrations of superoxide generating herbicides (eg. paraquat). Some of these mutants are expected to have a deregulated expression of oxidative stress responsive genes.

Fruit Ripening and the Production of High Value Products in Plants

X1-010 PRODUCTION OF HIGH-VALUE PROTEINS IN PLANTS, Jan Pen[1], Theo C. Verwoerd[1], Peter A. van Paridon[2], Albert J.J. van Ooyen[2], Peter J.M. van den Elzen[1], and André Hoekema[1,3], [1]MOGEN N.V., Einsteinweg 97, 2333 CB Leiden, The Netherlands, [2]Gist-brocades N.V., P.O. Box 1, 2600 MA Delft, The Netherlands, [3]Present address: Gist-brocades N.V., P.O. Box 1, 2600 MA Delft, The Netherlands.

The use of transgenic plant seeds as carrier of industrial enzymes is a new concept in plant biotechnology. Expression in seeds provides instant packaging of the enzyme in a stable and convenient form. Such enzyme-enriched seeds can be directly applied in the industrial process where the enzyme activity is required. Transgenic enzyme-containing seeds will be most compatible with those industrial processes where plant material is used, as in the production of animal feed. In this industry supplementation with enzymes to improve digestibility and utilization of nutrients is becoming common practice.

One enzyme applied in animal feed is phytase, which catalyzes the conversion of phytate into inorganic phosphate and myo-inositol. Phytate, the principal storage form of phosphorus in plant seeds, can barely be utilized by monogastric animals, calling for inorganic phosphate addition to the fodder to meet the animal's requirements. We have engineered active phytase from *Aspergillus niger* in tobacco seeds. The enzyme accumulated to 1% of total soluble protein in mature seeds. This level remained constant on storage of the seeds at room temperature for period of a year. Direct application of phytase-containing transgenic seeds to broilers showed that this supplementation obviated the need for phosphorus supplementation. Large scale application of phytase-containing transgenic seeds in animal feed will have a beneficial impact on the environment, especially in regions with intensive livestock farming, because diminished excretion of phosphorus will reduce eutrophication of surface waters.

X1-011 *Abstract Withdrawn*

Systems for Gene Transfer to Crops

X1-012 PRODUCTION OF INSECT RESISTANT CORN, Michael Fromm, Chuck Armstrong, Alfreda Blasingame, Sherri Brown, Dave Duncan, Dave Deboer, Beate Hairston, Arlene Howe, Sean McCaul, Margaret Neher, Mark Pajeau, Gregory Parker, Jay Pershing, Bill Petersen, Colleen Santino, Pat Sanders, Shirley Sato, Steve Sims, Tom Thornton, Monsanto Company, St Louis, MO 63198.

European Corn Borer (ECB) is an lepidopteran pest in corn in most of the corn belt. The insect is difficult to control with conventional insecticides since it bores into the plant soon after hatching. Since the severity of ECB infestations varies year to year and the insecticidal control is difficult, many farmers choose not to spray for ECB control despite the damage the insect causes. ECB is sensitive to Bacillus thuringiensis insecticidal crystal proteins (Bt protein). Monsanto has produced corn plants expressing a Bt gene, that prevent ECB damage to the plants. The status of corn transformation, glyphosate selection and plant tolerance, and the results of field trials of the insect resistant corn will be presented. The criteria for identifying a commercial line, such as insect control, normal genetics and lack of negative yield drag will also be discussed.

X1-013 DEVELOPMENT AND USE OF AN EFFICIENT TRANSFORMATION SYSTEM FOR BARLEY. Yuechun Wan and Peggy G. Lemaux, Department of Plant Biology and U.C. Berkeley/USDA-ARS Plant Gene Expression Center, University of California, Berkeley, CA 94720

The application of modern methods of genetic manipulation has been limited in cereals by the development of user-friendly transformation methodologies. We have developed a rapid, efficient and reproducible system to generate self-fertile, transgenic barley plants and it requires minimal tissue culture manipulation. A total of 91 independent transgenic callus lines were generated using microprojectile bombardment of three different target tissues, immature zygotic embryos, young callus and microspore-derived embryos. Stable integration of the introduced genes was confirmed by DNA hybridization analysis. The presence of the coat protein gene from barley yellow dwarf virus (BYDV), introduced on a separate plasmid, was confirmed in 86% of the events. More than 500 green, fertile transgenic plants were regenerated from 36 independent lines; 41 lines yielded only albino plants. The approach utilizing immature embryos resulted in approximately 8 independent events per 100 half-embryos and 57% of these lines yielded self-fertile green plants. Transmission of the introduced genes to R_1 progeny was confirmed by DNA hybridization and functional expression of *bar* in transgenic R_0 and R_1 plants was confirmed by spraying with the herbicide Basta™. Evidence of non-Mendelian segregation of gene expression in some lines was observed. The transgenic R_1 plants were in soil approximately 7 months after bombardment of the immature embryo. Transgenic R_1 and R_2 plants are currently being tested by our collaborators for their response to BYDV infection in the greenhouse (R. Lister/Purdue University and S. Wyatt/Washington State University) and will be examined in field trials in the summer of 1994 (P. Bregitzer/USDA-ARS Aberdeen and C. Qualset/University of California, Davis).

X1-014 FACTORS WHICH INFLUENCE THE *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF SOYBEAN, Jeffrey A. Townsend and Laurie A. Thomas, Pioneer Hi-Bred International, Inc., Johnston. IA 50131

We have developed a method for producing transgenic soybean plants by cocultivation of cotyledon explants with *Agrobacterium tumefaciens*. Factors have been identified which affect the process. Amoung the most important of these appears to be the induction of the *Agrobacterium* virulence genes using signal molecules in cocultivation conducted at low temperature and pH. Transformation effeciency is bacteria concentration dependent. Innoculation conditions have been defined which ensure consistently high frequencies of transformation. Under the improved coculture conditions soybean varieties show little variation in suseptibility to transformation. Antibiotic selection regimes during shoot and root organogenisis will be described which allow recovery of transformed plants from one percent of treated explants. A set of twenty independent transformants of a commercial variety, 9341, have been characterized by expression analysis of two chimeric genes introduced into the soybean chromosome. They were the ß-glucuronidase (GUS) gene from *Escherichia coli* and the methionine-rich seed storage protein (BNP) gene from Brazil nut, *Bertholletia excelsa*. The genes were introduced on a common T-DNA in binary plasmid pPHI1816. In histochemical analyses the T^0 plants expressed GUS in one of three patterns. Three of the twenty (0.15) had the enzyme localized in the epidermal or L1 cell layer. Five of the twenty (0.25) had the enzyme localized in other tissues, those deriving from the L2 cell layer. In twelve of the twenty plants (0.60) GUS was present in both L1 and L2 tissues. All plants transformed in the L2 layer transmitted the new traits to progeny. None of the L1 transformants did. Thus seventeen of twenty plants (0.85) were germline transformants. BNP accumulates in transgenic seed. PAGE separation of soluable seed protein and staining with Coomasie Blue allowed the identification of a major new protein unique to transgenic meal. The protein resolved to the same location as purified BNP and in Western blots reacted with a polyclonal antibody specific for it. The accumulation of BNP in soybean results in a significant increase (26%) in the level of methionine in the seed of transgenic plants. Several of the plants gave the same mean yield (bushel/acre) as the progenitor variety in field tests.

X1-015 MOLECULAR GENETIC IMPROVEMENT OF WHEAT, Indra K. Vasil[1], Vimla Vasil[1], Vibha Srivastava[1], Ana M. Castillo[1], and Michael E. Fromm[2], [1]Laboratory of Plant Cell and Molecular Biology, 1143 Fifield Hall, University of Florida, Gainesville, FL 32611-0692, and [2]Monsanto Company, 700 Chesterfield Parkway North, St. Louis, MO 63198.

Three methods of direct DNA delivery have been used to obtain transgenic cereals: a) permeation of protoplast membranes by polyethylene glycol treatment or electroporation, b) high velocity microprojectile bombardment, and c) electroporation of intact cells and tissues. Regenerable cell suspension and protoplast systems have been developed for wheat, and used to study transient expression and obtain transgenic calli. No transgenic plants could be recovered owing to the reduction or loss of regenerative ability during the long periods of time required for the establishment of such cultures, and the relatively low frequencies of regeneration and transformation. We have obtained fertile transgenic plants following the Biolistic delivery of DNA into long-term regenerable type C callus tissues, and demonstrated the presence, expression and Mendelian segregation of the introduced herbicide (Basta) resistance gene *bar* (Vasil et al. 1992 Bio/Technology 10:667-674). In order to overcome the difficulties faced in identifying and maintaining type C callus cultures, and the 12-15 months required for obtaining transgenic plants, we have recently introduced DNA directly into immature embryos 4 days to 2 months after culture. From the bombardment of 544 explants belonging to two spring and one winter cultivar of wheat, 7 transformed regenerable lines were selected. Thus far, R1 plants have been obtained from 4 of these lines. The presence and expression of the *bar* gene - which segregated in a Mendelian fashion in the sexual progeny - was demonstrated by enzyme (PAT) assays and Southern analysis in R0 and R1 plants. Transgenic plants could be obtained in as little as seven months.

X1-016 TRAVELS OF THE AGROBACTERIUM T-DNA TRANSFER COMPLEX: TUNNELING THROUGH BIOLOGICAL MEMBRANES, John Zupan[1], Vitaly Citovsky[1]*, Debra Warnick[2], and Patricia Zambryski[1], [1]Department of Plant Biology, Koshland Hall, University of California, Berkeley CA 94720, [2]Sandoz Crop Protection, Palo Alto, CA 94304. *Present address, Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794-5215

Agrobacterium plant cell transformation involves passage of the DNA transfer intermediate through the bacterial inner and outer membranes, and the plant cytoplasmic and nuclear membranes. The T-DNA has been proposed to be transferred as a DNA-protein complex, the T-complex, comprised of the single stranded (ss) T-strand, VirD2 at the 5' end, and cooperatively bound ssDNA binding protein, VirE2, along its length. The proteins of this complex presumably help to target the T-complex to different bacterial and plant cell receptors to mediate its transport to the plant nucleus. In support of this model, we have previously shown that both VirD2 and VirE2 proteins themselves can localize to plant cell nuclei. More recently, we have developed a method to directly test whether these proteins can mediate nuclear uptake of ssDNA. To date we have prepared fluorescently labelled ssDNA alone, or coated with VirE2 in vitro. These molecules are microinjected into individual plant cells, and nuclear uptake is monitored microscopically. Indeed, VirE2 coated ssDNA is transported into the plant cell nucleus, while DNA alone remains cytoplasmic. These results provide strong support for the proposed structural and functional domains of the Agrobacterium ssDNA-protein complex transfer intermediate. Additional studies aim to provide information on why monocots are recalcitrant to Agrobacterium DNA transfer. We assayed whether the block might reside in the nuclear localizing functions of VirD2 and VirE2. Nuclear transport of VirD2 and VirE2 GUS-fusion proteins in maize leaves and roots was compared to that in tobacco leaf protoplasts and whole root tissues. Interestingly, both proteins accumulated in maize leaf and tobacco protoplast nuclei, as well as nuclei of immature root cells. In contrast, both proteins remained cytoplasmic in mature roots of tobacco and maize. These studies point out that both maize and tobacco have similar requirements for nuclear uptake of proteins of the Agrobacterium T-complex. Initial results on migration of the T-complex through the first stage of the transport process, exit through the bacterial membranes, will also be presented.

Modification of Seed Protein Composition

X1-017 TRANSGENIC CROPS WITH IMPROVED AMINO ACID COMPOSITION, S. C. Falco, C. Beaman, C.-F. Chui, T. Guida, L. Hirata, T. Jones, S. Keeler, S. Knowlton, C. Kostow, M. Locke, J. Mauvais, S. McAdams, K. Reiter, J. Rice, C. Sanders, R. Schreiner, C. Wandelt, R. T. Ward, and P. Webber, E. I. DuPont de Nemours & Co., Wilmington, DE 19880-0402.

Our goal is to increase the amount of lysine, methionine, tryptophan and threonine in seeds. We are attempting to increase the biosynthesis and accumulation of the free amino acids or to express high levels of natural or synthetic storage proteins rich in these amino acids in seeds.

The biosynthesis of lysine in plants is regulated predominantly by feedback-inhibition of two enzymes, aspartokinase (AK) and dihydrodipicolinic acid synthase (DHDPS). The biosynthesis of threonine and methionine is also regulated by AK. We expressed the *E. coli* and *Corynebacterium* DHDPS enzymes, with an attached chloroplast transit sequence, in tobacco leaves using the 35S promoter. The *E. coli* DHDPS enzyme is 20X less sensitive to feedback inhibition by lysine than a typical plant enzyme and *Corynebacterium glutamicum* DHDPS is insensitive to lysine. Expression of either enzyme causes 100-fold increases in free lysine in young leaves. However, high level seed-specific expression of these enzymes, via the phaseolin promoter, has no effect on lysine content of the seeds. We also expressed the *E. coli* lysine-sensitive aspartokinase (AKIII), and a lysine-insensitive mutant AKIII, with chloroplast transit sequences attached, in tobacco leaves and seeds. In contrast to the DHDPS results, expression in leaves had no effect on free amino acid composition, but high level seed-specific expression of lysine-insensitive AKIII resulted in a 23-fold increase in the free threonine accumulated in the seeds. This suggested that expression of lysine-insensitive AKIII along with lysine-insensitive DHDPS would be needed to obtain over-production of lysine in seeds. Seeds expressing high levels of both the lysine-insensitive enzymes showed a small (2-fold) increase in the free lysine. Additionally, there was a striking accumulation of α-amino adipic acid, which is not normally found in tobacco seeds. α-amino adipic acid is an intermediate in a lysine catabolic pathway present in the endosperm of some seeds.

Two natural seed storage proteins rich in methionine, the 10kd zein of maize (30% met) and a related, but previously unknown, 20kd zein (38% met) have been used to increase the methionine content of seeds. Expression of these monocot proteins in the seeds of the dicot plants tobacco, Canola and soybean has been achieved using either the phaseolin promoter or a soybean β-conglycinin promoter. Alterations in the intracellular targetting signals of the proteins were tested to determine their effects on accumulation of the protein in mature seeds. The native targetting signal worked as well or better than any of the modifications.

As an alternative to natural proteins, we have designed proteins de novo to serve as seed storage proteins. The structure of these new proteins, designated complement proteins, is based on an α-helical coiled coil model. This structure permits considerable variation in the amino acid sequence, allowing diverse end-user-specific nutritional deficiencies to be complemented. The proteins may contain up to 43% lysine, 28% methionine and 7% tryptophan. Genes encoding such proteins were synthesized and the proteins were expressed in *E. coli* to test in vivo stability. A protein containing 31% lysine and 22% methionine was expressed in tobacco seeds using either the phaseolin promoter or a soybean β-conglycinin promoter, without any added intracellular targetting signal. The synthetic protein represented up to 2% of the total seed protein of transformants.

X1-018 ENHANCING NUTRITIONAL QUALITY WITH QUALITY PROTEIN MAIZE, Brian A. Larkins, Etti Or, Jeffrey E. Habben, Gloverson A. Moro, Dwight E. Bostwick, Joanne M. Dannenhoffer and Mauricio A. Lopes, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Discovery that the maize *opaque-2* (*o2*) mutation resulted in an endosperm with an increased percentage of lysine generated a great deal of interest in utilizing this gene to improve the nutritional quality of maize seed protein. However, the soft, starchy phenotype associated with the mutation caused the seed to be very susceptible to insect pests and mechanical damage. These problems, as well as the reduced yield and protein content, were largely responsible for preventing the so-called "high-lysine" corn from gaining wide acceptance. However, the existence of genes that modified the phenotype of *o2* mutants, resulting in a kernel with a higher protein content and a vitreous endosperm, led to the development of modified *o2* genotypes, known as Quality Protein Maize (QPM). QPM was created using a dual selection based on endosperm hardness and lysine content. QPMs typically have a normal phenotype and protein content, and a lysine concentration that is intermediate between normal and *o2*. To better understand the genetic and biochemical changes involved in QPM development, we have analyzed the genetics of the modifier gene system and studied the effects of these genes on protein synthesis and endosperm development. Using phenotypic analysis and RFLP mapping, we identified what appear to be two major modifier loci on chromosome 7. The primary effect of these genes is a two to three-fold increase in the synthesis of the gamma-zein storage protein. The regulation of the synthesis of this protein appears to occur at the posttranscriptional level, since modifier genes have little effect on the level of transcription of gamma-zein genes. The gamma-zein protein contains no lysine; rather, it is cysteine-rich and is found in the outer regions of protein bodies. The enhanced formation of disulfide bonds in maturing endosperm appears to be a factor contributing to the increased vitreous quality of QPM. The increased lysine content of QPM is a pleiotropic effect of the *o2* mutation, which leads to the increased synthesis of a number of endosperm proteins that contain lysine. Among the proteins that appear to be significantly increased by this mutation are: trypsin inhibitor, elongation factor 1-alpha, catalase, and several structural proteins. With these and other selectable markers, it should be possible to develop elite QPM germplasm for commercial utilization.

X1-019 MOLECULAR CONTROL OF GENE EXPRESSION IN SEEDS, Jost Muth, Stefan Lohmer, Philippe Gallusci, Gregorio Hueros, Martin Müller, Serena Varotto, Francesco Salamini and Richard D. Thompson, Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, D-50829 Köln, Germany.

The regulation of storage protein accumulation in maize endosperm is being studied using mutant lines and an isolated transcription factor, Opaque-2 (O2). The interaction between O2 and its target promoters, 22 kD zein and b-32 has been studied in tobacco leaf protoplasts [1], and in endosperm transformed by particle gun bombardment. Both promoters possess multiple binding sites for O2. The removal of individual binding sites has revealed cooperative effects which may be important in fine-tuning zein regulation in the kernel. In a deletion analysis of O2, two acidic domains have been identified which are responsible for the ability of the protein to trans-activate. The effect of treating endosperm with various N-regimes on zein gene expression [2] is being used to characterize an N-responsive component of the promoter. The mechanism by which the leader sequence of O2 mRNA reduces translational efficiency of O2 protein has been attributed to the presence of three short upstream open reading frames [3]. This effect may vary with the metabolic status of the cell, as shown for example in the yeast GCN4 system. A possible role for translational control in regulating zein deposition is under investigation.

1. Lohmer et al. (1991) EMBO J. 10: 617
2. Balconi et al. (1993) Plant J. 3: 325
3. Lohmer et al. (1993) Plant Cell 5: 65

Control of Gene Expression in Transgenic Plants

X1-020 FROM TARGETING SIGNALS TO WATER CHANNEL PROTEINS: AQUAPORINS IN THE TONOPLAST AND THE PLASMA MEMBRANE, Maarten J. Chrispeels, Christophe Maurel, T. Erik Mirkov and Mark J. Daniels. Department of Biology, University of California, San Diego, La Jolla CA 92093-0116 USA.

The tonoplast contains an abundant 27 kDa protein called TIP (tonoplast intrinsic protein), that occurs in different isoforms and belongs to a large family (the MIP family) of channel-like proteins found in bacteria, plants and animals. We identified these proteins as part of a search for targeting signals on tonoplast proteins. We have now discovered the function of γ-TIP, which is abundantly found in the roots and shoots of *Arabidopsis thaliana* by expressing the protein in *Xenopus* oocytes. *In vitro* synthesized mRNA was injected into individual oocytes, which were incubated for two days and shifted to a hypotonic medium. The change in diameter of the oocytes was then measured and at 30 second intervals and the rate of volume increase used to calculate the water permeability of the plasma membrane. γ-TIP increased the osmotic water permeability of oocytes 6 to 8-fold, just like the mammalian homolog CHIP28. The bacterial homolog GlpF did not increase the osmotic permeability of oocytes, but increased glycerol uptake, in accordance with its known function. Voltage clamp experiments showed that γ-TIP induced no electrogenic ion transport in oocytes, especially during an osmotic challenge that resulted in massive transport of water. Thus, the various members of the MIP family have unique and specific functions, and γ-TIP functions as a water-specific channel or aquaporin. Several of the TIP homologs are turgor responsive and are induced by desiccation. One of these, RD28 identified by K. Shinozaki, functions in the same way as γ-TIP: its expression in oocytes causes a 10-fold increase in osmotic permeability. Preliminary evidence indicates that desiccation-induced homologs may be plasma membrane proteins. The discovery of these aquaporins in the plant membranes opens up a new and exciting field of plant water relations research. The role of these proteins in the physiology of plants will be discussed.

(Supported by a grant from the US Department of Agriculture through the CRGO.)

X1-021 THE CRYIAb CODING REGION LIMITS CRY mRNA FORMATION IN TOBACCO, Marc Cornelissen[1], Piet Soetaert[1], Roel van Aarssen[1], Maike Stam[2], Veronique Gosselé[1] and Jan Dockx[3], [1] Plant Genetic Systems NV, J. Plateaustraat 22, B9000 Gent, Belgium, [2] Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, [3] University of Utrecht, Utrecht, The Netherlands.

The regulatory features of DNA and RNA sequences are mostly context dependent. Local context changes thus can affect gene regulation in a significant and yet unpredictable manner. In this way, transgenes may follow unexpected expression pathways, both because of cryptic regulatory signals and because of changes in the genetic background.

A well documented case of this sort is the expression of insecticidal crystal protein (*cry*) genes in plants. Chimaeric $P_{CaMV35S}$*cry* genes direct in tobacco mesophyll protoplasts mRNA levels of about one transcript per cell. We have found that this low cytoplasmic *cry* mRNA level is not due to a rapid turnover but rather results from a marginal import flow of *cry* messenger into the cytoplasm. In the case of *cry*IA(b), mRNA production in the nucleus hampers at several levels. First, we have some evidence suggesting that RNA polymerase II stalls at some 1000 nucleotides downstream of the transcription start site. Second, the *cry* precursor mRNA carries several cryptic processing sites. The absence of high cytoplasmic levels of any of these processed *cry* mRNAs suggests that these mRNAs are unstable and/or not efficiently made. Modification of some of these processing sites has a significant effect on accumulation of the full length mRNA in tobacco.

X1-022 PATTERNS OF HOMOLOGOUS GENE SUPPRESSION ELICITED BY TRANSGENES AND BY DEVELOPMENTALLY-IMPOSED TRANSGENE IMPRINTS, Richard Jorgensen, University of California, Davis. Ca. 95616-8587.

In petunia, most flower pigmentation patterns are determined by post-transcriptional control of chalcone synthase (CHS) gene expression, and so represent a level of pattern determination that can be superimposed onto cell-type-specific transcriptional controls. It was discovered that an ectopic chalcone synthase transgene elicits a variety of such patterns through post-transcriptional co-suppression of endogenous and ectopic CHS gene expression. It turns out that the type of pattern that is elicited by the transgene locus is determined by whether the transgene is a single copy or a tandem duplication, not by any "position effect". The most complex patterns depend on inverted duplications of the T-DNA element. All patterns are organized spatially in three dimensions and appear to be determined by one or more of several cell types: cells at petal edges, at petal junctions, and at the vasculature, each a determinant of corolla morphology. Single copy transgenes elicit only simple, stable patterns centered on petal junctions. Inverted repeats are required for patterns involving all three cell types and these are usually chaotic and unstable. The inheritance and somatic behavior of the developmental patterns that are elicited by a directly repeated transgene have been investigated in great detail; these patterns are determined by petal junctions and vasculature, are well organized, and are epigenetically metastable. From this direct repeat locus, a series of epiallelic variants have been identified, each eliciting a distinct morphological pattern. Most are germinally hertiable, reversible, and epimutable; each differs in its epimutability, i.e., its ability to change to another epiallele. New epimutations can be induced coordinately in adjacent cells or in neighboring meristems of a single plant, suggesting responsiveness to (as yet unknown) environmental factors. Also, different epialleles can interact, one causing heritable "paramutations" in the other. Taken together, these results recapitulate and extend the classic work of McClintock and Brink over thirty years ago on epigenetic phenomena in maize. Our long term goal is to identify the environmental, developmental and molecular factors that induce new epialleles, as well as to determine the mechanism by which the expression of the transgene(s) and the endogenous gene is co-suppressed.

X1-023 HOMOLOGY-DEPENDENT METHYLATION AND INACTIVATION OF TRANGENES IN TOBACCO, M.A. Matzke, F. Neuhuber, Y.-D. Park, P. Ambros, A.J.M. Matzke, Institute of Molecular Biology, Austrian Academy of Sciences, Salzburg, Austria

Homology-dependent gene silencing can occur when multiple copies of a transgene, or a transgene with homology to an endogenous gene, are present and interact in a plant genome[1]. We have been studying homology-dependent silencing phenomena that involve unlinked, partially homologous transgenes in tobacco. The effects we have observed are nonreciprocal, meaning only one locus involved in the interaction undergoes a change, and there is a clear correlation between inactivation of genes on the sensitive target locus and methylation in the promoters of the inactivated genes. Recent work has focused on the properties of transgene loci that are susceptible to inactivation, and the characteristics of "suppressor" loci that are able to inactivate in *trans* susceptible target loci. Susceptibility of a transgene locus to *trans*-inactivation is often associated with an inherent instability and a tendency to partial methylation, which is increased when the locus is homozygous and/or when an unlinked homologous transgene construct is present. Suppressor loci have been found to contain multiple, hypermethylated copies of the transgene construct. Reducing the degree of methylation within a suppressor locus decreases its ability to suppress. To account for these observations, a process termed "epigene conversion"[2], in which pairing between homologous DNA regions on a methylated suppressor locus and an unmethylated target locus leads to the imposition of methylation on the latter, is proposed.

1. Matzke, M. and Matzke, A. (1993) Ann. Rev. Pl. Physiol. Pl. Mol. Biol. 44: 53-76.
2. Sabl, J. and Laird, C. (1992) Am. J. Hum. Genet. 1171-1177.

X1-024 DNA METHYLATION AND GENE EXPRESSION IN TRANSGENIC PETUNIA, Heinz Saedler[1] and Peter Meyer[2], [1]Max-Planck-Institut für Züchtungsforschung, Köln, [2]Max-Delbrück- Laboratorium, Köln.

Instabilities in transgene expression are often associated with changes in DNA-methylation patterns. The maize A1-gene has been employed as a model system to study causes and consequences of transgene methylation in *Petunia hybrida*. The insertion of multiple copies of the A1-gene preferentially resulted in an increase in methylation within the promoter region. Single copy integration events became methylated after insertion into a hypermethylated chromosomal region, while integration of the transgene into a hypomethylated region did not cause immediate de novo methylation, thus representing a necessary, although not sufficient condition for stable expression of the transgene. The transgenic line R101-17 contains one copy of the A1-gene integrated into an unmethylated genomic region. In R101-17 an almost stable expression of the A1-gene could be observed in the greenhouse, but hypermethylation of the construct was induced when the plants were grown in the field. Analysis of epigenetic mutants of this line revealed that de novo methylation was limited to the transgenic region, while the hypomethylation pattern of the genomic integration region stayed unaltered. Hypermethylation of the transgene was associated with an increased resistance of nuclei against endonucleases, which argues for a condensation of chromatin within the transgene DNA. De novo methylation and chromatin condensation of the transgene did not only impair transcription of the transgene itself, but also influenced an homologous allel. When a hypermethylated allele was combined with a hypomethylated allele, de novo methylation was frequently induced in the previously hypomethylated homologue. This semi-dominant effect, that is reminiscent of certain paramutation phenomena, suggests that either the hypermethylated state of a transgene or its associated condensed chromatin structure is involved in the transinactivation of an allelic homologue. It seems likely that certain co-suppression phenomena are mediated via a similar interaction between ectopic homologous.

Alterations in Oil Content and Composition in Seeds

X1-025 ANALYSIS OF FATTY ACID BIOSYNTHESIS IN TRANSGENIC PLANTS WITH MODIFIED OIL COMPOSITION, Jan G. Jaworski[1], John B. Ohlrogge[2], Heeyoung Tai[1], Joachim Kopka[1] and, Dusty Post-Beittenmiller[3] 1) Chemistry Dept., Miami University, Oxford, OH 45056, 2) Dept. of Botany and Plant Pathology, Michigan State University, E. Lansing, MI 48524, 3) Plant Biology Division, The S.R. Noble Foundation, P.O. Box 2180, Ardmore, OK 73402

Modification of triacylglycerol composition of oilseeds of transgenic plants has been accomplished in a limited number of cases by introduction of new genes or supressing existing activities with antisense technology. In addition, major changes of triacylglycerol composition have been accomplished as a result of breeding programs. We are interested the analysis of both types of plants to address both basic and applied questions concerning regulation of fatty acid and lipid biosynthesis. For example, oilseeds with a high stearic acid tracylglycerol composition have been produced using antisense suppression of stearoyl-ACP desaturase, whereas preliminary analysis of soybeans bred to produce high in stearate demonstrated that these plants have normal levels of stearoyl-ACP desaturase. This suggests that analysis these soybean cultivars may reveal new mechanisms for regulation of the acyl composition in these oilseeds that will lead to new strategies for modification of that composition. In addition to carrying out *in vitro* analysis of target enzymes, we are developing techniques to measure the *in vivo* levels of key intermediates of the biosynthetic pathway, viz. acyl-acyl carrier proteins (acyl-ACPs) and acyl-CoAs. We have previously demonstrated that simple patterns of acyl-ACPS could be separated using urea-PAGE, leading to the demonstration, for example, that under some conditions, acetyl-CoA carboxylase is a key regulatory site of fatty acid synthesis. This analysis has been extended to include plants such as oilseeds with complex patterns of acyl-ACPs by replacing the urea-PAGE analysis with a technique based on GC-MS. Results of analysis of developing oilseeds will be discussed. In addition, progress on the transformation of plants with clones of 3-ketoacyl-ACP synthase III from spinach and *Arabidopsis thaliana* will also be presented. This enzyme catalyzes one of the initial reactions of fatty acid biosynthesis and is potentially rate limiting under some conditions.

X1-026 ENGINEERING OF MEDIUM-CHAIN FATTY ACID BIOSYNTHESIS IN CANOLA, Toni A. Voelker, Ann M. Cranmer, Aubrey Jones, Sharon Radke, H. Maelor Davies, Calgene Inc., 1950 Fifth Street Davis, CA 95616

Like the phospholipids found in cellular membranes, most seed reserve triacylglycerols (vegetable oils), contain predominantly C_{16} and C_{18} fatty acyl groups. However, developing seeds of plant species from several families commit to the production of large amounts of medium-chain fatty acids (C_6-C_{14}), which are then deposited in triacylglycerols. Even though oils with all conceivable compositions of medium-chain fatty acids are described in the plant kingdom, only very few species can be used for commercial harvesting. Currently, tropical trees are the exclusive source for these feed stocks, which are important for the food and detergent industry. No annual crop plant is available.

Previously, we have shown that a medium-chain specific acyl-ACP thioesterase (TE) from undomesticated California Bay, when expressed in the seeds of transgenic *A. thaliana*, leads to the synthesis of laurate (C_{12}) and myristate (C_{14}) fatty acids (1). This demonstrated for the first time *in vivo* a mechanism for the production of medium-chain fatty acids in plants. It also showed that the metabolic engineering of fatty acid chain length is possible.

We will present our strategy for the generation of a novel crop plant, transgenic canola (*Brassica napus*) producing up to 50% of its seed fatty acid as laurate (up from 0.02%). Discussed will be the impact on the resident long-chain fatty acids, triglyceride biosynthesis, models for the actual mechanism of medium-chain production in the plastids, and data from field trials.

More recently, using a PCR approach based on conserved AA sequences between the bay medium-chain specific TE and long-chain TEs from different plant species, we have cloned TE cDNAs from species which also accumulate medium-chain fatty acids. Our data clearly demonstrate that bay medium-chain TE has not evolved recently from the common long-chain TE, but from a previously undescribed enzyme of unknown function. We predict that this enzyme is present in all higher plants, including all species which produce only long-chain fatty acids. We will present sequences, activity profiles, evolutionary relationships, and expression studies with members of this novel class of enzymes.

Reference (1) Voelker, T.A., Worrell, A.M, Anderson, L., Bleibaum, J., Fan, C., Hawkins, D.J., Radke, S.E., Davies, H.M., (1992) SCIENCE, 257, p72-74.

Analysis of Genome Organization for Breeding and Gene Isolation

X1-027 TRANSPOSON TAGGING FOR GENE ISOLATION IN ARABIDOPSIS, Caroline Dean, Ian Bancroft, Anuj Bhatt, Paul Jarvis, Emily Lawson and Tania Page. AFRC, IPSR, Cambridge Laboratory, John Innes Centre, Norwich, UK.

An effective transposon tagging system, using the maize transposable elements *Ac* and *Ds*, has been developed in Arabidopsis. The transposition frequency of wild-type *Ac* is very low in Arabidopsis. The transposition frequency of these elements in Arabidopsis has been increased both by modifying the elements and isolating Arabidopsis mutants. The characteristics of transposition and transposition patterns of the *Ac/Ds* elements in Arabidopsis have been characterized in order to establish the most efficient way of using the system for tagging genes. 1300 families carrying transposed elements have been screened on agar, in the light and dark and in the greenhouse for segregating mutations. Over 35 mutations have been identified. Currently, we know that two are tagged with either *Ac* or *Ds* - *drl1* (deformed roots and leaves, no inflorescence produced) and *dif1* (determinate, infertile) and two others -*wlc1* (wavy leaves, cotyledons furled back) and *pcm1* (leaves initially wild-type, but turn pale green after *ca.* 3 weeks growth on culture medium) are at least closely linked to a t*Ds* and therefore are likely to be tagged. These and other mutations will be described and the current status of the frequency of tagged mutations presented.

X1-028 MOLECULAR APPROACHES TO FOREST TREE IMPROVEMENT, Ron Sederoff, David O'Malley, Ross Whetten, Ben Liu, Dario Grattapaglia, José Chaparro, Phil Wilcox, Henry Amerson, Steve McKeand, Floyd Bridgwater, Barbara Crane and Susan McCord.

With the advent of anonymous PCR based DNA markers, it has become possible to rapidly generate genomic maps for individual forest trees. These studies take advantage of the high levels of genetic diversity and heterozygosity in forest trees. Genomic maps have been obtained from individual open pollinated pines, and from a full sib cross of Eucalyptus. DNA markers can be used to dissect quantitative traits, and to locate QTLs for commercially important traits affecting growth and yield. Strategies are being developed to use molecular markers to predict performance in existing breeding and to accelerate gain.

Modification of Complex Carbohydrate Composition in Seeds

X1-029 MODIFICATION OF SOLUBLE CARBOHYDRATE COMPOSITION IN SOYBEAN,Phil Kerr[1], Scott Sebastian[1], Christine Wandelt[2], Natalie Hubbard[2], and John Pierce[1], DuPont Agricultural Products Department, [1]Crop Research Laboratory, Newark, DE 19714 and [2]Experimental Station, Wilmington,DE 19880-0402.

Traditional breeding and molecular biology techniques have been used in a program to modify the soluble carbohydrate composition of soybean. Although some variation in seed soluble carbohydrate content exists among commercial soybean varieites, typical values are 150 μmoles/g sucrose, 75 μmoles/g stachyose and 20 μmoles/g raffinose. Mutants have been identified that display a seed stachyose and raffinose content less than 50% of that seen in commercial varieties. Further, the sucrose content of these mutants is typically 25 to 30% higher than that seen among commercial varieties. Inheritance studies indicated that this mutant line contains a single recessive to codominant gene that confers the modified soluble carbohydrate phenotype. Hereafter this gene will be referred to as stc1.

In addition, genetic modifiers have been identified in certain elite lines that compliment the stc1 mutation. Combinations of the stc1 mutant and the modifier(s) have been identified that further reduce the stachyose and raffinose content of the seed to less than 15% of that seen in commercial varieties. The raffinose saccharide content of these lines is lower than that typically seen in a number of processed soy products (e.g., soyflour, soy protein concentrates, soymilk, tofu, etc.). Further, defatted, toasted meals produced from stc1 lines displayed a Nitrogen-corrected, True Metabolizable Energy content for broilers that was ca. 20% greater than that seen in meals produced from commercial varieties. Collectively, these results suggest that low stachyose lines have potential to provide a novel raw material for various aspects of the soy protein processing industry.

In a related project, galactinol synthase (UDP-D-galactose:inositol galactosyltransferase, E.C.2.4.1.123), a key enzyme in the raffinose saccharide biosynthetic pathway, has been purified and used to clone the gene from zucchini and soybean. The expression of the gene in soybean will be described.

X1-030 IMPROVING CROP PRODUCTS BY MANIPULATING METABOLISM, Ganesh Kishore, David Stark, Christina Nasrawi, Russell McKinnie and Gerard Barry, Food and Crop Quality Improvement Program, New Products Division, Monsanto Agricultural Group, 700 Chesterfield Parkway North, Chesterfield, MO 63198

Carbohydrate metabolism is critical for plant growth and development. The production of sucrose in the leaf tissue followed by its long distance transport and its subsequent conversion to hexose polymers within storage organs appears to be a fundamental metabolic process manifested in a wide range of plant species. A number of genes encoding enzymes of carbohydrate metabolism have recently been cloned and they serve as valuable tools to perturb carbohydrate metabolism in a tissue specific manner in plants. Recently, we have demonstrated that expression of the gene encoding the enzyme ADP glucose pyrophosphorylase in plant tissues substantially enhances starch content. The *E. coli glgC* and *glgC16* genes encoding the wild type and mutant ADPGPP enzymes, respectively, were used in these experiments and the proteins were targeted to the chloroplasts using the chloroplast transit peptide of the small subunit of RuBP carboxylase. In potato tubers, the wild type enzyme was not as effective as the mutant in enhancing starch content, suggesting that it is the allosteric regulation of the ADPGPP enzyme that plays the more critical role in regulating starch content of starch storage organs. The plant ADPGPP enzyme is regulated by inorganic phosphate (Pi) and 3-phosphoglycerate (3-PGA). The wild type *E. coli* enzyme is not very sensitive to inhibition by Pi but is activated substantially by 3-PGA. In view of the varying levels of starch enhancement observed with wild type and mutant *E. coli* enzymes, we conclude that activation by 3-PGA is the more critical parameter regulating starch content of plant cells. High starch tubers provide a convenient mechanism to reduce the fat content of the human diet since fried products prepared from these tubers absorb less oil during the frying process.

X1-030 FRUCTANS AND FRUCTAN ACCUMULATING TRANSGENIC PLANTS, Sjef Smeekens[1], Michel Ebskamp[1], Ingrid van der Meer[1], Richard Visser[2] and Peter Weisbeek[1], [1]Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands, [2]Department of Plant Breeding, Wageningen Agricultural University, P.O. Box 386, 6700 AJ Wageningen, The Netherlands

The best known non-structural storage carbohydrates in plants are starch and sucrose. Approx. 12 % of the Angiosperms have the capacity to synthesize a third type of storage carbohydrate, fructan. The ability to accumulate fructans is not distributed throughout the Angiosperms but is found in a limited number of taxa. Fructans are polyfructose molecules. Depending on the plant species the fructose units are linked by different types of glycosidic bonds. The specific advantages for plants to metabolize fructans is at present unclear as is the relation to other non-structural carbohydrate storage forms like starch and sucrose. We are interested in the role of fructans in plant metabolism and physiology. Our approach involves the introduction of fructan accumulation in normally non-fructan storing plants like potato, tobacco and *Arabidopsis*. These plants were modified to accumulate fructans by the introduction of microbial fructosyltransferase genes. Constructs were assembled in which the fructosyltransferase genes of either *Bacillus subtilis* (*sacB*) or *Streptococcus mutans* (*ftf*) were fused to targeting domains for different cellular locations. Sucrose is the substrate for fructosyltransferases and in plant cells sucrose accumulates in three location, cytosol, vacuole and apoplast. Constructs were placed under the control of the constitutive CaMV 35S promoter and introduced into plant tissue. The regenerated plants accumulate high MW (>5.10^6 Dalton) fructan molecules in which the degree of polymerization of fructose units exceeds 25,000. Fructan accumulation was detected in every plant tissue tested. Depending on the cellular location of the fructosyltransferase activity interesting phenotypes were observed. The fructan content in potato leaves can reach up to 30% of total non-structural carbohydrates on a dry weight basis. Also in microtubers induced on transgenic potato plants could fructan be detected. Our results demonstrate that non-fructan storing plants can be induced to synthesize and store fructans by introduction of bacterial fructosyltransferase genes. This modification affects photosynthate partitioning in leaves and microtubers and increases non-structural carbohydrate content in leaves. Both the fructan polymer and its hydrolysis products (oligofructan and fructose) are increasingly in demand for food and industrial uses. Two recent publications (1, 2) illustrate the potential of fructans in food and non-food applications.

1) A. Fuchs, Ed. (1993) Inuline and Inuline-containing Crops: Studies in plant science Vol. 3, Elsevier Science Publishers, Amsterdam.
2) M. Suzuki and N.J. Chatterton, Eds. (1993) Science and Technology of Fructans, CRC Press.

X1-032 MANIPULATION OF PHOTOASSIMILATE PARTITIONING IN TRABNSGENIC PLANTS, Uwe Sonnewald[1], Jens Lerchl[1], Gudrun Mönke[1], Ingo Wilke[1] and Rita Zrenner[1], [1]Institute of Plant Genetics and Crop Plant Research, Corrensstrasse 3, 06466 Gatersleben, Germany.

Growth and development of plants are dependent upon the energy gained by fixing carbon dioxide into carbohydrates during photosynthesis and the distribution of newly synthesised metabolites. Primary products of photosynthesis are starch and sucrose, the former being the storage form and the latter being the transport form of photoassimilates. Assimilates are mainly formed in photosynthetically active source leaves and are subsequently transported via the phloem system to sink tissues, which are photosynthetically inactive and dependent on the photoassimilate supply from the source tissues.

The development of individual sink organs might be dependent on the ability of the sink tissue to use the produced photoassimilates and / or the ability of the source tissue to export photoassimilates rapidly. The export capacity of source tissues might be regulated by carrier proteins modulating the phloem loading or by the partitioning of newly fixed carbohydrates between starch and sucrose.

Based on (partly) known biochemistry of carbohydrate metabolism we have tried to identify key steps controlling carbohydrate metabolism and partitioning in transgenic plants. Using reversed genetics we have manipulated the partitioning between starch and sucrose in source leaves by: (i) The inhibition of Calvin Cycle enzymes, (II) the inhibition of starch synthesis and (iii) the stimulation of sucrose synthesis. To investigate the ability of sink tissues to utilize photoassimilates, the hydrolysis of the imported sucrose and the conversion of metabolites to starch was manipulated. This was achieved by either the antisense strategy or heterologous overexpression of foreign enzymes.

Examples for antisense inhibition are: (i) the ADP-glucose-pyrophosphorylase, (ii) the plastidic aldolase, (iii) the cytoslic fructose-1,6-bisphosphatase, (iv) the pyrophosphate:fructose-6-phosphate-1-phosphotransferase, (v) the UDP-glucose pyrophosphorylase, (vi) the sucrose synthase; (vii) the acidic invertase and (viii) the sucrose-phosphate synthase. Examples for hetrologous overexpression are: (i) Yeast-derived invertase, (ii) spinach sucrose-phosphate synthase and (iii) *E. coli* inorganic pyrophosphatase. Using tissue- and organelle specific expression systems in transgenic plants. the influence of induvidual tissues, cells and compartments on carbon flow was analysed for a variety of biochemical and physiological parameters.

Host Plant Resistance to Pathogens and Insects; Dissection of Signal Transduction Pathways

X1-100 AGRICULTURAL BIOTECHNOLOGY FOR SUSTAINABLE PRODUCTIVITY: A PROTOTYPE PROJECT FOR INTERNATIONAL COOPERATION IN BIOTECHNOLOGY, Judith A. Chambers, USDA/RSSA Biotechnology Specialist, Office of Agriculture, Bureau for Research and Development, Agency for International Development, Washington, D.C. 20523

The promise of biotechnology, when applied in conjunction with traditional plant breeding techniques, offers renewed hope for the developing nations of the world as they attempt to simultaneously address the complex problems of food security, escalating population growth, and environmental degradation. While industrialized countries have been quick to capitalize on the potential benefits of this technology, less developed nations are at a comparative disadvantage in its application due to a lack of financial resources and well-developed private and public infrastructure. To address this growing imbalance, the U.S. Agency for International Development is sponsoring a unique initiative in agricultural biotechnology focused on agricultural production constraints important to the economies of less developed countries. This six year project, entitled Agricultural Biotechnology for Sustainable Productivity (ABSP), was awarded to Michigan State University, as the lead contractor, in September, 1991. The project supports collaborative research in plant micropropagation and genetic engineering among a number of diverse partners from the United States and developing countries, such as Indonesia, Egypt, Kenya and Costa Rica. Participating U.S. institutions, in addition to Michigan State, include ICI Seeds America, Inc., DNA Plant Technology, Cornell University, Texas A&M University and the Biotechnology Industry Organization. Crops targeted for improvement represent a widely divergent mixture including maize, potato, sweet potato, cucurbits, banana, pineapple, coffee and ornamental palm. ABSP also provides support for activities in biosafety and intellectual property in cooperation with USDA/APHIS and Stanford Law School, respectively. Currently, the project's active network, which sponsors regional and international conferences and publishes a quarterly newsletter, involves over 113 countries and 2000 institutions and individuals.

X1-101 THE PERFORMANCE OF TRANSGENIC LINES IN VARIETY REGISTRATION FIELD TRIALS, Alan McHughen, G.G. Rowland and F.A. Holm, Crop Development Centre, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, Canada

Before transgenic cultivars of crops can be commercialized, producers need assurance that the basic agronomic performance of the genotype is not dramatically different from their standard cultivars. Most reports of field tests of transgenic plants to date concentrate on the expression and efficacy of the transferred traits, while the activity of the remainder of the genome has been neglected. We have two transgenic linseed flax cultivars in national registration trials. These tests are designed to compare the agronomic and quality performance of candidate cultivars in many locations in competition with standard commercial cultivars and with other candidate cultivars. The performance in these trials is the basis for registration and commercial release. Regardless of the transgenic attributes, if a candidate cultivar does not compete favourably with current standard cultivars in these agronomic trials, it will not be accepted for registration as a new variety. Such parameters as seed yield, days to maturity, lodging, oil content, oil quality (ie. fatty acid composition), protein content and responses to relevant diseases are measured and the data reported. In four years of tests so far, the two transgenic lines are similar to the standard cultivars in agronomic and quality characteristics. Separate field tests measuring the efficacy of the transgene (conferring the ability for the plants to be grown on soil contaminated by chemical sulfonylurea residue) are also being conducted. Depending on the final data, one or both of these lines could be considered for registration in February, 1994.

X1-102 TRANSPOSON TAGGING OF THE TMV RESISTANCE GENE N USING THE MAIZE CONTROLLING ELEMENT Ac, B. Baker[a,b], S. Whitham[a,c], D. Choi[a,c], R. Hehl, C. Corr[a,b], [a]Plant Gene Expression Center, 800 Buchanan St, Albany CA 94710. Departments of [b]Plant Biology and [c]Plant Pathology, UC Berkeley

The dominant N locus of tobacco confers resistance to tobacco mosaic virus (TMV) by mediating a localized hypersensitive response (HR) at the site of viral infection. Our goal is to isolate and characterize the N locus as a starting point for elucidation of the mechanism of N mediated TMV resistance. We have isolated and characterized 25 TMV sensitive, mutant lines (HR-) from ~60,000 Nn, HR+ heterozygotes on the basis that they fail to respond to TMV infection with a characteristic HR. All characterized mutant lines were derived from NN tobacco parental lines that harbor the maize Ac transposon, and all HR- mutants characterized contain Ac transposons. Genetic analyses show that the HR- lines posses loss of function mutations of N. Molecular analyses, using RFLP markers tightly linked to N, show that 20 of the 25 mutants bear deletions in the chromosomal region of the N locus. All HR- lines bearing N-region deletions posses stable, TMV sensitive, HR- mutations. One non-deletion, HR- mutant line is genetically unstable and gives rise, in addition to TMV sensitive progeny, to fully TMV resistant, HR+ revertant progeny (1-10%), and to progeny displaying TMV induced, necrotic (HR+) sectors on a TMV sensitive (HR-) background. We have shown that this HR- mutant contains an Ac element tightly linked to N (Ac-N), and that the presence of this element is directly correlated with the TMV sensitive, HR- mutant phenotype as well as the potential to revert to the HR+ phenotype. We have isolated seven independent germinal, HR+ revertants from progeny of the the original unstable mutant, and all show germinal loss of Ac-N, whereas sibling progeny displaying TMV induced, necrotic (HR+) sectors on a TMV sensitive background, harbor Ac-N. Tobacco genomic DNA flanking Ac-N has been isolated and used to confirm that the presence and absence of Ac-N is correlated with the TMV-sensitive, HR- mutant and TMV-resistant, HR+ revert phenotypes respectively. These data suggest that we have transposon tagged the N locus using the maize Ac controlling element. The genomic sequences flanking Ac-N will be employed for isolation of the N locus (gene) from wild type NN tobacco for sequence and complementation analyses.

X1-103 ENGINEERING LETTUCE FOR RESISTANCE TO LETTUCE INFECTIOUS YELLOWS VIRUS, Maury L. Boeshore, Vicki A. Klaassen[1], Kim Carney, J. Russell McMaster, Paul F. Russell, Phyllis Himmel, James E. Duffus[2] and Bryce Falk[1], Asgrow Seed Company, Kalamazoo, MI 49001, [1]Department of Plant Pathology, UC Davis, Davis, CA 95616, and [2]USDA-ARS, U.S. Agricultural Research Station, 1636 East Alisal Street, Salinas, CA 93905

Lettuce infectious yellows virus (LIYV) causes a yellowing disease in lettuce, cucurbit, and other crops in the Southwestern US. No commercially useful genetic sources of resistance to LIYV in lettuce have been identified. The virus shows clostero-like morphology (long flexuous rods); the genome appears to consist of 2 single-stranded RNA molecules (~7.5kb and ~9kb). Successful efforts to use engineered resistance against closterovirues have not been reported. To protect plants against losses in the field, we are applying engineered resistance strategies to the LIYV problem in lettuce. We have identified, characterized, and engineered the coat protein gene of LIYV for plant expression. By *Agrobacteria*-mediated transformation we have transformed commercial varieties of lettuce with LIYV coat protein gene constructs. Evaluation of transgenic plants includes NPTII and coat protein gene expression, molecular characterization of transgenes, and resistance to LIYV challenge in the greenhouse.

X1-104 SMALL CYSTEINE-RICH ANTIFUNGAL PROTEINS FROM RADISH: ROLE IN HOST DEFENCE, Willem F. Broekaert[1], Franky R.G. Terras, Kristel Eggermont[1], Bruno P.A. Cammue[1], Valentina Kovaleva[2], Natasha V. Raikhel[2], Rupert W. Osborn[3], Anthea Kester[3], Sarah B. Rees[3], [1]F.A. Janssens Laboratory of Genetics, University of Leuven, B-3001 Heverlee, Belgium; [2]Plant Research Laboratory, Michigan State University , East Lansing, MI 48824-1312, USA, [3]ZENECA Agrochemicals, Jealott's Hill Research Station, Bracknell, Berks RG12 6EY, UK

Radish (*Raphanus sativus* L.) seeds were previously shown to contain two homologous 5 kDa basic, cysteine-rich proteins , called Rs-AFP1 and Rs-AFP2, which exhibit potent antifungal activity (Terras et al. 1992, J Biol Chem, 267, 15301-15309) . We now show by immunocytochemistry that Rs-AFPs are stored in the middle lamellae of the cell walls of different radish seed tissues. These proteins are preferentially released from their location during seed germination after disruption of the seed coat. The amount of released proteins is sufficient to create a microenvironment around the seeds in which fungal growth is inhibited. This phenomenon may be relevant to the protection of seeds germinating in the soil.

A cDNA encoding the Rs-AFP1 preprotein was isolated from a cDNA library prepared from mRNA of near mature seeds. Transcripts (0.6 kb) hybridizing with a Rs-AFP1 cDNA probe are present in near-mature and mature seeds, as well as in leaves infected with fungal pathogens, whereas such transcripts are barely detectable in healthy uninfected leaves. Hence, Rs-AFP-like proteins represent a novel class of pathogenesis-related proteins. Extracts of transgenic tobacco plants expressing Rs-AFP2 showed an up to 16-fold higher antifungal activity against *Fusarium culmorum* relative to extracts from control tobacco plants. The T2 generation of the transgenic plants is currently being tested for resistance to *Alternaria longipes*.

X1-105 SEQUENCE CONSERVATION OF A FAMILY OF DISEASE RESPONSE GENES AMONG PEA SPECIES AND THEIR EXPRESSION IN TRANSGENIC CANOLA, Stuart M. Brown, Sandhya Tewari, Brian Fristensky, Department of Plant Science, University of Manitoba, Winnipeg, MB R3T2N2, CANADA

Wild pea species such as *Pisum elatius, P. fulvum,* and *P. humile* are resistant to the fungal pathogen *Fusarium solani* pv *pisi* while the garden pea (*Pisum sativum* cv Alaska) is susceptible. Transcription of the drr49 disease response multi-gene family has previously been shown to be induced in pea by inoculation with *F. solani*. We observe variations in the patterns of expression of the multiple copies of drr49 in different pea species and in response to inoculation with different pathogens. We are investigating the correlation between the timing of induction of each drr49 gene copy and the expression of resistance or susceptibility in the plant.

Sequence analysis of drr49 genes from *P. elatius, P. fulvum,* and *P. humile* indicates that the 3 copies of drr49 previously cloned from *P. sativum* are present in each of these species with only approximately 1% divergence in both coding regions and introns.

Analysis of transgenic canola (*Brassica napus*) plants containing the *P. sativum* drr49a gene with its native promoter is underway to determine if this gene is induced by fungal infection in a heterologous system. Effects of the drr49 gene on resistance of transgenic canola to blackleg disease (*Leptosphaeria maculans*) is also being investigated.

X1-106 EXPRESSION OF *BACILLUS THURINGIENSIS* VAR. *KURSTAKI CRY*IA(C) SEQUENCES IN TRANSGENIC SOMATIC WALNUT EMBRYOS, Abhaya M.Dandekar, Gale H. McGranahan, Patrick V. Vail[1], Sandra L. Uratsu, Charles Leslie, and J. Steven Tebbets[1], Department of Pomology, University of California, Davis CA 95616 USA and [1]Horticulture Crops Research Labs., USDA/ARS, 2021 South Peach Ave, Fresno CA 93727

Commercial production of English or Persian walnuts (*Juglans regia* L.) in the United States exceeds 200,000 metric tons with a farm value of over $240,000,000. Losses in walnut production due to insect pests are significant despite the extensive chemical controls used. Codling moth (*Cydia pomonella*) is generally regarded as the key insect pest species. The main objective of this study is to achieve resistance to this pest. Purified insecticidal crystal protein fragments (ICPFs) of *Bacillus thuringiensis* encoded by *cry*IA(c) and *cry*IA(b) of this organism were shown to be lethal to codling moth. One of these genes, *cry*IA(c), was used to transform walnut somatic embryos using *Agrobacterium*-mediated transformation. A binary vector (pWB139) was used to introduce this gene which was expressed as a protein fusion with the kanamycin resistance gene from bacteria. Insect feeding trials revealed very low levels of *cry*IA(c) expression in transgenic shoots or embryos corresponding to each transgenic line obtained with pWB139. To correct this problem a full length synthetic version of one of these genes, *cry*IA(c) expressed with the cauliflower mosaic virus 35S transcript regulatory sequences (CaMV35S), was used to transform walnut somatic embryos resulting in 61 individual transgenic embryo lines. We observed that in 34% of these lines (21/61), designated as "class A", expression was high enough to kill almost all of the codling moth larvae (98% mortality). Twelve clones (20%) were designated "class B" and these showed a marked retardation of larval development and a mortality between 50-94%. Insect from the remaining 28 lines (46%), although transformed, was indistinguishable from the control. A detailed analysis of these lines will be presented.

X1-107 GENETIC ENGINEERING OF CROPS TO SCLEROTINIA RESISTANCE USING ARABIDOPSIS AS A MODEL, M.B. Dickman, T.L. Buhr, and A. Mitra, Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722

Among the economically important plant pathogens, Sclerotinia sclerotiorum has an unusually broad host range attacking a wide range of crop plants. Pathogenesis of S. sclerotiorum is complex and not well understood. Recent work in our laboratories has demonstrated the importance of oxalic acid (OA) in the infection process. S. sclerotiorum mutants specifically deficient in oxalic acid production are unable to infect host plants. We explored the possibility of using Arabidopsis as a model host due to its attractive genetic features. S. sclerotiorum readily infects Arabidopsis, and importantly the OA$^-$ mutants of the fungus are non-pathogenic. Thus, Arabidopsis is not only a suitable host for S. sclerotiorum, but pathogenesis is likely to proceed in a similar manner to that described for its natural hosts. Our aim is to assess the potential of introducing a gene encoding an oxalate degrading activity first in Arabidopsis and then in crop plants, to confer resistance to Sclerotinia. We have identified bacterial strains with such capability in a plate bioassay technique. Furthermore, using a plant bioassay, these bacterial strains protect Arabidopsis from Sclerotinia infection. Cosmid libraries of two bacterial strains, possessing OA degrading activity have been constructed. Using transposon mutagenesis and the OA plate assay, two OA$^-$ bacteria were generated. Southern analysis showed that Tn5 inserted into different restriction fragments indicating the mutants were not identical. The two mutants were electroporated with one of the cosmid libraries. Cosmids with identical restriction patterns complemented both mutants. We have truncated and subcloned the complementary fragment to 5 kb. Characterization of this DNA will be discussed.

X1-108 PROMOTERS AND THEIR USE IN THE EXPRESSION OF AGRONOMICALLY IMPORTANT GENES FOR GENETIC ENGINEERING OF INDICA RICE, **Gadab C. Ghosh Biswas, Joachim Wünn, Peter K. Burkhardt, Andreas Klöti, Johannes Fütterer and Ingo Potrykus, Institute of Plant Sciences, Swiss Federal Institute of Technology, CH-8092 Zürich, Switzerland.**

In our continuos effort to genetically engineer Indica rice for enhanced plant productivity, we have developed a protoplast transformation and regeneration system of Indica rice, cultivar IR43. Protoplasts were co-transformed with CaMV 35S promoter-*hph* chimeric gene conferring resistance to hygromycin and gus A gene driven by either wheat *rbcS* or wheat *cab* promoter in separate plasmid constructs. Over 60 percent of independent hygromycin selected callus clones could be regenerated to plants. Southern analysis confirmed the stable integration of the gus A coding sequence into the plant genome. Histochemical analysis showed that wheat *cab* promoter confers strong cell type specific expression of the gus A reporter gene in transgenic rice plants. Such promoter should be useful for insect resistance studies (particularly against yellow stem borer) in rice. For such insect resistance studies, we have introduced synthetic and truncated CryIA(b) gene of *Bacillus thuringiensis* into protoplasts and obtained several plants through selection. Expression of the CryIA(b) gene in transgenic rice either driven by the CaMV 35S promoter or by *rbcS* and *cab* promoters will be presented.

X1-109 ARCELIN : IMPLICATIONS FOR BRUCHID RESISTANCE

M-F. Grossi de Sa, C. de Souza, I. Gerhardt, P. Ferreira M. Azevedo, N. Harris and N. Paes. Mol. Biol. Lab, EMBRAPA/ CENARGEN - SAIN Parque Rural - 70849 - Brasilia - DF-Brazil

The mexican bean weevil, **Zabrotes subfasciatus**, and the bean weevil, **Acanthoscelides obtectus**, are the most important pests of stored beans (**Phaseolus vulgaris**). Cultivated **Phaseolus vulgaris** beans are infested by both species and damage of bruchids on beans is of considerable importance in Africa and throughout Latin America. Several wild form of **Phaseolus vulgaris** of Mexican origin were reported presenting high levels of antibiosis resistence to bruchids. Research conducted on the factors associated with resistence led to the hypothesis that the arcelin protein present in 10% of the wild **Phaseolus vulgaris** lines plays a role in the resistence to **Z. subfasciatus**. However, no information has been obtained for **A. obtectus**.

Our main objective is to obtain cultivated dry beans resistent to both species of bruchids **Z. subfasciatus** and **A. obtectus** via genetic engineering and to express differents arcelin in microorganism to be used in biological control of bruchids on stored beans. The arc1, arc4 and arc5 proteins were purified and used on antibiosis tests with both bruchids. We have produced polyclonal and monoclonal antibodies against arc-1 and Arc-5 and a detailed study of immunocytolocalization was carried out. This study show the presence of arcelin in the vacuolar protein body concentrated in cotyledon cells of wild bean seeds. We have constructed genomic and cDNA libraries of differents lines of wild **Phaseolus vulgaris** in order to isolate Arcelin genes.

EMBRAPA,CNPq, IFS

X1-110 PHYTOTOXIC COMPOUND(S) OF PYRENOPHORA GRAMINEA, CAUSAL AGENT OF BARLEY LEAF STRIPE.

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P. graminea culture filtrates and cell walls contain a phytotoxic compound(s) which, upon infiltration on barley leaves, reproduce symptoms of barley leaf stripe. This compound(s) has been partially purified by means of selective precipitation in acetone, dialysis and passages on gel-filtration column Sephacryl S-300-HR. In gel-filtration toxic activity is separated into two fractions, named PI and PII, that have a molecular weight of 250-300 kDa and 55 kDa respectively and that contain both glucidic and proteic moieties. Monosaccharide analysis revealed that either PI and PII contained different proportions of fucose, glucuronic acid, glucose and galactose. Glucuronic acid seems to be involved in toxic activity since ß-glucuronidase specifically inactivates the toxin.

The role of the proteic component of the toxin is less clear, as treatments with different protease only delay the appearance of necrotic stripes. PII is host-specific while PI is not.

The availaibility of the purified toxin open the possibility to determine whether it can be used to develop: 1) a method of screening in vitro for resistance to P. graminea; preliminary treatments with the purified toxin reduce barley leaf protoplasts viability of 45%, in the conditions used; 2) a method of rapid diagnosis of infection using antibodies raised against the toxin.

X1-111 CHARACTERIZATION AND EXPRESSION OF CLASS I METALLOTHIONEIN GENES IN COTTON,

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Metallothioneins (MTs) are low-molecular-weight, metal binding proteins which are widespread throughout the animal kingdom. Recently, genes which encode proteins with sequence similarity to class I MTs have been identified in several plant species, including *Mimulus guttatus*, maize, soybean, pea, barley and *Arabidopsis*. We have identified a cDNA clone (pGHR5) derived from an mRNA that is highly expressed in cotton roots and has sequence homology with other class I MT genes. Hybridization of cotton DNA with pGHR5 suggests class I MT is encoded by a small gene family with 6-8 members. Sequence differences between pGHR5 and other cotton MT cDNA clones indicate at least two closely related genes are expressed in cotton roots. When RNAs isolated from various cotton tissues (leaf, bracts, immature petals, stem, anthers, seeds, ovaries / pistils, ovules / immature fiber, pericarp and roots) were hybridized to pGHR5, the level of MT transcript was very low in all tissues other than root. Genomic clones have been isolated to further characterize the organization and structure of these genes. Preliminary analysis of the MT genomic clones suggest the linkage of several MT genes in the cotton genome. Results of transient expression assays will be presented that identify MT gene promoter sequences which confer high levels of gene expression in cotton roots.

X1-112 POLYPHENOL OXIDASE (PPO) EXPRESSION AND INSECT RESISTANCE IN TRANSGENIC TOMATO

Michelle D. Hunt and John C. Steffens. Department of Plant Breeding and Biometry, Cornell University, Ithaca, NY 14853

Polyphenol oxidases (PPO; E.C. 1.14.18.1 and E.C. 1.10.3.2) are nuclear-encoded, plastid-localized, bicopper metalloenzymes found in both photosynthetic and nonphotosynthetic tissues in angiosperms. PPOs catalyze the oxidation of phenols to quinones contributing to oxidative browning and melanization, the primary detrimental effects of PPO in the postharvest physiology and processing of crop plants. The PPO found in glandular trichomes of some Solanaceous species has been associated with the polymerization of insect entrapment exudate and, therefore, may play a role in plant defense against insect predation. However, a definitive biochemical function for PPOs in general is unknown. Proposed functions include participation in pseudocyclic photophosphorylation, oxygen regulation and/or plant insect resistance. Recent studies suggest that wounding induces the production of PPO in apical tissues thus supporting a defensive role. To address the issue of function, PPO cDNAs cloned from tomato and potato leaves have been sequenced, cloned into pBI121 in the sense and antisense orientations, and used in tomato transformation experiments to produce mutants which have modified PPO levels. Plants overexpressing PPO as well as functional nulls have been recovered and are being utilized in insect feeding experiments utilizing Colorado potato beetle and tobacco hornworm to test the hypothesis that PPOs are involved in insect resistance. Preliminary feeding results with the Colorado potato beetle indicate a significantly lower mortality rate and higher survivor weight in larvae fed PPO antisense leaves as compared to larvae fed leaves from nontransformed and PPO overexpressing plants.

X1-113 CHEMICAL DEFENSES IN CONIFERS. WOUND-INDUCIBLE MONOTERPENE BIOSYNTHESIS IN GRAND FIR

Efraim Lewinsohn, Mark Gijzen, Christopher L. Steele & Rodney Croteau, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340

Conifers accumulate resin as a physical and chemical defense against wounding, herbivores and pathogens. The volatile component of conifer resin (turpentine) consists primarily of monoterpene olefins. Grand fir (Abies grandis, Pinaceae) saplings and mature trees respond to stem wounding by the rapid and localized increase in monoterpene production, as determined by measuring the levels of monoterpene synthase activity in cell-free extracts. These enzymes catalyze the production of monoterpene olefins from geranyl pyrophosphate (GPP), and at least six distinct cyclases have been isolated from wounded grand fir saplings and characterized. Polyclonal antibodies were generated in rabbits against the major wound inducible monoterpene synthase, a 62 KDa monomeric enzyme that produces both (-)-α- and (-)-β-pinene from GPP. Antibodies directed against this pinene cyclase cross-reacted on Western blots with other monoterpene cyclases from grand fir that convert GPP to sabinene, limonene, and 3-carene, but not with functionally similar enzymes from other conifers. Wound-inducible de novo synthesis of monoterpene synthase proteins was also demonstrated by immunoblotting. A novel protocol for the isolation of mRNA from woody conifer stems was developed and used to construct cDNA libraries from wounded grand fir stems for studying the monoterpene synthase genes and their regulation at the transcriptional level. The expression of these genes in transgenic plants could increase plant resistance to pathogens and pests.

X1-114 SYNERGISTIC ACTIVITY OF CHITINASES AND ß-1,3-GLUCANASES ENHANCES FUSARIUM RESISTANCE IN TRANSGENIC TOMATO PLANTS,

J. Logemann, L.S. Melchers, H. Tigelaar, M.B. Sela-Buurlage, A.S. Ponstein, J.S.C. van Roekel, S.A. Bres-Vloemans, I. Dekker, B.J.C. Cornelissen, P.J.M. van den Elzen and E. Jongedijk, MOGEN Int., Leiden, The Netherlands.

Tobacco mosaic virus inducible proteins were isolated from inoculated leaves of Samsun NN tobacco and were tested for antifungal activity. *In vitro* studies demonstrated that specifically the class I (vacuolar) chitinases and class I ß-1,3-glucanases are potent inhibitors of various fungal species including Fusarium and that they act synergistically. In contrast, the tobacco class II chitinases and ß-1,3-glucanases, which are localized extracellularly, display *in vitro* no antifungal activity, neither alone nor in combination with other proteins. The simultaneous expression of class I chitinase (1.5-4% of soluble protein) and class I ß-1,3-glucanase (0.1-2% of soluble protein) in transgenic tomato plants resulted in a substantially increased resistance against *Fusarium oxysporum*. However, tomato plants that expressed either of these genes alone were not protected against *Fusarium oxysporum*. These observations support the idea that the combined expression of specific chitinases and ß-1,3-glucanases enhances fungal resistance in transgenic plants.

X1-115 FUNCTIONAL EXPRESSION OF THE *USTILAGO MAYDIS* KILLER TOXIN P4 IN PlANTS,

Susanne Logemann[1,2], Claudia Bollhoefer[1] and Jeff Schell[1], [1]Max-Planck-Institut fuer Zuechtungsforschung, Carl-von-Linne'-Weg 10, 50829 Koeln, Germany; [2]Institute of Plant Molecular Biology, Nonnensteeg 3, 2311 VJ Leiden, The Netherlands

Some natural occurring strains of the corn smut fungus *Ustilago maydis* secrete a toxic protein with the ability to kill other fungi (which do not produce that toxin) of the same or closely related species. Those strains carry mycoviruses, which consist of a segmented doublestranded RNA-genome, and code for the so called killer protein. In nature, the killer protein is translated as a larger precursor protein, posttranslationally maturated and secreted by the fungal cells. The (maturated) killer protein was designated as "Ustilin".

The aim of this project was the cloning and expression of *Ustilin* to obtain increased resistance in plants against fungal attack.

Ustilin of the strain UmVP4 was purified, characterised, cloned and some truncated forms of the protein were expressed in *E. coli* and transgenic tobacco plants as a model system.

A construct encoding 34 aa of the proteinase-inhibitorII leader peptide fused to the 105 amino acids of *Ustilin* (*PISS-Ustilin*) leads to a fusion protein which is supposed to be secreted, and by that, mimics the natural fate of the killer toxin. Tobacco plants expressing *PISS-Ustilin* export biologically active *Ustilin* out of the cell as shown by bioassays and Western-Blots of intercellular washing fluids.

X1-116 THE CHARACTERIZATION OF a 33 kD PROTEIN ASSOCIATED WITH CORN GENOTYPES RESISTANT TO FALL ARMYWORM, Dawn S. Luthe, Binghua Jiang and William P. Williams, Dept. of Biochemistry and Molecular Biology, Mississippi State University, Miss. State, MS 39762. USDA, Crop Science Research Laboratory, Agricultural Research Service, Mississippi State, MS 39762

The protein patterns of calli initiated from corn genotypes resistant and susceptible to fall armyworm were analyzed by 2-D gel electrophoresis. All of the resistant genotypes, including Mp704, had an intense protein spot of 33 kD, and none of the susceptible genotypes, including Tx601, had this protein. The 33 kD protein was excised from stained 2-D gels and was used to generate polyclonal antibody. *In vivo* labeling with Tran^{35}S label indicated the 33 kD protein was synthesized by the resistant but not by the susceptible corn calli. Western blot analysis confirmed that the protein was present in resistant, and absent from susceptible corn calli. When callus from the resistant genotype Mp704 changed from nonfriable to friable morphology it lost the 33 kD protein and its resistance to insect feeding. Amino terminal sequence analysis indicated that the 33 kD protein had about 48% homology with stem bromelain, a cysteine protease from pineapple. The protein appeared one month after embryos from Mp704 were cultured on MS medium supplemented with 2,4-D and zeatin. It was not present in the embryos cultured without the hormone, or in calli from the susceptible genotype Tx601. Unfortunately the protein could not be detected in the whorl, the feeding site of the insect. Analysis of callus from the F_2 generation of Mp704 x Tx601 indicated that larval weights and concentration of the 33 kD protein were highly and negatively correlated.

X1-117 ANALYSIS OF *B.T.* INSECTICIDAL CRYSTAL PROTEIN EXPRESSION IN TRANSGENIC BRASSICA OLERACEA: APPLICATIONS TO INSECT RESISTANCE MANAGEMENT. Timothy D. Metz[1], Ram Dixit[1], Anthony M. Shelton[2], Richard T. Roush[3] & Elizabeth D. Earle[1]. [1]Dept. of Plant Breeding, Cornell University, Ithaca, NY 14853; [2]Dept. of Entomology, New York State Agricultural Experiment Station, Geneva, NY 14456; [3]Dept. of Entomology, Cornell University, Ithaca, NY 14853.

Bacillus thuringiensis insecticidal crystal protein (*Bt* ICP) genes are being genetically incorporated into many crop plants in order to improve insect control. Unfortunately, the benefits of these genetically-transformed plants may not be realized due to the development of insecticide resistance. The diamondback moth, an important pest of crucifers worldwide, has already developed resistance to *Bt* sprays in the field, and there is concern that other insects may also develop resistance to *Bt* ICPs in transgenic plants. Principles of managing resistance to plant-incorporated insecticides must be developed if they are to have a commercially viable future. We are using the only case of field-evolved *Bt* resistance as a model system to study different options for resistance management. We report the production of transgenic broccoli expressing a *Bt* ICP. Flowering stalk explants of broccoli were inoculated with an *Agrobacterium tumefaciens* strain containing the *NPTII* gene and a *Bt cryIA(c)* gene optimized for plant expression. A total of 181 kanamycin-resistant plants were obtained from 5 broccoli lines. Initial screening of transformants for *Bt* ICP expression was conducted using 5-10 1st instar larvae of a *Bt*-susceptible diamondback moth strain; 112 of 162 kanamycin-resistant plants provided 100% insect mortality. Selected plants that gave 100% mortality of susceptible larvae were also assayed with 1st instar larvae from a strain of diamondback moth that had developed resistance to *Bt* in the field and with F_1 hybrids from a cross between the resistant and susceptible strains. *Bt*-resistant larvae showed >90% survival on all plants tested, whereas all F_1 larvae died. The ability of these transgenic plants to kill susceptible larvae while serving as a suitable host for resistant larvae makes them an excellent model for testing the various *Bt* resistance management strategies. Insect resistant progeny plants from suitable transformants are being used in greenhouse resistance management experiments. Hypocotyl and petiole segments from 21 day *in vitro* grown cabbage and broccoli plants were transformed with the same *Bt* gene under the control of a light inducible promoter. Transformants are currently being analyzed and may allow further tests of resistance management strategies involving control of gene expression.

X1-118 PHENOTYPIC CHARACTERIZATION OF A TOBACCO MERISTEMATIC MUTANT, Naderi M. and Berger P. H., Department of Plant, Soil and Entomological Sciences, Division of Plant Pathology, University of Idaho, Moscow, ID 83844-2339

The initial phenotypic characterization of a mutation in tobacco , *meristematic* (*mer*), is reported here. The mutation affects embryogenesis, cotyledon formation, leaf development and initiation, leaf vein pattern, and flower development. Embryonic development ranges from abnormal to lethal. Cotyledons are heart-shaped to seashell-shaped. Leaves progress from normal to cup-shaped with occasional abnormal initiation. Sometimes new shoots arise from the leaf midvein. Flowers develop from normal to compound with occasional fused floral parts. Conversion of stamen parts to petal-like structures is occasionally observed. Endosperm development is also affected in *mer* mutants. The mutant resulting in the *mer* phenotype has possibly affected plant hormone synthesis or transport. Initial results indicate that the *mer* mutant may be tagged with t-DNA.

X1-119 TRANSGENIC APPLE PLANTS CONTAINING LYTIC PROTEINS HAVE INCREASED RESISTANCE T *ERWINIA AMYLOVORA*. J.L. Norelli[1], H.S. Aldwinckle[1], L. Destéfano-Beltrán[2], and J.M. Jaynes[2]. [1]Department of Plant Pathology, Cornell Univ., Geneva, NY 14456. [2]Department of Biochemistry, Louisiana State Univ., Baton Rouge, LA 70803.

Cecropin B and attacin E are lytic proteins native to the hemolymph of *Hyalophora cecropia*, the giant silk moth. SB-37 and Shiva-1, substitution analogs of cecropin B, and attacin E possess a broad spectrum of activity against both gram negative and gram positive bacteria. The concentration of Shiva-1 necessary to kill 50% of *Erwinia amylovora* cells ranged from 28 to 197 nM for three different strains. Genes encoding SB-37, Shiva-1, and the naturally occurring attacin E have been cloned and coupled to plant promoters to facilitate their expression in plants. Apple has been transformed with DNA constructs encoding attacin E, SB-37, and Shiva-1 to determine the effect of lytic protein production in transgenic apple on susceptibility to the fire blight disease caused by *E. amylovora*.. The apple rootstock M.26, which is very susceptible to fire blight, was transformed with the gene for attacin-E to yield a transgenic line T1. Integration of the attacin E gene into the apple genome was confirmed by Southern analysis. Northern analysis of T1 indicated the presence of attacin E mRNA in plants inoculated with *E. amylovora*. After inoculation of *in vitro* grown plants of T1, M.26, and Liberty (resistant control cultivar) with *E. amylovora* , 50% of the plants were killed by 10^4 X 14, 4, and 16 cells of *E. amylovora*, respectively. In greenhouse trials T1 was significantly more resistant to fire blight than M.26 ($P<0.0001$). 21 days after inoculation, 30% of the shoot length of T1 was necrotic, compared with 57% of M.26 and 23% of Liberty. Greenhouse trials have indicated that there is no significant difference in the fire blight susceptibility of M.26 and an M.26 transgenic containing the vector plasmid pBI121. A field trial of T1 and control cultivars was planted in the spring of 1993 to evaluate the fire blight resistance and horticultural characteristics of this apple transgenic.

X1-120 A NEW CLASS OF TOBACCO CHITINASES HOMOLOGOUS TO BACTERIAL EXO-CHITINASES IS ACTIVE AGAINST FUNGI *IN VITRO*

S. Ohl, M. Apotheker-de Groot, J.A. van der Knaap, A.S. Ponstein, M.B. Sela-Buurlage, J.F. Bol, B.J.C. Cornelissen, H.J.M. Linthorst and L.S. Melchers, MOGEN Intl.nv, Einsteinweg 97, 2333 CB Leiden, NL

Pathogenesis-related (PR) proteins are thought to be involved in the general defense mechanism of plants against fungal pathogens. In a few cases increased resistance has been demonstrated in transgenic plants expressing one or a subset of the corresponding genes. Some of the PR proteins have been identified as glucanases and chitinases, lytic enzymes which can degrade major components of fungal cell wall.

Here we report on the identification and initial characterization of a new class of plant chitinases (class V) showing no sequence similarity to the previously identified chitinases (class I - IV) but sharing significant homology with some bacterial exo-chitinases.

Class V chitinases are encoded by a small multigene family and are induced by various stresses such as TMV infection, ethylene treatment, wounding and UV irradiation. Comparison of a cDNA clone and a genomic clone from two class V chitinases reveals a high degree of sequence identity.

From TMV infected tobacco leaves class V chitinase was purified and the enzymatic activity was tested on a number of chitin substrates. Unlike the bacterial chitinases the plant enzymes were endo-chitinases lacking detectable exo-chitinase activity.

In vitro class V chitinases were shown to inhibit the growth of *Trichoderma viride* and *Alternaria radicina* and in synergy with class I ß-1,3 glucanase were active against *Fusarium solani*.

The expression pattern and *in vitro* activity of this new class of plant chitinases strongly suggests a role in the plants' general defense mechanism against fungal pathogens.

X1-121 ISOLATION AND CONSTRUCTION OF THE cDNA CLONES FROM CUCUMBER MOSAIC VIRUS (CMV-K) RNAs, Kyung-Hee Paek[1], Shin Je Kim[3], Seok Yoon Kwon[1], Hye Sun Cho[1], Sang Hyeon Nam[2], and Yong Hwan Cho[2], [1]Genetic Engineering Research Institute, KIST, Taejon 305-606, [2]Breeding & Research Station, Hungnong Seed, Chungwon-Kun, 363-950, [3]Research Institute, Korea Kumho, Yeocheon, 287-1

The four main RNA species were isolated from purified CMV-K(Cucumber Mosaic Virus-Korea) and were separated on 1% agarose gel containing formaldehyde. Double-stranded cDNAs synthesized from RNA 1 to 4 were cloned in *Sma*I site of pUC 19, and the cDNA bank was screened with the probes from DSM (Deutche Sammlung von Mikroorganismen und Zellkulturen GmbH). We have isolated cDNA clones of RNA 1, 3 and 4. These putative clones were characterized and confirmed by Northern hybridization. Sequencing data of RNA 3 cDNA confirmed that CMV-K belonged to subgroup II of CMV. BY using a PCR technology, cDNAs corresponding to the movement protein, the inter-cistronic region and the coat protein were synthesized. Some of the cDNA clones were constructed both sense and anti-sense orientation in the plant expression vector, pMBP-1. Each construction was then transformed into *Agrobacterium tumefaciens* strain LBA4404 and is on the way for developing virus-resistant transgenic plants, especially hot pepper(*Capsicum annum* cv. Golden tower).

X1-122 THE "MISSING" CLASS I PR-4 PROTEIN FROM TOBACCO EXHIBITS ANTIFUNGAL ACTIVITY,

A.S. Ponstein, A.A. Bres-Vloemans, M.B. Sela-Buurlage, B.J.C. Cornelissen, and L.S. Melchers, MOGEN Int., Leiden, The Netherlands.

Tobacco plants respond to stress by the synthesis of a large number of pathogenesis related (PR) proteins. These proteins are subdivided into five distinct groups based on enzymatic, structural and immunological data. Within each group of tobacco PR proteins a division can be made between basic, vacuolarly targeted proteins and acidic, extracellularly targeted proteins, now generally referred to as class I and class II proteins, respectively.

In case of the PR-4 proteins no class I representative has been isolated so far. However, a class I PR-4 protein (further referred to as CBP) was recently purified in our lab. Analysis of the protein and corresponding cDNAs revealed that it contains a N-terminal chitin-binding (hevein) domain and a C-terminal domain showing homology to class II PR-4 proteins from tobacco PR-4a,4b), and tomato (PR-P2). Thus the whole protein resembles the putative WIN1 and WIN2 proteins of potato. CBP is localized intracellularly and is induced upon TMV infection and wounding, typical characteristics of class I PR proteins.

In vitro assays demonstrated that CBP exhibits antifungal activity toward *Trichoderma viride* and *Fusarium solani* by causing lysis of the germ tubes and/or growth inhibition. In addition it was shown that CBP acts synergistically with a tobacco class I chitinase and with a tobacco class I ß-1,3-glucanase against *F. solani* germlings.

X1-123 PEAR AND TOMATO POLYGALACTURONASE INHIBITOR PROTEINS: ANALYSIS AND EXPRESSION IN TRANSGENIC TOMATO, Ann L.T. Powell, Henrik Stotz, Guy D'hallewin, James Contos, John M. Labavitch, Alan B. Bennett, Depts. of Vegetable Crops and Pomology, Univ. of California, Davis, CA 95616

Polygalacturonase inhibitor proteins (PGIPs) are hypothesized to impede fungal infection of fruit or vegetative tissues. Two explanations for how PGIP functions are based on the *in vitro* observations: 1. pear PGIP is most effective against polygalacturonases (PGs) from fungi to which the fruit is most resistant and 2. oligosaccharide plant defense response elicitors result from the PG-PGIP interaction. To test the hypothesis that PGIPs are an impediment to fungal infection, we have characterized and cloned PGIPs from pear and tomato and have overexpressed pear PGIP in transgenic tomato plants. Transgenic plants expressing large amounts of PGIP provide the biological material for the analysis of the role of PGIP as a deterrent to pathogen invasion *in vivo*.

Glycoprotein inhibitors of fungal PGs have been purified and characterized in cell wall extracts from pear and tomato fruit. Analysis of purified tomato PGIP reveals both glycosylation and charge heterogeneity. PGIPs in pear and tomato are encoded by one or two genes. The coding sequences for pear (Stotz et al., Plant Phys. 102:133, 1993), tomato (Stotz et al., in prep.) and bean (Toubart et al., Plant J., 2:367, 1992) PGIPs show approximately 60% identity to each other. All three sequences have multiple glycosylation sites and encode potential signal peptides, indicating cell wall localization.

In pear, PGIP activity, protein, and mRNA are abundant in fruit, 100-fold lower in flowers, and barely detectable in leaves. PGIP activity is about 100-fold less abundant in tomato fruit than in pear, and in tomato leaves the activity is undetectable. After wounding or after infection of pear fruit by *B. cinerea*, no substantial induction of PGIP can be measured.

Two cultivars of tomato have been transformed with chimeric genes encoding pear PGIP. Active pear PGIP is expressed abundantly throughout transgenic plants when regulated by the 35S CaMV promoter or in the ripening fruit when regulated by the E8 tomato fruit-specific promoter. Western analysis of cell wall extracts has indicated that the pear PGIP is correctly targeted and glycosylated. Analysis of plants containing constructs with and without translational enhancer elements confirmed that abundant expression is dependent on the elements. Transgenic plants expressing pear PGIP are being analyzed to assess their resistance to infection by fungi whose PGs have been shown to be inhibited *in vitro* by pear PGIP and preliminary results of these experiments will be presented.

X1-124 ENGINEERING OF PROGRAMMED CELL DEATH AS A DEFENCE MECHANISM OF POTATO AGAINST LATE BLIGHT DISEASE, Günter Strittmatter[1], Margot Egen[1], Irmgard Rüntz[1], Jan Janssens[2], Chris Opsomer[2] and Johan Botterman[2], [1]Max-Planck-Institut für Züchtungsforschung, Department of Biochemistry, D-50829 Köln, Germany, [2]Plant Genetic Systems, B-9000 Gent, Belgium

Infection of potato with the pathogenic fungus *Phytophthora infestans* results in cultivar/race specific interactions, depending on the genotypes of the host cultivar and the fungal race. Incompatible interactions (host cultivar: resistant) are characterized by hypersensitive cell death of the host tissue in the vicinity of infection sites, thereby, blocking the spread of fungal hyphae. No comparable response is established in compatible interactions (host cultivar: susceptible) which finally lead to the development of late blight disease. In order to improve the resistance of susceptible potato cultivars, we try to engineer an artificial programmed cell death in compatible host/pathogen interactions by rapid and strictly localized synthesis of a cytotoxic compound.

Expression studies with chimeric promoter/GUS gene constructs in transgenic potato revealed a 273 bp long *cis*-acting regulatory region from a potato PR gene, *prp1-1*, sufficient to mediate strictly localized transcriptional activation at infection sites during the initial stages of compatible interactions. This regulatory sequence did not respond to various abiotic stimuli and its activity was not detectable in any non-infected tissue except the root tips.

Potato plants have been transformed with a construct combining the 273 bp promoter fragment with the barnase gene from *Bacillus amyloliquefaciens* encoding a highly cytotoxic RNase. Simultaneously, the barstar gene (encoding a specific inhibitor of barnase) under control of a constitutive promoter has been introduced into these plants, to minimize the detrimental effects of background barnase expression in non-infected tissue. In transgenic plants, the level of barnase expression is expected to exceed the level of barstar expression only in the vicinity of fungal infection sites, thereby, causing strictly localized death of host cells. According to several phytopathological evaluations, in three out of 24 primary transformants harboring the two-component system the efficiency of fungal sporulation was reduced after infection with *Phytophthora infestans*. The correlation of this novel phenotype with the presence and expression level of the barnase gene is being tested by genetic and molecular analysis of the different transgenic lines.

X1-125 ISOLATION AND CHARACTERIZATION OF A ß-1,3-GLUCANASE GENE IN APPLE. Jyothi Thimmapuram and Schuyler S. Korban, Department of Horticulture, University of Illinois, Urbana, IL 61801.

ß-1,3-glucanase is one of the pathogenesis-related proteins that has been shown to be correlated with disease resistance in plants. An apple genomic library was constructed using Sau3A digested 'Golden Delicious' DNA cloned into BamHI digested Charon35 Lambda cloning vector. The library was screened using a maize ß-1,3-glucanase cDNA clone as a probe. Out of 8 positive clones isolated, at least 2 clones appeared to be different. A restriction enzyme map of one of the clones (σAG7142) will be presented. A 2.1 Kb EcoRI/HindIII fragment which hybridized to the maize ß-1,3-glucanase cDNA probe was subcloned into a plasmid vector pMOB. The recominant pMOB plasmid was used to generate transposon-mediated deletions which are being sequenced. The sequence of this clone will also be presented.

X1-126 FIELD TRIAL RESULTS OF TRANSGENIC SQUASH AND CANTALOUPE PLANTS CONTAINING MULTIPLE VIRUS RESISTANCE, David M. Tricoli, Kim J. Carney, Rosaline Deng, J. Russell McMaster, John F. Reynolds, Dave Groff[1], Keisha Hadden[1], Maury L. Boeshore, Paul F. Russell and Hector D. Quemada, Asgrow Seed Company, Kalamazoo, MI 49001, [1]Asgrow Seed Company, Tifton, GA 31794

We have produced transgenic squash and cantaloupe plants with multiple virus resistance. The squash lines were created by transforming a proprietary inbred line with a trivalent cassette containing the cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and the watermelon mosaic virus-2 (WMV-2) coat protein genes. The transgenic inbred line was crossed to the appropriate non-transgenic parental lines to create virus resistant commercial hybrids. A transgenic cantaloupe hybrid was produced by crossing two transgenic parental inbred lines, one containing a CMV-coat protein construct and the other containing a ZYMV-WMV-2 bivalent coat protein construct. Transgenic squash and cantaloupe hybrid seeds were germinated in the greenhouse and challenged with a mixed inoculum consisting of a 1/10 w/v dilution of CMV, ZYMV, and WMV-2. Inoculated seedling were transplanted to the field and evaluated for resistance over the course of the growing season. Evidence of multiple virus resistance will be presented.

X1-127 CHARACTERIZATION OF A BACTERIAL SYSTEM CAPABLE OF DEGRADING DICAMBA AND EVALUATION OF ITS POTENTIAL IN THE DEVELOPMENT OF HERBICIDE-TOLERANT CROPS, Donald P. Weeks†, Xiao Zhuo Wang†, Patricia L. Herman†, Ju Yang* and David Hage*, †Department of Biochemistry and *Department of Chemistry, University of Nebraska, Lincoln NE 68583-0718

A number of field and vegetable crops have been genetically engineered for tolerance to a variety of existing herbicides. One strategy for developing such plants is the insertion into target crops of genes from microbial systems which encode enzymes capable of inactivating a particular herbicide. We are exploring the potential of using such a strategy to develop plants that are tolerant to treatment with the widely used, inexpensive, and environmentally-friendly herbicide, dicamba (trade name, Banvel). The bacterial system we are characterizing is derived from *Pseudomonas maltophilia*, strain DI-6, which is capable of degrading dicamba to CO_2 and water (Cork and Krueger, Adv. Appl. Microb. 36: 1-66). We have developed analytical methods with capillary zonal electrophoresis to assay the degradation of dicamba *in vivo* and *in vitro*. These methods have allowed us to confirm that the first step in dicamba degradation is the production of 3,6-dichlorosalicylate (3,6-DCSA), a compound with no herbicidal activity. As a first approach in developing methods for identifying and cloning the dicamba demethylase gene that is responsible for the conversion of dicamba to 3,6-DCSA, we have generated mutants of strain DI-6 which have lost the ability to grow on dicamba as a sole carbon source, but retain the ability to grow on 3,6-DCSA. Two libraries of both genomic and plasmid DNAs from DI-6 have been created in vectors which allow conjugative transfer from the *E. coli* host strain, S17-1, to DI-6 or other Pseudomonas strains which lack the ability to degrade dicamba. Preliminary experiments suggest that it may be possible to recover the dicamba demethylase gene(s) by genomic complementation of DI-6 mutants or other Pseudomonas strains. Recovery of the demethylase gene(s) will allow modification of the gene for expression in plants and evaluation of herbicide tolerance.

X1-128 REGULATION OF THE TOMATO *hmg2* PROMOTER IN RESPONSE TO PATHOGEN ATTACK IN TRANSGENIC PLANTS. Deborah L. Weissenborn, Xueshu Yu, David N. Radin, Jonathan D. Eisenback, and Carole L. Cramer. CropTech Development Corporation, Blacksburg, VA 24060 and Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Application of molecular technologies has yielded significant new information on mechanisms involved in pathogen recognition, signal transduction, and defense gene activation, and has provided novel strategies for engineering enhanced disease resistance. We are using these approaches to analyze regulation of HMG CoA reductase, a key enzyme mediating the production of terpenoid defense compounds. HMG CoA reductase is encoded by four genes in tomato; the isogene *hmg2* has been most strongly identified with defense reponse. In order to monitor *hmg2*-specific gene expression, DNA constructs fusing the *hmg2* promoter to the GUS reporter gene have been used to transform tobacco and tomato. In these transgenic plants, *hmg2*:GUS expression is induced by wounding and bacterial, fungal, or viral attack. Interestingly, *hmg2* is also highly expressed in roots of plants inoculated with root-knot nematodes (*Meloidogyne incognita* and *M. hapla*). In these susceptible interactions, initial *hmg2*:GUS expression is associated with the onset of feeding. By seven days post-inoculation, *hmg2*:GUS expression is evident throughout the developing root galls. In addition to localized wound- and pathogen-incuded expression, *hmg2* is also expressed in hypocotyl tissues, trichomes, and pollen of unstressed plants.

X1-129 ISOLATION OF *ARABIDOPSIS* MUTANTS AFFECTED IN THE ESTABLISHMENT OF BIOLOGICALLY INDUCED SYSTEMIC ACQUIRED RESISTANCE. Robin K. Cameron, Richard A. Dixon and Christopher J. Lamb*. Plant Biology Division, Noble Foundation, P.O. Box 2180, Ardmore, Oklahoma 73402. *Plant Biology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, LaJolla, California 92037.

Throughout this century, it has been demonstrated that a primary or immunizing infection with a necrotizing pathogen can render plants resistant to subsequent infections by other pathogens. This phenomenon known as Systemic Acquired Resistance (SAR) is associated with the expression of SAR genes (PR proteins-glucanase, chitinase) and increased levels of salicylic acid which has recently been shown to be necessary for the establishment of SAR in tobacco. However, little is known about the sequence of events which occur between the initial immunizing infection and the onset of SAR. In order to understand the events which lead to and maintain SAR we have developed an *Arabidopsis*-SAR system. Using the pathosystem of Staskawicz and Ausubel, plants were immunized with avirulent *Pseudomonas syringae* pv *tomato (Pst)* and when challenged with virulent Pst (2 days later), remained symptomless, that is resistant to the infection. Moreover, this resistance was shown to be systemic and protected the plants against other bacterial pathogens. The *Arabidopsis rps-201C* mutant's(suseptible to *Pst* DC3000*avrRpt2,* Staskawicz) limited ability to establish SAR will also be presented. We are in the process of using this *Arabidopsis*-SAR system to screen for mutants affected in the ability to produce SAR. To date ~10,000 T-DNA tagged (Feldman, Ohio State) plants have been screened and 200 SAR putants have been selected. Of these 200 putant plants, a number have passed two additional SAR screens (in the M2 and M3 generations). The initial characterization of these mutants will be reported.

X1-130 A NOVEL LONG DISTANCE SIGNAL IS REQUIRED FOR SYSTEMIC ACQUIRED RESISTANCE, Leslie Friedrich*, Bernard Vernooij*, Alison Morse*, Roland Reist+, Rachida Kolditz-Jawhar+, Eric Ward*, Scott Uknes*, Helmut Kessmann+ and John Ryals*, *Ciba-Geigy Biotechnology, P.O. Box 12257, Research Triangle Park, N.C. 27709, +Ciba-Geigy Ltd., CH-4002, Basle, Switzerland

Infection of plants by necrotizing pathogens induces systemic resistance to subsequent pathogen infection. This systemic acquired resistance (SAR) is thought to be triggered by a vascular-mobile signal that moves systemically from the site of infection. Salicylic acid (SA) has been shown to accumulate in infected leaves and its exogenous application can induce resistance and SAR-related gene expression in various plant systems. To determine if the mobile SAR signal is SA, we carried out grafting experiments using transgenic plants that express a bacterial SA-degrading enzyme. Transgenic tobacco rootstocks, although unable to accumulate SA, are fully capable of delivering a signal that renders non-transgenic scions resistant to further pathogen infection. This result indicates that a translocated signal other than SA is responsible for triggering SAR.

X1-131 METALLOTHIONEIN LIKE GENES IN HIGHER PLANTS, Toru Fujiwara, Keishiro Takahashi, Ichiro Kawashima, Tatsuya Horiguchi, Eiichi Akahoshi and Mitsuo Chino, Department of Agricultural Chemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Organisms including mammals and fungi carry genes encoding proteins responsible for heavy metal detoxification called metallothionein. In case of higher plants, peptides called phytochelatins, products of an enzymatic reaction but not of translation of products, are induced and involvement of these peptides in detoxification of heavy metals are suggested. On the other hand, several groups including ours cloned genes from higher plants that are similar to metallothionins. We cloned homologous genes from soybean and *Arabidopsis* using oligomers corresponding to conserved regions of methallothionein genes as probes. Positive cDNA and genomic clones were identified and their sequences determined. The genes contain regions rich in cysteine which is characteristic to metallothionein. The upstream regulatory region of the clone from *Arabidopsis* contain sequences which is identified as a responsible *cis* acting element for induction by cupper treatment. The gene cloned from *Arabidopsis* is a single copy gene. Analysis using transgenic Arabidopsis carrying the upstream regulatory sequences::*gusA* fusion gene suggested that genes expressed in older part of plants and its expression is induced by heavy metal treatment in the tip of roots. Possible involvement of these genes in heavy metal detoxification will be discussed.

X1-132 **ISOLATION AND CHARACTERIZATION OF *ARABIDOPSIS THALIANA* MUTANTS WITH ALTERED RESPONSES TO AUXIN TRANSPORT INHIBITORS**

Christine Garbers, Alison DeLong, Carl R. Simmons and Dieter Söll, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520-8114.

Auxin transport is believed to play a role in mediating tropic responses and to regulate developmental processes. Auxin transport inhibitors like naphthylphthalamic acid (NPA) inhibit gravitropic curvature of roots and shoots as well as normal root elongation. However very little is known about auxin transport at the molecular level. From a collection of T-DNA mutagenized *Arabidopsis* lines, we have isolated four independent mutants resistant to NPA by screening for seedling growth in the presence of NPA. One mutant shows five-fold increased resistance to NPA in a root curling assay. Three other mutants show NPA-dependent root curling in the same assay. The latter mutant class also shows NPA resistance in the root waving assay (for assay see Okada and Shimura *Science* 250:274-276). However, hypocotyl lengths of these mutants are greatly reduced by high concentrations of NPA. Analyses of the T-DNA insertions and their cosegregation with the mutant phenotypes are in progress.

X1-133 **ANALYSIS OF GENE ACTIVITY IN NEMATODE-INDUCED FEEDING STRUCTURES, USING PROMOTER-GUS FUSIONS AND PROMOTER TAGGING.**

Oscar J.M. Goddijn, Frederique M. Vanderlee, Joke C. Klap and Peter C. Sijmons. MOGEN International nv, Leiden, The Netherlands.

The formation of nematode feeding structures in plant roots after infection with cyst or root-knot nematodes is accompanied by severe changes in gene regulation. To monitor these changes at the molecular level, a variety of promoter-*gus*A fusions were introduced into *Arabidopsis* and analysed for GUS activity after infection with either *Heterodera schachtii* or *Meloidogyne incognita*. Strikingly, promoters which are highly active in root cells, such as those of the CaMV35S, *rol*A-D and *nos* genes, are down-regulated inside the feeding cells of both root-knot and cyst nematodes. A common feature of most of these promoter sequences is the presence of a binding site for the transcription factor ASF1. Currently, we are investigating if this factor is involved in regulating gene-activity in nematode feeding structures.

Furthermore, a large number of transgenic *Arabidopsis* plants were generated using *Agrobacterium tumefaciens* harbouring a binary vector with a promoterless GUS gene located at the right border sequence. Using this approach we were able to tag regulatory sequences that give rise to high GUS activity inside the nematode feeding structures. After isolation with inverted PCR, these tagged sequences are now subject to a more detailed analysis and are being reintroduced into *Arabidopsis* . The latest results from both approaches will be summarized on the poster.

X1-134 MOLECULAR BIOLOGY OF CHLOROPHYLL BIOSYNTHESIS AND ITS MANIPULATION BY EXPRESSION OF ANTISENSE RNA, Bernhard Grimm, Hans-Peter Mock & Livio Trainotti, Institut für Pflanzengenetik und Kulturpflanzenforschung, 06466 Gatersleben, Germany

The ability to synthesize tetrapyrroles such as haem and chlorophyll is fundamental to all forms of life. In both animals and plants these complex compounds are assembled from eight molecules of 5-amino-levulinate (ALA). Light mainly exerts its regulatory effect by controlling two different steps in the chlorophyll biosynthesis: The rate limiting step of ALA formation and the conversion of protochlorophyllide. We have cloned and sequenced full-length cDNA clones encoding enzymes of all three steps of ALA-syntehsis and various steps of the porphyrin synthesis. Although latter enzymes required for the catalytic conversion of ALA to Mg-protoporphyrin are most likely present in non-rate limiting amounts in higher plants, their expression shows also response to light.

Gene expression of these enzymes was inactivated in transgenic plants by introducing their cDNA sequences in reverse orientation behind the CaMV S35 promoter. The expression of these antisense genes allows to study the indispensability of each enzyme and the significance of its function in pigment formation as well as control in the chlorophyll synthesizing pathway. Transgenic plants containing each one of these antisense genes showed differences in chlorophyll reduction and variegation pattern. Chlorotic phenotypes were observed if transgenic plants were transformed with antisense genes for enzymes of the ALA-synthesizing pathway. Antisense gene expression for enzymatic steps in porphyrin synthesis results in necrotic or pale green plants. Latter effects primarily base on the accumulation of porphyrin intermediates or interference of the regulated expression of other pigment synthesizing enzymes. Consequences of inactivated chlorophyll synthesis on the formation of the photosynthesis apparatus and the chloroplast biogenesis is currently investigated.

X1-135 IDENTIFICATION OF A COTTON SPECIFIC PATHOGEN RECOGNITION PROTEIN; C. Lan[1], C.-S. Chen[1], T.-Y. Feng[2], and Y.-S. Zeng[3]; [1]Department of Biology, Fu Jen Catholic University, Hsinchuang, Taipei 242, Taiwan, ROC; [2]Institute of Botany, Academia Sinica, Nankang, Taipei 115, Taiwan, ROC; [3]Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031, PRC

A protein R isolated from the *Fusarium* wilt-resistant cotton cultivars (*Gossipium hirsutum*) was able to associate with spores of *Fusarium oxysporum* f. sp. *vasinfectum* and to inhibit the spore germination in a race-specific fashion. The R protein from cotton plants inhibited spore germination of Fov, but not that of tomato wilt fungus, *F. oxysporum* f. sp. *lycopersici*. Because all the plants used were from a pathogen-free cotton field, the R protein may be constitutively present, but not due to the induction of pathogen challenge. Together with some other indirect evidences we are tempting to suggest that this protein is a recognition molecule in the very first step of host-pathogen contact.

Our data indicated that the R protein might be composed of several monomers and be a glycoprotein. R protein was able to bind to Con-A sepharose and be eluted by mannose-NaCl solution. R proteins that bound to spores was also effectively dissociated by 0.5M mannose solution. The molecular weight of the supposed monomer was about 31KD. The optimal pH value for the inhibitory action of the R protein was 6.5 to 7.0. The R protein was heat labile. While the R protein was incubated at 37°C for 10 min, the bioactivity was completely abolished. Several kinds of divalent cations, such as Co^{++}, Ni^{++}, and Mn^{++}, could enhance the bioactivity of the R proteins. The protein extracts from the wilt-susceptible cotton cultivars contained R-like protein S that could associate with *Fusarium* spores, but had no inhibitory effect.

X1-136 REGULATORY GENES CONTROLLING FLOWER PIGMENTATION IN PETUNIA. Francesca Quattrocchio, John Wing, Joseph Mol and Ronald Koes, Dept. of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands.

In petunia at least four regulatory genes (*an1*, *an2*, *an4*, and *an11*) control pigmentation in different parts of the plant. By using a combination of RNA gel blot analysis, transcription run-on assays, and transient expression assays we showed that these regulatory genes control the transcription of a subset of structural genes from the anthocyanin pathway. $an1^-$, $an2^-$ and $an11^-$ mutants could be complemented by transient expression of the maize regulatory genes *Lc* and *C1* delivered together via particle bombardment. The same combination of *Lc* and *C1* induces pigment accumulation in young leaves, indicating that *Lc* and *C1* are both necessary and sufficient to produce pigmentation in petunia cells that normally do not express pigmentation genes. This suggests that the regulatory proteins in both species are functionally similar. Furthermore, in transient expression assay, the *Lc* gene alone can complement an $an2^-$ mutant indicating that *an2* encoces or controls a transcriptional factor similar to Lc. We have cloned a petunia cDNA (*jaf 13*) encoding a helix-loop-helix transcriptional factor with high homology to Lc and its snapdragon homologue delila. *jaf13* is expressed in pigmented flower tissues of wild-type petunia, but is completely down regulated in the $an2^-$ mutant used in the complementation experiments. A further characterization of the jaf 13 gene will be presented.

X1-137 ELUCIDATION OF THE ETHYLENE-ACTIVATED TRANSDUCTION PATHWAY, Vered Raz and Robert Fluhr, Department of Plant Genetics, The Weizmann Institute of Science, Rehovot, ISRAEL.

Ethylene plays a regulatory role in different physiological and developmental processes in plants. In the defense response ethylene induces the accumulation of a subset of PR (pathogenesis related) proteins in leaves. Two pathways for the induction of PR proteins have been identified in tobacco plants. The two pathways differ in their dependency for ethylene and calcium for the induction of PR proteins. The ethylene signaling pathway is transduced via protein phosphorylation events. The phosphorylation events and the expression of PR proteins induced by the ethylene dependent pathway are down regulated by the kinase inhibitors H-7 and K252a. In addition, the ethylene signaling pathway is up regulated by okadaic acid, a phosphatase *type-1 and type-2A* inhibitor. The expression of PR proteins in the ethylene independent pathway is not regulated by phosphorylation events involved in the ethylene dependent pathway. Our results point to an essential role for calcium and protein phosphorylation events in the ethylene signaling pathway in tobacco leaves. Using the reverse transcriptase-PCR technique we have isolated a novel protein kinase whose expression level increased following ethylene treatment. Immonoprecipitation experiments reveal perturbation in the *in vitro* kinase activity and the *in vivo* phosphorylation state of this kinase in an ethylene dependent manner. The relevance of these changes to the ethylene signaling pathway is being studied.

X1-138 THE RESPONSE OF ARABIDOPSIS THALIANA ECOTYPES TO TOSPOVIRUS INFECTION, Joachim Schiemann and Anja Matzk, Federal Biological Research Centre for Agriculture and Forestry, Institute for Biochemistry and Plant Virology, Messeweg 11/12, D - 38104 Braunschweig

Although several plant resistance genes are agronomically important and led to the production of more resistant cultivars, the molecular mechanisms underlaying these resistances are not known. There is a growing interest in exploring variation in virus resistance and symptom formation in Arabidopsis thaliana bacause the genetics and molecular biology of this small crucifere allow the identification and cloning of genes responsible for various phenotypes.

Important prerequisites for a large ecotype and mutant screening are high reproducibility of virus infection and easy detection of different symptoms caused by the virus. Among a wide range of single-stranded RNA viruses we found tospoviruses (Tomato Spotted Wilt Virus, Impatiens Necrotic Spot Virus) to fulfil these prerequisites. Both viruses cause highly reproducible stunting, leaf crinkling, and partially leaf-mottling, while INSV causes death in a few ecotypes.

54 ecotypes were inoculated with TSWV and screened for differences in symptom expression. No virus symptoms were observed with 12 ecotypes. All other ecotypes showed symptoms in 5 to 80 percent of the inoculated plants. The presence of TSWV in plants was proved by means of TAS-ELISA using monoclonal antibodies against the nucleocapsid protein and an envelope glycoprotein of TSWV. The virus was detected in all plants expressing symptoms and in a few symptomless plants. No TSWV was detected in 8 ecotypes. A genetic analysis of the symptomless ecotypes has been started.

X1-139 ASCORBATE PEROXIDASE EXPRESSION IN TOBACCO IS REGULATED BY H_2O_2, Ashima Sen Gupta, Robert P. Webb, A. Scott Holaday and Randy D. Allen. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131

In addition to elevated levels of Cu/Zn superoxide dismutase (SOD), transgenic tobacco plants that express a chimeric gene for chloroplastic Cu/Zn SOD (SOD^+) have elevated, constitutive levels of ascorbate peroxidase (APX) and Fe and Mn SOD isoforms. This coordinate expression of SOD and APX isoforms in transgenic SOD^+ plants provides a SOD to APX ratio that is equivalent to that in wildtype plants. We are interested in identifying the signal(s) that are responsible for the induction of endogenous genes in response to SOD transgene expression. Since the product of superoxide dismutation is H_2O_2, solutions of various H_2O_2 concentration were tested to determine if they could affect APX expression in transgenic SOD^+ and wildtype tobacco plants. Induction of APX activity in wildtype plants occurred over a narrow range of H_2O_2 concentrations with peak activity at 40 mM H_2O_2. At this concentration, induction was detectable within 60 min. of treatment and reached a maximum within 120 min. No increase in SOD activity, catalase activity or glutathione reductase activity was detected in H_2O_2 treated leaf samples. Treatment of transgenic SOD^+ plants with H_2O_2 did not result in any additional induction of APX activity. These results clearly indicate that APX expression is responsive to H_2O_2. The lack of response in transgenic SOD^+ plants indicates that the H_2O_2-APX induction system of these plants may be saturated. These results support the hypothesis that the constitutive increase in APX in SOD over-expressing plants is mediated by a putative increase in H_2O_2 production due to the high SOD activity. However, they do not explain the constitutive increase in Mn and Fe SOD that also occur in these plants.

X1-140 OXIDATIVE BURST IN PLANT CELLS: ACTIVATION AND FUNCTION

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Treatment of soybean cell cultures with a variety of elicitors or plant pathogenic bacteria causes a rapid insolubilization of pre-existing proline-rich proteins (PRP) in the plant cell wall. This insolubilization precedes transcription-dependent defence responses such as the synthesis of phytoalexin antibiotics or lytic enzymes. The cross-linking of PRP is mediated by a H_2O_2-dependent peroxidase reaction leading rapidly to a reinforcement of the plant cell wall. Overexpression of a soybean PRP-gene under the control of the CaMV 35S promoter results in a high expression of the transgene in *Arabidopsis thaliana* and tobacco. The resistance of these transgenic plants towards pathogenic microorganism is currently under study.

In order to dissect the activation process of the plasma membrane NAD(P)H-oxidase we are studying components of the signal transduction pathway for oxidase activation and biochemical characterization of the oxidase prior to molecular cloning. This should allow new insights into one of the most rapid plant defence responses so far.

X1-141 DISSECTION OF SIGNAL TRANSDUCTION LEADING TO DISEASE RESISTANCE IN ARABIDOPSIS

Kris Weymann, Danielle Chandler, Terrence Delaney, Eric Ward, Scott Uknes and John Ryals, Department of Molecular Genetics, Ciba Agricultural Biotechnology, 3054 Cornwallis Road, Research Triangle Park, NC 27709

Infection of plants with a necrotizing pathogen can result in enhanced systemic resistance against subsequent pathogen infection. This physiological immunity, known as systemic acquired resistance (SAR), has been well characterized in tobacco, cucumber, and *Arabidopsis*. Although salicylic acid is known to be involved in the signal transduction pathway leading to the onset of SAR gene expression and SAR, the pathway in general is not well understood. In order to dissect the SAR signal transduction pathway, mutagenized *Arabidopsis* plants were screened by analysis on RNA gel blots for constitutive and non-inducible expression of SAR genes. We have identified two broad classes of mutants: constitutive immunity, (*cim*) and no immunity (*nim*) mutants. Some of the *cim* lines are disease lesion mimics. Phenotypic and molecular characterization of mutant lines will be presented.

X1-142 AUXIN-RESPONSIVE PROMOTER ELEMENTS,

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Auxin, a class of plant hormones, which affects a wide range of growth and developmental processes, has a rapid and selective effect on gene expression. In order to study the molecular mechanisms by which auxin controls plant development a number of auxin responsive genes have been isolated and characterized. However, comparison of the promoter regions of these diverse genes has not revealed any significantly similar DNA motifs which might be involved in auxin responsive transcriptional activation. To identify auxin response DNA regulatory elements and their functional interactions, we initially conducted a detailed analysis of the bidirectional mannopine synthase promoter from *Agrobacterium* T DNA. This promoter controls an intricate pattern of tissue and organ specific expression in transgenic plants and is auxin-inducible. We cloned deletions and rearrangements of the *mas* promoter upstream of a β-glucuronidase gene and tested these constructs in transgenic plants for their pattern of expression and auxin response. This analysis defined a number of auxin responsive elements and demonstrated that both the combination and orientation of elements influences transcriptional activation. A 35 bp auxin response element (AuxRE-1) was sufficient to control an intricate pattern of expression in developing flowers and seedlings. This pattern of expression was very similar to that of the soybean GH3 auxin responsive gene. Comparison of the *mas* and GH3 promoters identified two DNA sequences, ACTTTTG and CGCAAxxxxTGACGTAA, as putative auxin response motifs. The characterization of AuxRE sequences and the *trans*-acting factors which bind to these promoter sites will provide a way of identifying the molecular mechanisms by which auxin mediates developmental processes.

Yield Improvements Through Genetic Analysis and Heterosis; Fruit Ripening and the Production of High Value Products in Plants

X1-200 COORDINATE EXPRESSION OF OXIDATIVE STRESS PROTECTIVE ENZYMES IN TRANSGENIC PLANTS THAT OVER-EXPRESS CHLOROPLASTIC CU/ZN SUPEROXIDE DISMUTASE, Randy D. Allen, Ashima Sen Gupta, Robert P. Webb and A. Scott Holaday. Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131

Introduction, into tobacco plants, of a chimeric gene that encodes chloroplast localized Cu/Zn superoxide dismutase (SOD) from pea results in significant increases in their resistance to oxidative stress produced by paraquat treatment and to photoinhibition caused by low temperature and high light intensity[1]. These plants (SOD^+) exhibit a three-fold increase in total SOD activity. Analyses of endogenous enzymes of the superoxide and H_2O_2 scavenging pathway of these plants showed that levels of ascorbate peroxidase (APX) activity, in both cytosolic and chloroplastic compartments, were more than three-fold higher in SOD^+ plants than in non-expressing control plants (SOD^-). In addition, activities of chloroplastic Fe SOD and mitochondrial Mn SOD were also induced by approximately three-fold in SOD^+ plants. However, activities of dehydroascorbate reductase, monodehydroascorbate reductase and glutathione reductase were virtually identical in SOD^+ and SOD^- plants. Large increases in the levels of APX and Mn SOD mRNAs, were also seen in leaves of SOD^+ plants but Fe SOD mRNA levels were nearly identical in SOD^+ and SOD^- plants. These results indicate that expression of various SOD and APX isoenzymes are coordinately regulated in tobacco plants. The regulatory systems that control these genes are able to maintain the balanced expression of SOD and APX isoforms in transgenic plants that over-express chloroplastic Cu/Zn SOD, apparently by using both transcriptional and post-transcriptional mechanisms.

[1]Sen Gupta et al. 1993. PNAS 90: 1629-1633.

X1-201 UNDERSTANDING THE ROLES OF HEAT SHOCK PROTEINS IN ACQUIRED THERMOTOLERANCE OF WHEAT PLANTS THROUGH MOLECULAR GENETIC ANALYSIS, Chandrashekhar P. Joshi and Henry T. Nguyen, Department of Agronomy, Horticulture and Entomology, Texas Tech University, Lubbock, Texas 79409.

Temperate crop plants such as wheat are vulnerable to heat stress during their growth periods that severely affect their grain yield and quality. A mild heat shock makes these plants resistant to a more severe heat stress and this phenomenon of acquired thermotolerance coincides with the massive synthesis of heat shock proteins (HSPs). The most noteworthy is low molecular weight (LMW) HSPs which are encoded by multigene families and are most abundant in plants. No protective functions have yet been assigned to this group of structurally conserved HSPs. Our main objective is to establish the genetic relationships between expression of LMW HSPs and thermotolerance in wheat. We have employed a divergent selection of F_2 plants derived from a single cross between thermotolerant 'Mustang' and thermosusceptible 'Sturdy'. A substantial genetic variability in acquired thermotolerance has been observed among 250 F_2 plants using triphenyl tetrazolium chloride (TTC) cell viability assay. Advanced generations (F_6) differing significantly in thermotolerance are being produced on the basis of TTC assays from this population. The *in vitro* translation of poly (A)+ RNAs from heat shocked seedlings revealed several quantitative and qualitative differences in LMW HSPs between 'Mustang' and 'Sturdy'. Several LMW HSPs are uniquely present in 'Mustang' which are absent in 'sturdy'. The *in vitro* hybrid selection/ translation using three cDNAs encoding different classes of LMW HSPs indicated that HSP17, HSP18 and HSP26 family in wheat consists of at least 10, 14 and 2 members, respectively. We have isolated representative cDNAs encoding different members of LMW HSPs from these families. This will assist in isolation and identification of gene-specific probes that are instrumental in establishing association between a specific LMW HSP and thermotolerance traits in F_6 plants using northern blot hybridizations. In order to understand the relationship between quantitative variations of HSP accumulation and thermotolerance, we have also produced polyclonal antibodies against three classes of LMW HSPs. The studies with the kinetics of LMW HSP mRNAs and HSP accumulation during heat stress regime indicate that heat shock response in 'Mustang' is faster as compared to 'Sturdy'. These data are expected to provide valuable insights into the mechanisms of acquired thermotolerance in crop plants in relation to the production of LMW HSPs.

X1-202 THE TRANSFORMATION OF TOBACCO AND PETUNIA WITH SPINACH TEMPERATURE STRESS GENES, Claudia Kaye and Charles Guy, Environmental Horticulture, University of Florida, Gainesville, Fl. 32608

Cold acclimation and freezing tolerance in plants is associated with altered gene expression and the synthesis of several distinct high molecular weight proteins. Many of these proteins are also linked to water-stress response. cDNAs for three of the genes that are up-regulated during cold-acclimation have been isolated from spinach. *CAP 85* is related to the Group 2 LEAs; *CAP 160* has properties similar to those proteins found in the LEA family, but it does not show any amino acid sequence similarity with either the LEAs or any known antifreeze or ice nucleation proteins; *CAP 79* is a member of the HSP70 family with sequence similarity suggesting that it is the molecular chaperon, BiP (immunoglobulin heavy chain binding protein). Spinach BiP mRNA is up-regulated in response to cold temperatures but is not expressed during water stress.

The coding sequence of each of these genes either in the sense (*CAP 85* and *CAP 160*) or in both the sense and antisense (*CAP 79*) has been placed under the control of the nearly constitutive cauliflower mosaic virus promoter. These heterologous genes have been transferred by *Agrobacterium* mediated transformation into tobacco and Petunia. Tobacco is extremely sensitive to freezing, being killed as soon as ice forms in the tissue. Petunia can tolerate some freezing but has no capacity to increase tolerance to freezing during cold acclimation. The transgenic plants will be analyzed for the expression of the introduced genes.

X1-203 GENETIC MANIPULATION OF LIGNIN BIOSYNTHESIS, Weiting Ni, Murray Ballance and Richard A. Dixon Plant Biology Division, The Samuel Roberts Noble Foundation, P.O. Box 2180, Ardmore, OK 73402.

Lignin is a major polymer of secondarily thickened plant vascular tissue and fibers. Besides providing mechanical support, lignin has significant protective functions in plants. Due to its chemical durability the content of lignin is negatively correlated with forage digestibility. Caffeic acid O-methyltransferase (COMT), an early enzyme in lignin biosynthesis, methylates caffeic acid to form ferulic acid, a precursor of the conyferyl and sinapyl alcohol monomers of dicotyledonous angiosperm lignin. We have introduced antisense constructs of the alfalfa COMT gene under the control of the CaMV 35S promotor into tobacco and alfalfa plants. Some of the regenerated tobacco plants exhibited significant reduction in lignin content, particularly in the younger parts of the stems but the monomer composition of the lignin in these plants was apparently unchanged. The tobacco plants with reduced lignin showed no apparent phenotypical difference from control plants. In preliminary studies, transgenic alfalfa plants did not exhibit a significant change in lignin content. More detailed studies on these transgenic alfalfa plants are in progress. A possible alternative pathway of lignin biosynthesis in alfalfa may exist which bypasses COMT by methylating caffeoyl CoA to produce feruloyl CoA. We are currently testing this possibility.

X1-204 CHARACTERIZATION OF A PHYTOCHROME/MALE STERILE/ PHOTOPERIOD MUTANT IN RICE. Oard, J. H., Department of Agronomy, Louisiana State University, Baton Rouge, LA 70803

Hypocotyl mutants in *Arabidopsis*, tomato, and cucumber have provided valuable insights into the role(s) of phytochrome in gene regulation. The EMS-derived rice mutant ST1 displayed 9-18% and 0-18% pollen fertility under long days (14-15 hr during pollen development) in California and Louisiana field plots, respectively. Fertility increased five fold to 90% when tillers of field-grown material were transplanted and grown under a 12-hr daylength in a growth chamber. Pollen fertility was reduced ten fold to 9% when tillers from the growth chamber plants were transplanted and grown under summer field conditions. These results show that pollen fertility in ST1 can be altered by different photoperiod treatments. Genetic analysis in crosses with normal, fertile cultivars indicated pollen fertility in ST1 was conditioned by two, recessive nuclear genes. Under a 10-hr daylength during panicle initiation, fertility was high (94%) after a 10-day cycle of 5 min pulses of red light given 3 hr after the beginning of the dark period. In contrast a treatment of red followed by far-red light resulted in low fertility of 3%. High levels of fertility at 90% were restored by a red/far-red/red light regime. Northern analysis of ST1 exposed to the red/far-red treatments is currently underway. Taken together the data indicate that the ST1 mutant can serve as a useful tool to examine the potential role of phytochrome in pollen fertility and photoperiod responses.

X1-205 ANALYSIS OF TRANSGENE EXPRESSION DRIVEN BY THE MAIZE ZM13 PROMOTER IN TRANSGENIC MAIZE, James C. Register III, Philip J. Bell[1], Douglas A. Hamilton[2], Nicole S. Higgs, Joseph P. Mascarhenas[2], and Andrew J. Greenland[1], ICI Seeds, Slater, IA 50244, [1]Zeneca Seeds, Jealott's Hill Research Station, Bracknell, Berkshire RG12 6EY England, [2]Department of Biological Sciences, State University of New York at Albany, Albany, NY 12222.

The maize Zm13 gene is expressed in a pollen-specific manner (Hamilton *et al.* [1989] Sex Plant Reprod 2, 208.) and its 5' flanking region directs pollen-specific expression of a marker gene in transgenic tobacco (Guerrero *et al.* [1990] Mol Gen Genet 224, 161.). We have produced and analyzed transgenic maize plants containing a Zm13 promoter-*uidA* transcriptional fusion. These plants had high levels of GUS activity in mature pollen, but not in leaves. Three generations of transformants have been analyzed for one line of transgenic plants. Detailed analysis of ZM13 promoter regulation in these plants is underway.

X1-206 EXPRESSION OF CHLOROPLAST LOCALIZED MN SOD IN TRANSGENIC COTTON, Norma L. Trolinder and Randy D. Allen, USDA-ARS, Route 3, Box 215, Lubbock TX 79401 and Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131

Transgenic plants that over-express superoxide dismutase (SOD) have been developed in several laboratories. Although there are exceptions, these plants have been shown to have increased protection from oxidative stress caused directly by treatment with paraquat or indirectly through stress exposure. We have begun to test whether this approach is applicable for increasing stress tolerance in cotton. We used a chimeric gene developed by Dr. C. Bowler that contains coding sequences for the mature Mn SOD subunit from *Nicotiana plumbaginifolia* fused with a chloroplast transit peptide sequence from an *Arabidopsis thaliana* RUBISCO gene. This coding sequence is expressed under the control of a CaMV 35S promoter. The chimeric Chl-Mn SOD gene construct was transferred to cotton plants via an *Agrobacterium*-mediated transformation procedure. Regenerated transgenic cotton plants were analyzed for the expression of novel SOD isoforms using a non-denaturing polyacrylamide gel negative staining technique. A unique SOD isoform was identified in extracts of several transgenic SOD cotton plants that correlated with tobacco Mn SOD. Suspension cultures derived from these plants were analyzed for growth in media that contained elevated levels of NaCl or polyethylene glycol (PEG). The increase in dry weight in PEG containing cultures was approximately two fold higher for cultures from transgenic SOD plants than for control plants. However, growth in NaCl containing cultures was similar for transgenic SOD cells and for control cells. Further tests of the oxidative stress resistance of transgenic cotton plants that express chloroplastic Mn SOD are currently under way. These include characterization of their paraquat resistance, analysis of photosynthesis during and after exposure to high light intensity and low temperature and drought tolerance.

X1-207 FLORAL EXPRESSION OF THE *ARABIDOPSIS UBC6* GENE. Felicity Z. Watts, Neil Butt, Anthony L. Moore, School of Biological Sciences, University of Sussex, Falmer, Brighton, E. Sussex, U.K. BN1 9QG.

We have isolated an *Arabidopsis* gene (*UBC6*) encoding a member of the ubiquitin-conjugating protein family. The gene comprises six exons and five introns and encodes a protein of approx. 21 kDa. The predicted protein sequence has high levels of sequence identity with the wheat UBC4 protein. *UBC6* promoter-*GUS* gene fusions have been constructed in order to investigate the expression of the *UBC6* gene. GUS activity is observed in anthers (but not pollen) and in developing embryos after pollination. Antisense constructs have now been made and are being used to identify a potential role for the UBC6 protein, e.g. to determine whether the protein is involved in senescence, response to stress or in seed viability.

X1-208 REDUCED ETHYLENE SYNTHESIS AND SUSPENDED FRUIT RIPENING IN TRANSGENIC TOMATOES EXPRESSING S-ADENOSYLMETHIONINE HYDROLASE, Richard K. Bestwick, Xin Good, Wendy Wagoner, Dan Langhoff, Jill Kellogg and James Stamp, Agritope, Inc., 8505 S.W. Creekside Place, Beaverton, OR 97005-7108

We have utilized a gene from bacteriophage T3 that encodes the enzyme S-adenosylmethionine hydrolase (SAMase) to generate transgenic tomato plants that produce fruit with a reduced capacity to synthesize ethylene, delayed ripening and extended shelf-life. S-adenosylmethionine (SAM) is the metabolic precursor of 1-aminocyclopropane-1-carboxylic acid, the proximal precursor to ethylene. SAMase catalyzes the conversion of SAM to methylthioadenosine and homoserine. To restrict the presence of SAMase to ripening fruit, the promoter from the E8 gene of tomato was used to regulate SAMase gene expression. Transgenic tomato plants containing the -1100 E8 promoter bore fruit that expressed SAMase during the breaker and orange stage of fruit ripening but ceased expression when fully ripened. Plants containing the -2300 E8 promoter expressed SAMase at four-fold higher levels during the post-breaker phases of fruit ripening and had a substantially reduced capacity to synthesize ethylene. Several transgenic lines bore fruit that did not ripen past the orange stage of fruit ripening. These fruit do not soften and have been stored at room temperature for three to six months. Second generation plants homozygous for the SAMase gene have undergone extensive field trial evaluations. Field grown fruit demonstrated reduced ethylene synthesis and suspended ripening which correlated to the stage at which the fruit were harvested. Firmness was measured for six weeks postharvest and shown to be twice that of controls. Fruit from these field trials demonstrated excellent postharvest storage characteristics.

X1-209 FRUIT RIPENING REGULATED LOW MOLECULAR WEIGHT HEAT-SHOCK PROTEINS of PEACH , Ann M. Callahan and Reuben A. Cohen, USDA-ARS, Appalachian Fruit Research Station, 45 Wiltshire Rd., Kearneysville, WV, 25430

The function of low molecular weight heat shock proteins (LMW HSP) which accumulate during stress is unknown. They are found in most organisms after heat stress and can accumulate to as much as 1% of the total proteins (Vierling, Ann. Rev. of Plant Phys. Plant Mol. Biol. 1991). We are interested in their role during fruit ripening. We would like to use this information to potentially enhance keeping qualities of peach fruit. To this end, a peach cDNA encoding a LMW HSP, Pphsp17.3, has been isolated that appears to be associated with fruit ripening. It was isolated from a ripe fruit cDNA library and sequenced (703 bases). It has 65-69% predicted amino acid identity with class I LMW HSPs. The cDNA detects an RNA that accumulates at the beginning of fruit softening. The RNA also accumulates in fruit and leaves after heat stress. A small family of HSPs was detected during fruit ripening and heat shock treatment of leaf and fruit using a pea antiserum to Pshsp18.1 (courtesy of Dr. E. Vierling). At least 10 proteins were detected of which some were fruit specific, some leaf specific, some heat shock specific and some ripening specific. Based on this data, we hypothesize that LMW HSPs are involved in fruit ripening.

X1-210 EXPRESSION OF ANTIBODY GENES AGAINST HUMAN HEPATITIS-B VIRUS IN TOBACCO PLANTS

Seok Yoon Kwon[1], Shin Je Kim[2], Hyo Jeong Hong[1], Hosull Lee[2], Chang H. Chung[2], and Kyung-Hee Paek[1], [1]Genetic Engineering Research Institute, KIST, Taejon 305-606 and [2]Research Institute, Korea Kumho, Yeocheon, 287-1

Chimeric kappa-chain and gamma-chain gene (pCKS2 and pCHS2) of antibody against human hepatitis-B virus were ligated to *Xba*I site of plant expression vector, pBKS-1. Each plasmid DNA containing the chimeric gene was then mobilized from *E. coli* to *Agrobacterium tumefaciens* strain LBA4404 by direct DNA uptake method. The chimeric antibody genes were introduced into tobacco by *Agrobacterium* Ti plasmid-mediated transformation. Tobacco leaf disks were cocultured with *Agrobacterium tumefaciens* LBA4404 carrying kappa-chain and gamma-chain gene. The putative transformants were selected on the kanamycin containing medium. Shoots that were induced on shoot induction medium were analysed by Northern blotting and Western blotting to confirm the expression of kappa-chain and gamma-chain gene, respectively. In the future, we are planning to produce assembled functional IgG antibody against human hepatitis-B virus by sexual crossing of transgenic tobacco plants expressing kappa-chain and gamma-chain gene.

X1-211 EXPRESSION OF CANDIDATE ORAL VACCINE ANTIGENS IN TRANSGENIC PLANTS,

Hugh S. Mason, Jian-Jian Shi, Tariq Haq, and Charles J. Arntzen, Institute of Biosciences and Technology, Texas A&M University, 2121 W. Holcombe Blvd., Houston, TX 77030

Transgenic plants present promising possibilities for the economical, large-scale production of high value proteins such as growth factors, antibodies and blood proteins. We are experimenting with the expression of candidate vaccine antigens in plants, with the goal of using edible plant tissues for production and delivery of oral vaccines. Using *Agrobacterium*-mediated transformation, we have transferred antigen genes to tobacco, lettuce, tomato, and potato plants. To date we have successfully produced transgenic plants that express hepatitis B surface antigen (HBsAg), Norwalk virus capsid protein (NVCP), and *E. coli* heat-labile enterotoxin B subunit (LTB). The HBsAg and NVCP assemble into sub-viral particulate structures with sedimentation coefficients of approximately 50*S* and 40*S*, respectively. After purification by immunoaffinity chromatography, the particles were visualized by negative staining and transmission electron microscopy as spherical objects with diameters of about 20 nm. The occurrence of these antigens as particles may be critical for their use as oral vaccines, because protein aggregates are likely to be more resistant to digestive processes and more immunogenic. The LTB isolated from transgenic plants bound to ganglioside, which indicates that it is competent to form characteristic pentamers and bind to gut mucosal cells. Immunogenicity testing of the plant-derived antigens in animals is in progress.

X1-212 PRODUCTION AND RECOVERY OF ADDED VALUE PROTEINS IN ALFALFA

Dennis Mathews, Sandra Austin-Phillips, Mark Shahan, Richard Burgess, Richard Straub[1], Richard Koegel[1,2], Richard Amasino[3], University of Wisconsin Biotechnology Center, [1]Agricultural Engineering Dept., [2]USDA Dairy Forage Research Center, [3]Biochemistry Dept., University of Wisconsin, Madison, WI 53706

We are investigating the feasibility of expressing and recovering added value proteins in alfalfa. Critical to the feasibility of this process is the achievement of high level accumulation and efficient, low cost recovery. As a model protein we are using the thermostable α-amylase from *Bacillus licheniformis* which is used commercially in the starch liquefication process. It has been demonstrated that active *B. licheniformis* α-amylase can be expressed in plants (Pen et al., Bio/Technology 10:292-296). We have made a series of α-amylase constructs in an attempt to compare the effect on overall accumulation and activity of targeting this protein to the following intracellular compartments: cytoplasm, endoplasmic reticulum, vacuole, apoplast. In an attempt to facilitate recovery the α-amylase has been modified by the addition of six histidine residues at the carboxy terminus. This allows for affinity purification using metal chelate resins. We are investigating schemes to purify α-amylase from crude homogenates of transgenic alfalfa using a simple two-step purification scheme involving heat treatment and affinity binding. Such a scheme could be generally applicable to other thermostable proteins expressed in plants.

X1-213 THE BARLEY LTP2 PROMOTER YIELDS HIGH LEVEL OF GUS EXPRESSION IN THE ALEURONE LAYER OF DEVELOPING GRAINS OF TRANSGENIC RICE.

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The aleurone layer of immature and germinating barley seeds contains two non specific Lipid Transfer Proteins of 10 and 7 kDa, encoded by the genes *Ltp1* and *Ltp2*, respectively. As demonstrated by *in situ* hybridization analysis, the Ltp2 gene is expressed exclusively in developing grains, from the onset of aleurone cell differentiation (9 dap) to about mid-maturation stage (30 dap). Based on the presence of a signal-peptide sequence in the putative Ltp2 protein, as well as evidence from other systems, we propose that LTP2 is involved in the synthesis of the lipid layer covering the outside of the cereal aleurone cells. Using the particle bombardment method, we demonstrate that the Ltp2 promoter is active in the aleurone layer of developing barley seeds. When transformed into rice, a 845 bp fragment of the Ltp2 promoter drives strong expression of the Gus-reporter exclusively in the aleurone layers of immature transgenic rice seeds. The promoter of Ltp2 contains consensus MYB and MYC protein binding sites similar to those found in the promoter of the maize aleurone specific gene Bz1. Electrophoretic mobility shift assays demonstrate that the putative MYB-binding sequence is specifically recognized by the chicken c-Myb protein, indicating a possibe role for this element in the regulation of aleurone cell gene expression. Functional analyis of the Ltp2 promoter to identify the element(s) involved in cereal aleurone specific gene expression is underway. The usefulness of the Ltp2 promoter in plant breeding strategies using molecular approaches will be discussed.

Systems for Gene Transfer to Crops; Modification of Seed Protein Composition

X1-300 RECOVERY OF TRANSGENIC *GLADIOLUS* FOLLOWING PARTICLE BOMBARDMENT,

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Our focus is the application of biotechnology to ornamental plants. As part of this program, we have established a transformation system for *Gladiolus*, a commercially-important monocotyledonous ornamental. Regenerable callus and cell suspension cultures of the commercial cultivar "Jenny Lee" have been established. Transient ß-glucuronidase (GUS) expression levels from the *uid*A gene were measured following particle bombardment of these cultures to optimize efficiency of DNA delivery . Surprisingly, we have found that gene promoters which are typically most efficient in dicots (*e.g.*, CaMV 35S) are more active in *Gladiolus* cell cultures than the monocot-derived promoters tested (*e.g.*, rice *Act1*) which usually express more highly in transformed monocots. Phosphinothricin (PPT)-resistant *Gladiolus* calli were recovered following bombardment with a phosphinothricin acetyl transferase (PAT)-expressing plasmid and selection on PPT-containing medium. Transformation was confirmed by the detection of GUS activity and a PAT gene-specific PCR product in transformed calli. After PPT-resistant calli were moved to a regeneration medium, large numbers of transgenic *Gladiolus* plantlets were obtained. The establishment of an efficient transformation system for *Gladiolus* will permit the introduction of new phenotypic traits with appeal to growers and consumers alike.

X1-301RECOVERY OF TRANSGENIC PEANUT *(ARACHIS HYPOGAEA L.)* PLANTS FROM ELITE CULTIVARS UTILZING *ACCELL®* TECHNOLOGY,

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Transgenic plants of Florunner and Florigiant, two of the most widely cultivated peanut cultivars, have been developed using the ACCELL® gene delivery method. Shoot meristems of mature embryonic axes were bombarded with gold beads coated with DNA encoding β-glucuronidase (*gus*), phosphinothricin acetyl transferase (*bar*), and tomato spotted wilt virus-nucleocapsid protein (*tswv-np*) genes. Transgenic shoots were identified by screening for GUS expression, and independent transformants were recovered from both cultivars. Molecular analysis of two of these transformants in R0 and R1 generations demonstrated the stable integration of the foreign genes into the plant genome. One transgenic plant had one to two copies of the genes integrated into the genome of its progeny, whereas the other had multiple copies. *Gus* and *bar* genes exhibited predictable segregation ratios in the R1 and R2 generations and were genetically linked. Integration of the *bar* gene conferred resistance to BASTA™, a wide-spectrum herbicide, applied at 500 ppm of active ingredient. Resistance of the transgenic plants to tomato spotted wilt virus is currently being tested under greenhouse conditions. The ACCELL® method is expected to be an effective transformation system for a wide variety of commercial cultivars.

X1-302 A NOVEL AND POTENTIALLY UNIVERSAL METHOD OF PLANT TRANSFORMATION WITHOUT THE REQUIREMENT OF TISSUE CULTURE. Gangamma Chowrira, Vani Akella, and Paul F. Lurquin, Department of Genetics and Cell Biology, Washington State University, Pullman, WA 99164.

The conventional methods of plant transformation (i.e., *Agrobacterium* mediated, biolistic gun) require tissue culture which is expensive, time consuming, labor intensive and can result in somaclonal variations. We describe a novel method of transforming plants which involves *in vivo* electroporation of intact nodal bud. Following electroporation, buds develop normally. Seeds originating from treated buds are then used to screen for stable transformants. Using this technique we have obtained stable transformation in peas, cowpea, and lentils. The reporter gene used for transformation was a modified *E.coli uidA* gene coding for beta-glucuronidase (GUS) containing a plant intron, under the control of the CaMV 35S promoter and the nos terminator. Transformed plants were identified by GUS activity and stable transformation was confirmed by Southern and Northern blot analysis in R_1 individuals. Transformation of soybean, *Arabidopsis*, and wheat is in progress.

X1-303 PRODUCTION OF TRANSGENIC WHEAT BY PARTICLE BOMBARDMENT USING METHOTREXATE AS A SELECTION AGENT, Lyle Crossland,Susan Jayne,Yin-Fu Chang,Janet Reed, John Nahory,Susan Armour*,Jeff Stein,Tina Grater,Dawn Miner,Leslie Walker**,Jim Wong***
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Fertile transgenic wheat plants have been obtained using a helium-powered particle gun to deliver DNA to friable, embryogenic callus cultures. Expression of the dihydrofolate reductase (dhfr) gene from E. coli allowed for the efficient selection of transformants on medium containing methotrexate. Protocols for the establishment of callus cultures, transformation, and plant regeneration, as well as data on transgene expression in progeny, will be presented.

X1-304 FACTORS INFLUENCING TRANSFORMATION FREQUENCY AND SINGLE COPY T-DNA INTEGRATIONS IN RAPESEED, Brigitte Damm, Theo C. Verwoerd, Peter J.M. van den Elzen and Jacob B. Bade, MOGEN, Einsteinweg 97, Leiden, The Netherlands
Various rapeseed transformation procedures have been documented using Agrobacterium tumefaciens and different types of explant sources. We performed a series of rapeseed transformation experiments using hypocotyl segments since this explant material has been reported being most suitable. We aimed at determining optimal experimental conditions enabling (a) the routine regeneration of large numbers of transgenic plants using a simple and rapid protocol, (b) the efficient use of four selectable marker genes, and (c) the obtention of transgenic plants with only one copy of T-DNA integrated in their genome. To this end, transformation experiments were performed using different binary vectors carrying either the nptII, the hpt, the pat or the als gene, all in combination with the gus-intron reporter gene. Length of preculture, concentration of the agrobacteria, length of incubation and cocultivation as well as preculture and cocultivation media were varied. GUS expression was monitored early after transformation and a tight correlation was found between the number of GUS expressing cells at cut ends of the explants and the number of regenerated stably transformed calli and shoots. Thereafter, the early screening of GUS expression could be used for rapid detection of further protocol improvements. It was also found that culturing the explants in liquid medium on a rotary shaker during the early steps of transformation, i.e. preculture and cocultivation, was a major improvement over existing protocols. Direct selection for the presence of all four selectable markers resulted in high frequencies of calli and shoots expressing the gus gene. We are now analyzing groups of transgenic plants at the molecular level in order to be able to determine the influence of experimental treatments on the number of T-DNA copy integration.

X1-305 TRANSFORMATION OF THE FORAGE GRASS CAUCASIAN BLUESTEM VIA BIOLISTIC BOMBARDMENT-MEDIATED DNA TRANSFER. C.I. Franklin, A.T. Trieu, J. Ponsamuel and D.V. Huhman
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The forage grass caucasian bluestem (*Bothriochloa caucasica* L.)is well adapted to the soil and weather conditions in the south central U.S., and it is an obligate apomict. Seeds of an obligate apomict contain embryos that are produced asexually from the maternal tissue. Improvement of an apomictic species through conventional breeding is very difficult, because it cannot be used as a female parent to produce a hybrid. Application of biotechnological techniques to the improvement of this forage species is practical and valuable. A major drawback of this forage species with respect to the nutritional quality is its low protein content. The nutritional value of caucasian bluestem (CBS) may be improved by introducing gene(s) coding for specific storage protein(s). As an initial step towards this goal, we have developed a transformation system for CBS via particle bombardment-mediated DNA transfer. We have optimized conditions for DNA delivery into CBS embryogenic callus using the plasmid pBARGUS. In transient GUS assays the plasmid pAct1-D, containing a rice actin promoter-intron-GUS fusion, produced at least twice the number of GUS expressing cells than pBARGUS. As pAct1-D lacks a selectable marker, we cloned the bar gene driven by 35S promoter contained in pBARGUS into pAct1-D to construct pNF93-1. Using this construct we have regenerated CBS plants showing bialaphos resistance. Molecular analysis to confirm the stable integration of the foreign gene in these plants are now in progress. Also, attempts are now in progress to introduce the soybean vegetative storage protein genes *vsp*A or *vsp*B driven by rice actin promoter into CBS.

X1-306 PARTICLE GUN TRANSFORMATION OF CREEPING BENTGRASS (*Agrostis palustris*) USING THE *BAR* GENE, Christina L. Hartman, Lisa Lee, Nilgun Tumer, AgBiotech Center, Rutgers University, Cook College, New Brunswick, NY 08903
We have developed regeneration and particle gun transformation methods for creeping bentgrass, a type of turfgrass used for golf greens. Embryogenic callus lines of Emerald, Putter, and Southshore have been transformed using the BioRad Helium gun. Transgenics plants have been obtained from the varieties Emerald, Putter, and Southshore. Plants were recovered from both plate and liquid selection. Four experiments of 12-14 filters yielded a total of 105 plants that survived the initial spray concentration. Of these, 26 survived the higher spray rate. Transgenics were further confirmed by PCR, Southerns, and northerns.

X1-307 APPROACHES TO ESTABLISH A MERISTEM TRANSFORMATION SYSTEM USING WHEAT AS A MODEL.
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Meristems can develop into fertile plants directly without going through tissue culture and therefore provide a genotype independent regeneration system. Direct gene transfer by particle bombardment could lead to integrative events in meristem cells producing sectorial chimeras, from which transgenic offspring can be expected. Such transformation approach must be directed to L2 cells which give rise to the gametes. Shoot apical meristems of immature embryos [1] and seedlings [2], as well as flower meristems [3], were used for transformation experiments. Optimisation of particle delivery and cell survival was tested by direct observation of particle penetration and using visible marker genes (*gus; Bperu* and *C1*). Meristems grew normally after bombardment and showed transgenic sectors in all three approaches. A selectable marker gene (*bar*) is being used for the screening of seeds obtained from bombarded shoot apical meristems of immature embryos or seedlings. Attempts to obtain pollen grains or ovaries from flower meristems cultured after bombardment are in progress.

References:
(1) Iglesias, V.A., Gisel, A., Bilang, R., Leduc, N, Potrykus, I., and C. Sautter. Transient expression of different marker genes in meristem cells of wheat immature embryos after ballistic micro-targeting. Planta, in press
(2) Bilang, R., S. Zhang, N. Leduc, V.A. Iglesias, A. Gisel, J. Simmonds, I. Potrykus and C. Sautter. Transient gene expression in vegetative shoot apical meristem of wheat after ballistic micro-targeting. The Plant Journal 4: in press
(3) Leduc, N., V.A. Iglesias, R. Bilang, A. Gisel, I. Potrykus and C. Sautter. Gene transfer to inflorescence and flower meristems using ballistic micro-targeting (Submitted)

X1-308 EFFECTS OF A NEW EXPRESSION MEDIUM (YM BROTH) ON THE TRANSFORMATION EFFICIENCY OF AGROBACTERIUM TUMEFACIENS CELLS USING ELECTROPORATION, Jhy-Jhu Lin, Life Technologies Inc., (GIBCO/BRL), Molecular Biology Research and Development, Grovemont Circle, Gaithersburg, MD 20877.
The introduction of recombinant plasmid DNAs into *Agrobacterium tumefaciens* LBA4404 cells was achieved by electroporation using a field strength of 16.7 kv/cm. The transformation efficiency was $>5x10^6$ cfu/μg. Different expression media such as LB broth, LB broth + 1% mannitol, S.O.C. broth, M9 broth, and YM broth have been examined for the optimal expression of *A. tumefaciens* LBA4404 cells after electroporation. Among these media, YM broth is the best medium for expression of *A. tumefaciens* cells. The difference in transformation efficiency between YM broth and LB broth was about 40 fold. Different expression times were also investigated to optimize the transformation efficiency of *A. tumefaciens* cells. With a three hour expression time, the transformation efficiency was consistently two fold higher than with a one hour expression period. However, the amount of viable cells after three hours of expression was the same as after one hour expression.

X1-309 ANALYSIS OF TRANSFORMED PLANTS PRODUCED BY POLLEN ELECTROTRANSFORMATION,
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The development of plant gene transfer procedures that are effective in the uptake and expression of stably introduced plasmids into intact agronomic crops is the goal of many research groups. We have developed a procedure that electroporates functional DNA into germinating pollen which is then used to pollinate receptive flowers. GUS expression, CAT assays, PCR amplification, and Southern analysis of plants produced from the seed of these pollinated flowers indicates that we have incorporated foreign DNA into these plants without the necessity of tissue culture or regeneration procedures. Optimization of biological parameters such as the duration of the pollen germination, optimal electroporation conditions, and optimal seed production, together with the use of appropriate promoters is necessary for each plant species tested. Initial successful results in tobacco are being evaluated in corn and alfalfa to determine the usefulness of this procedure in multiple agronomic crops.

X1-310 TRANSFORMATION OF GRAPE (VITIS VINIFERA L.) SOMATIC EMBRYOS AND REGENERATION OF TRANSGENIC PLANTS, R. Scorza[1], J.M. Cordts[1], D.W. Ramming[2] and R.L. Emershad[2], [1]USDA-ARS Appalachian Fruit Research Station, 45 Wiltshire Rd., Kearneysville, WV 25430, [2]USDA-ARS, HCRL, 2021 South Peach Avenue, Fresno, CA 93727

Transgenic grape plants were regenerated from somatic embryos derived from immature zygotic embryos of seedless grape (Vitis vinifera L.) selections. Somatic embryos were bombarded twice with 1 µm gold particles using the Biolistic PDS-1000/He device (Bio-Rad Laboratories) and immediately exposed to Agrobacterium tumefaciens strain C58/Z707 containing binary plasmid pGA482/GG (Ling, et al., Plant Cell Reports, 1991: 189-194) or pCGN 7314 (Mante et al., *BioTechnology*, 1991: 853-857). Following a 2-day co-cultivation period and subsequent washing of the embryos, secondary embryos were allowed to proliferate on Emershad and Ramming Medium (ERM) for 6 weeks before being subjected to selection on ERM containing 40 µg/ml kanamycin. Transgenic embryos were identified after 3-5 months under selection and allowed to germinate and develop on Woody Plant Medium containing 1 µM BAP, 1.5% sucrose, 0.3% activated charcoal, and 0.75% agar. Integration of the foreign genes was verified by growth in the presence of kanamycin and positive GUS and PCR assays. To date 10 transgenic clones have been identified.

X1-311 PARTICLE GUN BOMBARDMENT OF EMBRYOGENIC BAHIAGRASS CALLUS CULTURE, Robert G. Shatters, Jr.[1], Richard A. Wheeler[1], Peggy G. Lemaux[2] and S.H. West[1], USDA, ARS, Dept. of Agronomy, [1]University of Florida, Gainesville, Florida 32611, University of California-Berkeley/USDA-ARS Plant Gene Expression Center and Department of Plant Biology, 800 Buchanan Street, Albany, California 94710

Bahiagrass is an important forage grass in the southeastern United States. Procedures were developed to produce an embryogenic callus culture from germinating seeds of Tifton 9 bahiagrass, a high biomass yielding variety. Four percent of the seeds produced callus that gave rise to embryos and about 2% produced highly prolific embryo masses. These cultures we bombarded with 1 A gold particles coated with the pAHC25 plasmid. This plasmid expresses the BAR gene encoding resistance to the herbicide bialiphos and the GUS gene both under promotion of a separate ubiquitin promoter. Cultures incubated for 18 hours after bombardment showed transient expression of the GUS gene. The bombardment was optimized to obtain the greatest number of individual transient GUS expressing regions. Bombarding the same tissue 4 times increased the number of transient expressing events 3-fold over single bombardments. Bombarded embryogenic callus was plated on medium containing 1 mg L^{-1} bialiphos. After 4 weeks, viable callus was transferred to 3 mg L^{-1} bialiphos. Data on expression and activity of the BAR gene product will be presented.

X1-312 GENE TRANSFER AND REGENERATION OF TRANSGENIC PLANTS IN FORAGE GRASSES, *German Spangenberg, Zeng-yu* Wang, Jutta Nagel and Ingo Potrykus, Institute for Plant Sciences, Swiss Federal Institute of Technology, CH 8092 Zürich, Switzerland

First transgenic plants of Festuca arundinacea (tall fescue), F. rubra (red fescue) and F. pratensis (meadow fescue) have been obtained by direct gene transfer to protoplasts isolated from embryogenic suspension cultures. Selectable marker genes (hph and bar) driven by constitutive promoters (CaMV 35S, rice actin1) transferred to protoplasts by the polyethylene-glycol method, allowed selection for and recovery of hygromycin (Hm) and phosphinotricin (PPT) resistant calli with overall frequencies between 10^{-4} and 10^{-6}. Co-transformation experiments by mixing hph and bar vectors with one plasmid bearing uidA as reporter gene revealed co-expression of selectable and reporter genes in 17% to 34% of the cases. Plants were regenerated from independent Hm and PPT resistant calli, screened by PCR and grown until maturity under biosafety greenhouse conditions. Their transgenic nature was demonstrated by genomic Southern hybridization analysis, enzyme assays and in situ hybridization to metaphase chromosomes. Similar experiments on direct gene transfer to morphogenic protoplasts are currently being performed for Lolium perenne (perennial ryegrass) and L. multiflorum (Italian ryegrass). In addition, protoplast-independent direct gene transfer methods, e.g. "particle inflow gun"- and "microtargeting"-mediated microprojectile bombardment to embryogenic cultures and to vegetative and floral meristems, respectively, are being explored for the production of transgenic ryegrasses. Reproducible gene transfer systems are expected to be soon in place for fescues and ryegrasses and show potential for achieving genetic engineering objectives defined for particular production systems, which will presumably include first specific nutritional improvements and pest and pathogen protection. Both, gene addition and down-modulation of existing genes are being followed to improve nutritional qualities of fescues by genetic engineering and thus could complement or enhance current conventional breeding efforts for these species.

X1-313 MENDELIAN INHERITANCE OF TRANSGENES IN BARLEY

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The finnish elite cultivar of malting barley, *Hordeum vulgare* cv. *Kymppi*, was genetically transformed by bombarding a 35S-*nptII* recombinant gene into immature embryos or microspore derived callus. Shoots regenerating after bombardment were screened for NPT-II activity by a dot blot assay and those which gave a positive signal were subjected to analysis by PCR to detect the presence of the transferred gene. PCR was performed at the stage of seed set, on the rachides remaining after the seeds were removed. The primary transformants were chimeric and the transferred gene was detected in only part of their spikes. Isolated embryos from spikes that gave a positive signal were germinated and subjected to more rigorous testing for their transformation by Southern blot and NPT-II gel assay. These plants would be genetically homogenous and the inheritance of the transgene to their offspring would be expected to be regular. After selfing, the ratio of transgenic to non-transgenic offspring was shown to follow the rule of Mendel. Although the transferred gene could be detected in multiple copies, the analyzed transgenic plants, derived from three primary transformants, were all shown to propagate the transgenes as a single genetic locus. The integration pattern of the transgene, as revealed by the Southern analysis and characteristic for each transformed line, was inherited unaltered. The 35S-*nptII* gene was expressed in 65% of the second generation transgenic offspring. No reduction in fertility was observed in transgenic barley.

X1-314 TRANSGENIC CEREALS: RAPID AND EFFICIENT TRANSFORMATION OF WHEAT

Rubén H. Vallejos, Silvia Altabe, Gerardo Cervigni, Lelia M. Orsaria, Juan Pablo Ortiz, Hugo R. Permingeat, Gabriela Rossi, Martín I. Reggiardo and Marcelo A. Spitteler. Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Suipacha 531, 2000 Rosario, Argentina.

We have achieved the stable transformation of maize and wheat using a gun-powder home-made microparticle accelerator. Bombardment were performed on embryogenic calli using plasmids DNA which contained the reporter GUS gene and the selector gene, either Hpt that confers resistance to hygromycin or Bar that confers resistance to bialaphos.

Resistant fertile plants of both cereals were obtained after a short period of selection, grown to maturity in the greenhouse and crossed for obtaining their progeny. The presence of the heterologous genes in several of these plants and in their progenies including the second generation was confirmed by molecular analysis and histochemical assays. PCR and Southern blotting with genomic DNA from transgenic plants of both cereals showed a Mendelian segregation of the foreign genes. Maize transformation was achieved with a commercial genotype and with the A188/B73 hybrid with an efficiency of 0.6 (transformant per bombarded explant). Transformation of wheat was achieved with several cultivars and hybrids in only 30 weeks and an efficiency of up to 4.7%.

X1-315 *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF CARNATION (*DIANTHUS CARYOPHYLLUS* L.) USING LEAF EXPLANTS.

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The transfer of genes into carnation *(Dianthus caryophyllus* L.) was accomplished by inoculation of leaf explants with a disarmed strain of *Agrobacterium tumefaciens*. This strain was harboring a binary vector carrying genes which encode neomycin phosphotransferase II (NPT II) and ß-glucuronidase (GUS) between T-DNA border sequences. Explants were cocultivated for 5 days and subsequently transfered to regeneration medium containing 100 mg/l kanamycin. Leaf explants were regenerated according to the procedure described by Van Altvorst et al. (Scientia Horticulturae, 51 (1992) 223-235). Transgenic plants were able to root in the presence of 200 mg/l kanamycin. Transformation has been verified by the expression of the functional *gus* gene in carnation leaf tissue, by PCR and Southern analysis.

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X1-316 EFFICIENT PRODUCTION OF FERTILE TRANSGENIC BARLEY PLANTS,

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A rapid, efficient and reproducible system to generate large numbers of independently transformed, self-fertile, transgenic barley (*Hordeum vulgare* L.) plants is described. Immature zygotic embryos, young callus and microspore-derived embryos were bombarded with either one plasmid containing *bar* and *uid*A, or in combination with another plasmid, containing a barley yellow dwarf virus coat protein (BYDVcp) gene. A total of 91 independent bialaphos-resistant callus lines expressed functional phosphinothricin acetyltransferase, the product of *bar*. Integration of *bar* was confirmed by DNA hybridization in the 67 lines analyzed. Cotransformation frequencies of 84 and 85% were determined for the two linked genes (*bar* and *uid*A) and for two unlinked genes (*bar* and the BYDVcp gene), respectively. More than 500 green, fertile transgenic plants were regenerated from 36 transformed callus lines; albino plants only were regenerated from 41 lines. R_0 plants in 25 lines (3 plants per line) were analyzed by DNA hybridization and all contained *bar*. Most contained the same integration patterns for the introduced genes (*bar, uid*A and the BYDVcp gene) as their parental callus lines. Transmission of the genes to R_1 progeny was confirmed in the five families analyzed by DNA hybridization. A germination test of immature R_1 embryos on bialaphos-containing medium was useful for selecting individuals that were actively expressing *bar* although it was not a good indicator of the presence/absence of *bar*. Expression of *bar* in some progeny plants tested was indicated by resistance to the herbicide Basta™. The transgenic R_1 plants were in soil approximately 7 months after bombardment of the immature embryo.

X1-317 PROTOPLAST CULTURE AND INTERGENERIC ASYMMETRIC SOMATIC HYBRIDIZATION IN FORAGE GRASSES,

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An efficient system for green plant regeneration from protoplasts in different Festuca (tall, meadow and red fescues) and Lolium (Italian, perennial and hybrid ryegrasses) species, based on single-genotype derived embryogenic cell suspensions, cryopreservation for long-term storage of established suspension cultures and protoplast bead-type culture using nurse cells, has been worked out. Protoplast plating efficiencies were in the range 10^{-3} - 10^{-4}. Regeneration of over 150 green plants from protoplasts each in L. multiflorum, L. perenne and L. x boucheanum was achieved. Fully fertile plants have been regenerated from protoplasts in F. arundinacea, F. pratensis and L. multiflorum. Genetic stability of protoplast- and cell suspension-derived plants from F. pratensis and L. multiflorum was evaluated by a cytogenetic, RFLP and RAPD analysis. Limited newly induced genetic variation among independent protoplast-derived fescue and ryegrass plants was detected. Thus, the required tissue culture basis for somatic hybridization and protoplast-dependent gene transfer methods has been established for these recalcitrant graminaceous species. First flowering asymmetric somatic hybrid plants for an intergeneric graminaceous monocot combination have been regenerated after fusion between recipient F. arundinacea and X-ray irradiated (10-500 Gy) L. multiflorum protoplasts. Independent, genotypically and phenotypically different, asymmetric somatic hybrid Festulolium plants were recovered. Their hybrid nature was demonstrated by chromosome counts, quantitative dot blot and in situ hybridizations using nuclear species-specific repetitive dispersed DNA sequences. Irradiation of parental protoplasts favoured an unidirectional elimination of most or part of donor chromosomes. An RFLP analysis of the organellar composition of asymmetric somatic hybrid clones revealed a biparental organellar transmission (≤100 Gy) and a bias towards recipient-type organelles (>100 Gy). Experiments on somatic hybridization for the transfer of traits such as improved persistence and disease resistances from sexually incompatible genetic origins and for the transfer of cytoplasmic male sterility are in progress.

X1-318 STABLE TRANSFORMATION OF WHEAT (*TRITICUM AESTIVUM* L.) BY MICROPROJECTILE BOMBARDMENT, J. Troy Weeks, Olin D. Anderson and Ann E. Blechl, Agriculture Research Service, United States Department of Agriculture, Western Regional Research Center, Albany, CA 94710

The recently developed biolistic method for gene transfer to intact cells has emerged as a simple and promising alternative for cereal transformation. In our attempts to transform wheat, we have established a system to improve selection efficiencies and to reduce the number of non-transformants. Calli derived from immature embryos were bombarded with microprojectiles coated with DNA containing the BAR and *uidA*(GUS) genes, each under control of the maize *Ubi1* promoter. Selection with the herbicide bialaphos resulted in multiple independent lines of resistant callus and fertile wheat plants. Stable integration of functional DNA has been confirmed by marker gene enzyme activity, DNA analysis, herbicide resistance, and sexual transmission to progeny. Progress is reported on the optimization of the microprojectile system for increased transformation frequency.

X1-319 CHARACTERIZATION OF A NOVEL OPAQUE MUTATION IN MAIZE, Dwight E. Bostwick, Etti Or and Brian A. Larkins, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

Zeins account for approximately 50-60 percent of the total protein in mature maize endosperm. Mutations that affect the synthesis of these proteins often result in mature endosperm that is soft and chalky compared to wild type. However, the molecular basis for what causes the mutant phenotype is unknown. We have recently characterized a novel EMS induced mutation that gives an opaque endosperm phenotype. This mutation acts as a single recessive gene and is lethal in the homozygous condition. The mutant endosperm has an unusual zein profile since it shows a significant reduction in the amount of 27- and 54-kDa γ-zein proteins compared to wild type. Based on ELISA analysis, the level of γ-zein protein is reduced by approximately 50 percent in mature endosperm compared to wild type. In developing endosperm, the reduction in γ-zein was apparent as early as 20 days after pollination (DAP). The level of γ-zein mRNA accumulation is also reduced by approximately 50 percent by 20 DAP. The two-fold difference in mRNA accumulation between the opaque and vitreous endosperm was relatively constant throughout endosperm development (up to 36 DAP). This opaque mutant has been crossed to the inbred W22+ and is currently being mapped by RFLP and bulk-segregant analysis. Initial results indicates that the mutation is located on the long arm of chromosome seven.

X1-320 THE INTRACELLULAR TRAFFICKING OF HORDEIN POLYPEPTIDES IN BARLEY ENDOSPERM CELLS, Verena Cameron-Mills[1], K. Björn Rechinger[2], and David J. Simpson[2], Carlsberg Research Center[1] and Department of Physiology, Carlsberg Laboratory[2], DK-2500 Valby, Denmark.

The major prolamin storage proteins of barley endosperm, B, C, D and γ hordein are synthesized coordinately in the developing grain and accumulate as protein bodies in the vacuoles. Immunocytochemistry with hordein-specific monoclonal antibodies localizes B, C, and γ hordein to the globular component and D hordein to the reticular component. The hordein polypeptides are targeted to the endoplasmic reticulum (ER) by a signal peptide, but they are first detected by immunocytochemistry in vesicles of the Trans Golgi Network (TGN), revealed by OsO_4-ferricyanide-staining. The Golgi vesicles subsequently fuse with endosomes, which can be up to 1 μm in diameter. The oligomerization of the hordein polypeptides during condensation in the Golgi bodies appears to be defined, with γ hordein polypeptides localized at the periphery. The involvement of intermolecular disulfide-bond formation between hordein polypeptides in the ER, their transport through the Golgi body and acidification of the TGN are being investigated in vivo with specific inhibitors including dithiothreitol, Brefeldin A and monensin.

The absence of detectable hordein globules in the ER indicates that there is no direct transport from the ER to the vacuole as proposed for prolamin in the wheat endosperm. Knowledge of the transport pathway of hordein polypeptides to the vacuole and their targeting motifs will be essential for any future attempts at improving storage protein composition in barley.

X1-321 CHARACTERIZATION OF THE ASPARTATE KINASE-HOMOSERINE DEHYDROGENASE GENE FAMILY IN SOYBEAN, Joan S. Gebhardt and Benjamin F. Matthews, USDA Agricultural Research Service, Plant Molecular Biology Laboratory, Beltsville, MD, 20705-2350

Aspartate kinase (AK) catalzyes the first reaction common to the biosynthesis of the amino acids lysine, threonine, isoleucine, and methionine. Homoserine dehydrogenase (HSDH) is the branch-point enzyme leading to threonine, isoleucine, and methionine biosynthesis. Two partial cDNAs encoding one bifunctional protein possessing both AK and HSDH activities have been isolated from soybean cDNA libraries. The 5' end of the cDNA encodes a chloroplast transit peptide consistent with the biochemical localization of these enzymatic activities. Differential screening of genomic libraries and Southern blots suggested the presence of additional genes encoding AK or HSDH activities. Genomic clones representing the cDNA and a second bifunctional AK-HSDH have been identified. Sequence analysis of these clones has demonstrated a high amount of similarity between the two gene copies within the AK and HSDH coding regions and the chloroplast transit peptides. This similarity includes the positions of introns within the AK and HSDH coding sequences and the sequences encoding the transit peptides. The introns are of different sizes in the two gene copies but share some sequence similarity, particularly at the intron/exon borders. Southern blot hybridizations suggested the presence of a third AK gene in the soybean genome. A portion of this third tentative AK gene was amplified from size-fractionated restricted genomic DNA. Sequence analysis of this fragment has demonstrated the presence of AK coding sequences similar to the two bifunctional AK-HSDH genes. It is not known whether this third AK gene also contains HSDH coding sequences.

X1-322 ANALYSIS OF THE PLEIOTROPIC EFFECTS OF THE *opaque-2* MAIZE MUTATION ON NON-ZEIN ENDOSPERM PROTEINS, Jeffrey E. Habben, Brenda G. Hunter and Brian A. Larkins, Department of Plant Sciences, University of Arizona, Tucson, AZ 85721

The Opaque-2 gene encodes a basic domain/leucine zipper (b-ZIP) transcriptional factor that binds the promoters and regulates the expression of the 22-kD α-zein genes and a ribosomal inactivating protein. While the reduced synthesis of these proteins in *opaque-2* mutants is not unexpected, the increased synthesis of several non-zein proteins and their mRNAs is not readily explained based on what is known about the Opaque-2 transcription factor. One ancillary effect of this elevated non-zein synthesis is an increase in the lysine content of the *opaque-2* endosperm.

We have previously demonstrated that the majority of lysine-containing proteins from subcellular fractions of *opaque-2* endosperm are soluble. Our objective in this study was to identify cDNAs corresponding to these soluble proteins by raising an antiserum against them and using it to screen an endosperm, cDNA expression library. After obtaining polyclonal antisera from rabbits and immunoscreening the library, we identified more than 3000 immunoreactive plaques. We randomly purified and sequenced 90 of these clones. Among the cDNAs we isolated were genes for carbohydrate metabolism (sucrose synthase), amino acid metabolism (aspartate aminotransferase) and protein synthesis (60s ribosomal protein). RNA dot blot hybridization analysis with these clones revealed significant variation in the levels of transcripts between normal and *opaque-2* endosperm, including several mRNAs that are elevated 1.5 to 3-fold in *opaque-2* and that likely encode proteins responsible for the enhanced lysine content. By using a molecular approach to identify the non-zein proteins we hope to determine the pleiotropic effect of the *opaque-2* mutation and better understand the basis for the improved nutritional quality of the mutant.

X1-323 MOLECULAR CLONING AND EXPRESSION OF ASPARAGINE SYNTHETASE FROM SOYBEAN, Cleo A. Hughes[1,2] and Benjamin F. Matthews[2], [1]Morgan State University, Department of Biology, Baltimore, MD 21239 and [2]USDA/ARS Plant Molecular Biology Laboratory, Beltsville, MD 20705

Asparagine plays an important role in plant growth and development because of its involvement as a nitrogen transport compound in higher plants. Asparagine synthesis is mediated by the enzyme asparagine synthetase (AS) (EC 6.3.5.4), which catalyzes the ATP-dependent transfer of the amide group of glutamine (or ammonia in some cases) to aspartate, producing asparagine and glutamate.

AS has not been characterized extensively at the biochemical level because of its extreme instability in vitro. Therefore, expression of the soybean AS gene in E. coli would allow us to obtain large quantities of the AS protein for purification, antibody production, and kinetic analysis. A full-length cDNA clone (SAS2) encoding AS was identified from a soybean cDNA library constructed from poly $(A)^+$ RNA isolated from light-grown leaves. The region corresponding to the predicted mature soybean AS protein was amplified by the polymerase chain reaction (PCR). The 5' oligonucleotide primer contained the proposed glutamine binding site with an EcoRI site at the extreme 5' end. The inverse oligo-nucleotide corresponded to a region 3' of the stop codon along with a BamHI site. These primers were designed to place the SAS2 gene in-frame with the lacZ gene of the pUC18 vector. Plasmid DNA from positive colonies was isolated and used to transform an AS deficient E. coli strain. Complementation experiments revealed that the soybean gene was expressed functionally in E. coli. This was also confirmed in preliminary AS assays using HPLC which directly measured the synthesis of asparagine.

X1-324 CLONING AND EXPRESSION OF GENES ENCODING ASPARTATE AMINOTRANSFERASE IN SOYBEAN Benjamin F. Matthews, Greg Wadsworth, Joan S. Gebhardt, Barbara Wilson, U.S. Department of Agriculture, ARS, Plant Molecular Biology Laboratory, Beltsville, MD 20705

In plants aspartate aminotransferases (AAT; EC. 2.6.1.1) play an important role in hydrogen shuttles, carbon shuttles and nitrogen distribution. AATs reversibly transfer an amino group from glutamate to oxaloacetate to form aspartate and α-ketoglutarate. We have identified a family of at least five isoforms of AAT in soybean which can be separated and visualized on agarose gels. These isoforms are named AAT-1 through AAT-5 according to their migration on gels from slowest to fastest. Soybean AATs are differentially expressed in different organs during soybean development. We have localized three of these isoforms to cellular compartments. AAT-1 is localized to glyoxysomes, AAT-4 to the mitochondria, AAT-5 to the chloroplast. We have identified cDNA clones encoding AAT-4, AAT-5 and a cytoplasmic form. The cDNA encoding AAT-4 and AAT-5 contain sequences for putative transit polypeptides, whereas the third cDNA clone does not appear to encode a transit polypeptide sequence. The coding regions of each clone representing the mature AAT protein have been functionally expressed in E. coli. Antibody has been made to AAT-4 and to AAT-5 purified from E. coli and each antibody is specific for the proper AAT isoform as indicated in immunoprecipitation reactions.

X1-325 EFFECTS OF *opaque-2* MODIFIERS ON NORMAL MAIZE GENOTYPES, Gloverson Moro*, Mauricio A. Lopes*, Bruce Hamaker+, Brian A. Larkins*. * Dept. of Plant Sciences, University of Arizona, Tucson, AZ 85721. + Dept. of Food Science, Purdue University, West Lafayette, IN 47907

The influence of *opaque-2* modifier genes on *opaque-2* mutants have been extensively studied. However, no information is available regarding their effects on normal genotypes. The *opaque-2* modifier genes were backcrossed into a normal background, using Pool 34 QPM as recurrent parent and W64A as the *Opaque-2* donor. After five rounds of backcrossing (BC5), plants went through a sequence of self-pollinations and the seeds were visually selected to eliminate the *opaque-2* allele. In BC5-F3 generation, thirteen families were tested for the presence of the *opaque-2* allele by Southern analysis, and all were found to be homozygous for the wild-type allele. The zein profile of these families indicated a γ-zein content intermediate between Pool 34 and W64A and showed a reduction in α-zein content compared to W64A. An intermediate amount of the zein fraction (measured by Kjeldahl) and an increased content of cysteine in the "modified normal" genotypes supports these observations. The expression of modifier genes appears to depress non-zein protein content and, as a consequence, the absolute amount of lysine (as % of flour). In contrast, the hardness of the kernel appears to be increased by the action of the modifier genes. Hardness measurements correlated with the cysteine content, suggesting that γ-zein plays a role in endosperm hardness. Interestingly, density was not affected by the introduction of modifiers in the normal genotypes, in contrast to what was observed in *opaque-2* backgrounds.

X1-326 MOLECULAR MODELLING OF METHIONINE ENHANCEMENT IN THE BEAN SEED STORAGE PROTEIN ß-PHASEOLIN, Norimoto Murai, John M. Dyer, and Jeffrey W. Nelson, Departments of Biochemistry, and Plant Pathology & Crop Physiology, Louisiana State University and LSU Agricultural Center, Baton Rouge, LA 70803-1720

Phaseolin is the predominant seed storage protein of common bean (*Phaseolus vulgaris*) and has a low content of sulfur-containing amino acids, essential amino acids for human nutrition. To improve the nutritional quality of bean seeds, we have used a protein engineering approach to increase the methionine content of ß-phaseolin. The complete three-dimensional structure of phaseolin was generated from α-carbon coordinates using molecular mechanic calculations. This structure was used as a template to simulate modifications aimed at increasing the methionine content of phaseolin (*J. Protein Chemistry* **12**, in press). Two types of modifications were tested: Insertion of a methionine-rich looping sequence to turn, loop, and helix regions, and replacement of 10 variant, hydrophobic amino acids with methionine in each of the β-barrel structures. Results of molecular dynamics show that the insert is well accommodated in turn and loop regions of the protein, but not within an α-helix. Analysis of individual replacements with methionine revealed that methionine can effectively replace conserved or variant leucine, isoleucine, and valine residues. Substitution of all 10 residues in each barrel shows that replacements made in the amino-β-barrel cause greater destabilization than replacements in the carboxyl-β-barrel, however both structures are less stable than wild-type. We are now testing these predictions by constructing these mutations. Preliminary results demonstrate that phaseolin proteins bearing inserts at turn and loop regions of the protein are expressed in *E. coli* whereas insertion into the α-helix causes complete loss of the gene product. Structural stability of these *E. coli*-expressed proteins will be analyzed using biophysical probes (*J. Protein Chem* **11**, 281-288).

X1-327 *OPAQUE-2* MODIFIERS ACT POSTTRANSCRIPTIONALLY AND IN A POLAR-MANNER ON γ-ZEIN GENE EXPRESSION, Etti Or, Scott K. Boyer and Brian A. Larkins. Department of Plant Sciences, University of Arizona College of Agriculture, Tucson, AZ, 85721

The maize *opaque-2* mutation dramatically increases the lysine content of the grain. However, the soft, starchy endosperm of the mutant results in increased susceptibility to insects, pathogens and mechanical damage. These problems prevented the *opaque-2* from becoming of widespread agronomic importance. In certain genetic backgrounds, genes were identified that converted the soft endosperm of the *opaque-2* mutant to a hard, vitreous phenotype. The primary biochemical change associated with the expression of these genes is a two- to three-fold increase in the synthesis of the 27-kD γ-zein protein. To investigate the mechanism of modifier genes activity we examined the level of γ-zein mRNA and protein synthesis during early stages of development in normal, *opaque-2* and modified *opaque-2* near isogenic lines. The activity of the modifier genes significantly increased the level of γ-zein protein and mRNA as early as 16 days after pollination. At this stage transcription of γ-zein genes was reduced to 50% in both *opaque-2* and modified *opaque-2* genotypes compared to the wild type. It appears, therefore, that the modifiers regulates γ-zein synthesis through a posttranscriptional mechanism.

Analysis of transcripts from the two nearly identical genes (A and B) encoding the 27-kD γ-zein protein showed that in the modified *opaque-2* mutants accumulation of A over B transcript is greatly enhanced during endosperm development. Our results suggest that the product of the *opaque-2* modifier genes increases the stability or translation of the A gene mRNA, leading to enhanced synthesis of the 27-kD γ-zein protein.

X1-328 A BARLEY MUTANT IN WHICH STORAGE PROTEIN ACCUMULATES IN THE ENDOPLASMIC RETICULUM (ER) INSTEAD OF THE VACUOLE, K. Björn Rechinger, Verena Cameron-Mills and David J. Simpson, Department of Physiology, Carlsberg Laboratory, DK-2500 Valby, Denmark.

Hordein, the storage protein of barley, is deposited in protein bodies in the vacuole of developing endosperms. When several mutant varieties deficient in B or γ hordein polypeptides were analyzed by immunocytochemistry, the γ3 hordein deficient variety Nevsky revealed a dramatic change in hordein polypeptide targeting, the hordein storage proteins being largely deposited in the lumen of the rough ER (RER). The parent line of Nevsky is unavailable, but the variety Nutans has an identical hordein composition, based on SDS-PAGE and western blot analysis, except for the presence of γ3 hordein. The ultrastructure of developing endosperm of Nutans resembled that of Carlsberg II, where hordein is not detected in the RER. Prolamin polypeptides are water insoluble and may form aggregates within the RER shortly after synthesis. Retention of hordein in RER in the absence of γ3 hordein suggests that γ3 hordein maintains the prolamin storage polypeptides in a transport competent state. γ3 Hordein is unique among the sulphur-rich hordein polypeptides in forming only intramolecular disulfide bridges. Although encoded by the *Hor5* locus on chromosome 5 (together with γ1 and γ2 hordein), alignment of B hordein, γ hordein and γ gliadin (wheat prolamin) amino acid sequences reveals a closer homology of γ2 hordein to γ gliadin than to γ3 hordein, which appears almost as distantly related as B hordein. When the database was searched for the cysteine motif characteristic for γ3 hordein, analogues were found only among the temperate cereals (wheat, rye, oats and barley), which also deposit prolamin in vacuoles in contrast to the tropical cereals (maize, rice and sorghum), which show storage protein deposition in the ER.

We are currently analyzing the F1 and F2 generations of crosses of Nevsky with other genotypes in order to determine if a genetic linkage exists between the presence of γ3 hordein and vacuolar transport of prolamin. The accumulation of storage protein in the RER may influence physical properties of the endosperm matrix (soft vs vitreous) or malt modification.

X1-329 EXPRESSION OF SYNTHETIC HIGH LYSINE SEED STORAGE PROTEINS CAN SIGNIFICANTLY INCREASE THE ACCUMULATED LEVELS OF LYSINE IN MATURE SEEDS OF TRANSGENIC CROP PLANTS. Janet A. Rice, Sharon J. Keeler, Lee T. Hirata, Carole Beaman, Patricia Webber, Todd Jones, Sean McAdams and S Carl Falco. Agricultural Products, Biotechnology Division, E. I. Dupont de Nemours, Experimental Station, Wilmington, DE 19880

We have designed protein molecules based on an alpha helical coiled coil structure. These proteins can be tailored to meet specific end user requirements to complement nutritionally unbalanced seed meals. In particular, proteins may contain up to 43 molar percent of the essential amino acid lysine. Genes encoding such proteins were constructed using synthetic oligonucleotides and tested for stability by expression in an *E. coli* model system. A protein containing 31% lys and 22% met (CP3-5) and another containing 35% lys, 24% met and 4.5% trp (CP534) were expressed in transgenic tobacco seeds utilizing the seed specific bean phaseolin and soybean β-conglycinin promoters. Both promoters provided a level of expression in the mature transgenic tobacco seeds which resulted in the accumulation of up to 2% of total seed protein as CP3-5 protein and significantly increased the total lysine content of the seeds. Several of these transgenic lines were followed for three generations to determine the stability of gene expression. The β-conglycinin CP3-5 gene continued to provide stable expression of the high lysine phenotype through three generations although the phaseolin promoter constructs failed to increase lysine content in the second and third generations. The bean phaseolin promoter with the CP3-5 coding region was also transferred to soybean and expression of the protein was detectable in the heterozygous R1 seed at levels up to 0.8% of extractable protein. Similar constructs utilizing seed specific promoters were transferred to corn. The transgenic corn accumulated the CP3-5 protein in tissue culture.

X1-330 CLONING AND EXPRESSION OF THE SOYBEAN GENE ENCODING DIHYRDODIPICOLINATE 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 aspartate B-semialdehyde and pyruvate, catalyzed by the enzyme dihydrodipicolinate synthase (DS), which is encoded by the nuclear DapA gene. DS activity undergoes feedback inhibition by lysine, and this inhibition limits lysine synthesis. Cloning of the soybean DapA gene is of potential economic importance because soybeans are a major agronomic source of lysine, and the genetic engineering of the DapA gene may be a way of increasing lysine synthesis in soybeans and other crop plants. The DapA gene had not been previously cloned from dicots. We have cloned the DapA gene of soybean (G. max var. Century) using PCR. Portions of the soybean DapA gene were amplified and cloned using degenerate PCR primers based on the sequences of monocot DapA genes. DapA sequences were amplified from soybean genomic DNA and cDNA. Inverse PCR was used to clone additional sequences within the DapA open reading frame. 3' RACE PCR was used to amplify the 3' end of the DapA gene. The sequence encoding the 5' end of the mature DS protein was obtained from DapA clones from a soybean genomic DNA library in lambda GEM11. PCR primers corresponding to the 5' and 3' ends of the region encoding the mature DS protein were used to amplify the gene from soybean cDNA. The gene was cloned into pUC18, making pUC18DS, which expressed DS in E. coli. The DS enzyme encoded by the pUC18DS was feedback inhibited by lysine like the wild type soybean DS enzyme. pUC18DS was mutagenized with EMS, and selected for growth on (S)-2-aminoethyl-L-cysteine (AEC), a toxic lysine analog. Some AEC resistant clones expressed a mutant DS activity that was less sensitive to feedback inhibition by lysine.

X1-331 **DELETION ANALYSIS OF A 2S STORAGE PROTEIN PROMOTER OF *BRASSICA NAPUS* IN TRANSGENIC TOBACCO.** Stålberg K, Ellerström M, Ezcurra I and Rask L; Uppsala Genetic Center, Department of Cell Research, Swedish University of Agricultural Sciences, Box 7055, S-750 07 Uppsala, Sweden.
Oil seed rape (*Brassica napus*) contains two major storage proteins the 1.7S albumin and the 12S globulin napin and cruciferin respectively. The sole purpose of these proteins is by definition, to serve as a nitrogen and sulfur source for the developing seedling during and after germination. The storage proteins totally dominate the seed content, in oil seed rape they constitute 80% of total protein content of the mature seed. The promoter and upstream region of the *Brassica napus* 2S storage protein gene napA were studied to identify cis-acting sequences involved in seed specific developmental expression. Fragments generated by successive deletions of the 5' control region of the napA gene were fused to the reporter gene β-glucuronidase (GUS). By use of PCR technique, the 5' ends of the fragments were chosen at sites that separated elements previously shown to bind nuclear proteins and at sites that separated conserved elements found to be important in the regulation of other genes. These constructs were used to transform tobacco leaf discs. Analyses of GUS activities in mature seeds from the transformed plants indicated that there were both negatively and positively acting sequences in the napin promoter.

X1-332 DEREGULATION OF THE ASPARTATE KINASE AND DIHYDRODIPICOLINATE SYNTHASE ENZYMES INVOLVED IN THE ASPARTATE PATHWAY, Marc Vauterin, Valérie Frankard, Marc Ghislain, Michel Jacobs. Plant Genetics, Free University Brussels, Paardenstraat 65, B-1640 Sint-Genesius Rode, Belgium.
Considering AK and DHDPS, the two aspartate pathway main enzymes, and their regulatory function through feedback-inhibition by their own products, the following progress has been achieved. Firstly, an *Arabidopsis thaliana* AK-HSDH cDNA and genomic clone including its promoter have been isolated and characterized, resulting in the identification of transitpeptide and mature protein, and localisation of several regulatory elements such as opaque-2 and GNC4 binding sites in the promoter region. The regulation of the AK-HSDH gene-expression is currently studied. The effector of the feedback regulation of the AK-HSDH protein has not yet been identified since no functional rescue occurs in an AK deficient *E.coli* mutant. Sequence identity suggests it is the plant homologue of the *E.coli thr*A gene. Hence, several scenarios have been set up to isolate cDNAs encoding for a functional lysine sensitive AK. The availability of this cDNA will open the way to clone the AK-LT gene of an *A. thaliana* mutant, characterized by a lysine insensitive AK. Secondly, a poplar DHDPS cDNA has been isolated by direct genetic selection in an *E. coli dapA*$^-$ mutant. Comparison between the poplar and maize DHDPS cDNAs made a PCR-based cloning of an *Arabidopsis thaliana* DHDPS cDNA and a genomic clone possible. The *Arabidopsis* DHDPS promoter is currently analysed. By probing a genomic library of the *Nicotiana sylvestris* RAEC mutant with available DHDPS cDNAs, a feedback-insensitive DHDPS enzyme has been obtained: comparison with the wildtype *N. sylvestris* DHDPS revealed two nucleotide changes substituting an asparagine into isoleucine. The RAEC DHDPS mutant gene as well as in vitro mutagenized DHDPS sequences will be expressed in transformed plants, either in combination with a lysine insensitive AK or not, in order to determine the effect of the mutant genes on DHDPS and AK activity and the accumulation of lysine and other amino acids in plant tissues.

X1-333 SEED-SPECIFIC EXPRESSION OF THE SULFUR-RICH BRAZIL NUT 2S ALBUMIN IN TRANSGENIC *Vicia narbonensis* PLANTS. D.R. Waddell, I. Saalbach, T. Pickardt, F. Machemehl, S. Hillmer, O. Schieder and K. Müntz. Institut für Pflanzengenetik und Kulturpflanzenforschung, 06466 Gatersleben, Germany.

The nutritional quality of legume seeds is limited by their low content of sulfur-containing amino acids. We are attempting to improve their nutritional quality by introducing the gene of the sulfur-rich 2S albumin (18% methionine) of Brazil nuts (*Bertholletia excelsa*) into their genome by using *Agrobacterium*-mediated gene transfer.

The 2S-albumin gene was completely synthesized according to the cDNA sequence of the pre-polypeptide published by Altenbach (1987). We have inserted this gene into binary vectors under the control of two different promoters: 1) the cauliflower mosaic virus 35S promoter and 2) the seed-specific legumin promoter of *Vicia faba*. Both chimeric genes have been successfully introduced into *V. narbonensis* plants.

Under the control of the CaMV 35S promoter, the highest level of expression was observed in roots (0.3% of total protein). Much lower levels were observed in the seeds. In contrast, in 8 independent transgenic plants containing the gene under the control of the seed-specific promoter, high levels of expression (1 to 2% of total protein) have been observed in the seeds of all of the plants. The genes have been inserted at different locations in the genome and are inherited stably. We have been able to demonstrate Mendelian segregation in some cases. We believe this is the first report of the transfer and high level tissue-specific expression of the Brazil nut gene in a grain legume.

Control of Gene Expression in Transgenic Plants; Alterations in Oil Content and Composition in Seeds

X1-400 ANALYSIS OF TRANSGENIC SOYBEAN CONTAINING A SOYBEAN CYTOSOLIC GLUTAMINE SYNTHETASE PROMOTER FUSED TO A ß-GLUCURONIDASE CODING SEQUENCE, Tai-Sheng Cheng[1], John G Streeter[1], Desh-Pal Verma[2], John J. Finer[1] [1]Department of Agronomy, [2]Department of Molecular Genetics, and Ohio State Biotechnology Center, The Ohio State University, Wooster, OH 44691

The promoter from a cytosolic glutamine synthetase (GS) gene from soybean was fused to the *E. coli* ß-glucuronidase (GUS) reporter gene and introduced into embryogenic soybean (*Glycine max* Merr. cv Chapman) cultures via particle bombardment. The expression of the reporter gene was examined at various stages of embryo and plant development using both fluorometric and histochemical GUS assays. Expression of the GUS gene was highest in proliferative embryogenic cultures but declined as the embryos developed. Histochemical GUS assays of roots from regenerated plant tissues indicated localization of GUS activity in the vascular system. Expression of GUS in leaf tissue of transgenic plants was not restricted expression to the vascular tissue but appeared mottled throughout the leaf lamina. Native GS activity was not affected by the introduction of the additional copy of the glutamine synthetase promoter fused to the GUS gene. Ammonia and nitrate induction of the GS promoter:GUS fusion is currently under investigation using both primary transformants and progeny plants.

X1-401A 22-BP SEED-SPECIFIC ELEMENT OF THE PEA LECTIN PROMOTER BINDS BZIP PROTEINS. Sylvia de Pater[1,2], Khanh Pham[1], Fumiaki Katagiri[2], Johan Memelink[1], Jan Kijne[1] and Nam-Hai Chua[2]. [1]Institute of Molecular Plant Sciences, Leiden University, The Netherlands and [2]Laboratory of Plant Molecular Biology, Rockefeller University, New York.

We have shown that a trimer of a 22-bp fragment of the promoter of the seed-specific pea lectin gene confers high expression in seed of transgenic tobacco. The temporal and spatial regulation of expression mediated by the 22-bp element is very similar to the expression pattern conferred by a 2000-bp fragment of the lectin promoter. The 22-bp fragment contains a binding site for the cloned basic domain/leucine zipper (bZIP) proteins TGA1a and Opaque-2 (O2). Both O2 and TGA1a bound to the odd base palindromic C-box sequence, ATGAGTCAT, present within the 22-bp fragment. The 22-bp fragment also contains the sequence CACGTA, which contains the ACGT core usually found in binding sites for bZIP proteins. However, this sequence did not significantly contribute to bZIP protein binding. The binding affinity of TGA1a for the odd base palindromic sequence was low relative to a high affinity C-box (ATGACGTCAT). By contrast, O2 strongly bound to the odd base C-box; the affinity was comparable with that to high affinity G-(GACACGTGTC) and C-boxes. We conclude that the presence of an ACGT core sequence is not a prerequisite for high affinity binding of O2.

X1-402 ANALYSIS OF AN EPIDERMAL SPECIFIC LECTIN GENE FROM PEA IN TRANSGENIC ALFALFA, Michael S. Dobres, Dipanwita Maiti, Karen Thum, Sevnur Mandaci. Dept. of Bioscience and Biotechnology, Drexel University, Philadelphia PA 19104

The epidermis serves a dual role as a protective and growth controlling tissue layer. As the outer most layer of the plant, it presents a first line of defense against invading pathogens. It is also believed to play an important structural and developmental role in the process of organ formation and expansion. We are examining the expression of a pea lectin gene (*Blec*) that is highly expressed in the pea shoot apex but is absent or undetectable at very low levels below the bud. Genomic Southern analysis and the isolation of four independent cDNAs indicate that *Blec* is transcribed by a multigene family. Its deduced amino acid sequence indicates that it is a calcium and manganese dependent lectin similar to the pea seed lectin and Concanavalin A. *In situ* hybridization studies reveal that its mRNA is highly specific to the meristematic epidermal cells flanking the apical meristem: *Blec* RNA is undetectable in the protodermis of the apical meristem and first accumulates in the derived epidermal cells. This is interesting for two main reasons: 1) It defines a gene regulatory transition from protodermal to epidermal cells. 2) It suggests a function for *Blec* within developing epidermal cells of the shoot apex. We have isolated a pea genomic clone for *Blec* and have demonstrated that it is correctly expressed in transgenic alflalfa. We are currently using the transgenic alfalfa system to map the sequences conferring its temporal and spatial pattern of transcript accumulation within the shoot apex.

X1-403 RIBOZYME CLEAVAGE OF TOBACCO MOSAIC VIRUS SEQUENCES AND INHIBITION OF TMV REPLICATION IN TOBACCO PROTOPLASTS. Brent V. Edington*, Richard S. Nelson#, Richard A. Dixon#, Kenneth G. Draper*, and Yajing Yang*, *Ribozyme Pharmaceuticals Incorporated, 2950 Wilderness Place, Boulder, Co. 80301, #Noble Foundation, P.O. Box 2180, Ardmore, OK. 73402.

The catalytic region of a hammer-head ribozyme has been targeted to cleave TMV genomic RNA at nucleotide position 2467 by forty nucleotides of complementary sequence. This ribozyme cleaves purified positive strand TMV producing fragments of the expected size. Active ribozyme, inactive ribozyme, and antisense constructs have been produced which are driven by the 35S promoter of CaMV and the expressed RNA terminated by the rbcS 3' polyadenylation signal. Using PCR, the ribozyme sequence and the nonpoly A flanking sequences that would be expressed in cells from such a construct and the ribozyme sequence alone, have been placed 3' to a T7 promoter and expressed *in vitro* . Transcribed ribozymes from these T7 transcription units have been used to evaluate the efficiency of this ribozyme alone or the ribozyme with flanking sequences as it is expressed in the cell. The ribozyme with flanking sequences is less efficient catalytically than the ribozyme alone. Constructs expressing ribozymes, inactive ribozymes, or antisense sequences and a construct expressing TMV target sequences were electroporated into tobacco protoplasts and the resulting target sequence levels evaluated. Also, ribozyme, inactive ribozyme, and antisense expressing constructs were introduced into tobacco protoplasts concurrent with TMV. Accumulation of TMV coat protein in protoplasts expressing ribozymes, inactive ribozymes, or antisense sequences was assessed.

X1-404 A MODEL AND EXPERIMENTAL DESIGN FOR QUANTITATIVE ANALYSIS OF GENETIC AND ENVIRONMENTAL VARIATION OF T1 TRANSGENIC PLANTS. Yonatan Elkind, Binyamin Nir and Talia Nadler-Hassar. Department of Field Crops, Vegetable and Genetics, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

High levels of variation in transgene expression between independent transformants are observed in most transformation experiments. The aim of this study was to develop a model for the quantitative estimation of the genetic and environmental variance components in the first generation (T1) of transgenic plants, in which the transgene effect is considered as a source of genetic variation. The experimental population consisted of at least two cuttings of each T1 independent transgenic plants (ITP). Forty-two ITPs of tobacco containing 35S-GUS, four cuttings of each, were grown in a randomized block design, and GUS activity (GA) in the leaves was determined. Log (LGA) scale transformations eliminated the scale effect and the data met the model's assumptions. For GA and LGA the inter-ITP variance was 28% and 72% of the total variance in the experiment, respectively. The opposite was true for the intra-ITP variance, which was reduced from 58% to 18%. Broad sense heritability 0.32 and 0.80 for GA and LGA, respectively. The experimental design allowed partitioning of the phenotypic variance in T1 transgenic plants into genetic and environmental components. According to the original scale (GA), most of the phenotypic variance was due to environmental variance; the common tendency to interpret this variance as an outcome of position effect leads to incorrect findings. In the present example, after scale transformation, the genetic component was 80% of the phenotypic variance. Detailed analysis of the distribution of LGA between ITP revealed three discrete levels of expression due to position effect and additional normal distribution due to environmental effects.

X1-405 MODIFICATION OF TRANSGENE ACTIVITY IN PLANTA AND SELECTIVE ELIMINATION OF MARKER GENES FROM TRANSGENIC PLANTS Andrew Goldsbrough* and John I Yoder. Department of Vegetable Crops, University of California Davis, Davis Ca 95616 and *Plant Breeding International Cambridge, Maris Lane, Trumpington, Cambridge CB2 2LQ, UK.

We have developed an *Agrobacterium* binary vector transformation system which utilizes the transposition functions of maize Ac/Ds transposable elements to reposition transgenes within the plant genome subsequent to a primary transformation event. The practical applications of this system are several: 1) It enables the production of progeny plants which display a range of different types of gene expression as a function of the genomic position of the transposable element insertion; 2) It provides a tag system for possible regulatory sequences at or near the site of transposable element insertion; and 3) It allows for the elimination of specific transgene sequences - such as a selectable marker gene - subsequent to the initial transformation event. The possible benefits of the elimination of selectable marker genes are that it would allow re-transformation using the same marker gene; it may lead to greater stability of gene expression following re-transformation; and from a commercial point of view it would alleviate both the need to assess human and environmental safety issues concerning particular selectable marker genes and possible consumer fears of the same genes.

The system is demonstrated using T-DNAs which incorporate a Ds element containing the GUS gene under control of either full-length or minimal 35S promoters, a transposase gene and the NptII selectable marker gene. Progeny plants were recovered which segregate for the reciprocal presence and absence of Ds/GUS and NptII sequences. Progeny with Ds/GUS elements at different and multiple locations were identified which showed different levels, both quantitatively and qualitatively, of GUS expression, demonstrating the utility of the system to generate transposition-mediated expression variation.

X1-406 RIBOZYMES AND COAT PROTEIN MEDIATED CMV RESISTANCE IN MELON, Véronique Gruber, Gaëlle Baudot, Catherine Ollivo, Philippe Lénée, Pascual Perez and Sofia Ben Tahar, BIOCEM Les Cézeaux/groupe LIMAGRAIN, 24, av. des Landais, 63170 AUBIERE (FRANCE)

Plant disease caused by virus is one of the most serious problem hindering agricultural productivity resulting in loss of quality and low output. An important virus pest with respect to crop losses, is the cucumber mosaic virus (CMV) which is known to infect over 750 species of plants, particularly in the Cucurbitaceae family. Natural resistance to CMV has been isolated in melon through classical plant breeding methods. Nevertheless, this resistance is multigenic and linked to undesirable traits.To obtain melon plants resistant to CMV infection that can be easily used in breeding schemes, CMV (strain I17F) coat protein (CP) gene and ribozymes designed against different sites of the CMV coat protein (polyribozyme) have been introduced into melon through a routine technique for melon transformation that has been developped in the laboratory. More than 60 progenies of transgenic melons expressing either CP gene or polyribozyme have been tested for CMV resistance. Twenty-three progenies from each line have been mechanically inoculated with the mediterranean strain TL28 of CMV. The virus levels have been quantitated by ELISA analysis.

The results demonstrate that protection against CMV was achieved in 2 and 1 transgenic melon lines expressing CP gene and polyribozyme respectively. The type of protection conferred with CP gene or polyribozyme was quite different. Coat protein mediated protection has shown tolerance rather than complete resistance. A tolerant plant is characterized by the presence of symptoms followed by recovery of the plant that developped new leaves without symptoms. Several cycles of recovery can occurred depending on climatic conditions. The T2 progenies of the 2 tolerant transgenic melon lines expressing the CP gene have presented resistance to CMV infection. ELISA analysis revealed a rather high level of virus in these plants that therefore appear as virus sinks. Otherwise, the results show also that CP levels are not correlated with the degree of CP mediated protection. In contrast, polyribozyme mediated protection has appeared rather as complete resistance. Recovery phenomenon was quite unusal. The transgenic melon line expressing the polyribozyme was totally resistant to CMV infection. The T3 progenies showed complete resistance to CMV infection. Therefore, the resistant gene is genetically heritable. ELISA analysis detected very little virus. Melon lines containing this resistant gene are currently tested in field trial in South of France.

X1-407 REVERSAL OF SENSE-SUPPRESSION OF PAL ACTIVITY IN CALLUS OF TRANSGENIC TOBACCO CONTAINING A HETEROLOGOUS PAL GENE.

Nadler-Hassar Talia, Mano Hagit, Jacqueline Pallas*, Christopher J. Lamb* and Yonatan Elkind. Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76-100, Israel. *Plant Biology Laboratory, Salk Institute for Biological Studies, San Diego, CA, USA.

Transgenic tobacco plants containing a heterologous (bean) phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) gene modified by inclusion of the CaMV 35S enhancer sequences in its promoter, exhibited reduced levels of PAL activity and soluble phenylpropanoids. The steady state level of the bean PAL2 mRNA level was high while the native tobacco PAL genes showed reduced levels when compared to the control plants. The objective of this study was to analyze gene expression and enzyme activity in callus obtained from transgenic lines. A five to ten fold increase of PAL enzyme activity over the control was observed in callus derived from sense-suppressed transgenic plants that exhibit five to fifty folds reduced PAL activity. The over-expessing callus had a brown coloration, probably resulting from increased level of phenolic compounds. The RNA steady state level of the internal tobacco genes did not differ from the wild type control. Plants regenerated from the callus exhibited reduced PAL activity and sense suppression of the tobacco PAL gene. It can be concluded that it is possible to over-express bean PAL in callus, while in whole plant sense suppression is observed both on the enzyme activity level and on the steady state level of the internal genes. These results might suggest that sense-supression of bean PAL in transgenic tobacco plants is a result of regulatory mechanisms at the intact plant level.

X1-408 CONTROL OF GENE EXPRESSION IN TRANSGENIC PLANTS USING A CHEMICALLY INDUCIBLE PROMOTER, Ian Jepson, Philip Bell, Simon Bright, David Holt, Susan Wright and Andrew Greenland. ZENECA seeds, Jealott's Hill Research Station, Bracknell, Berkshire, RG12 6EY.

Several isoforms of glutathione S-transferase (GSTs) are known to exist in maize. They represent a family of enzymes which conjugate glutathione to a range of xenobiotic compounds, leading to their detoxification. Glutathione S-transferase isoform II is of particular interest because of its role in herbicide detoxification, and inducibility by safener treatment. We have purified GST II to homogeneity by FPLC and glutathione affinity chromatography. Specific polyconal antisera to both the 27kD and 29kD subunits have been raised. In addition, internal peptide sequence has been obtained. We have isolated full length clones from a safener induced cDNA library using immuno- and oligonucleotide screening techniques. The expression patterns of GST II-27 and GST-29 will be described. Corresponding genomic sequences have been isolated for GST II-27, and their structure compared with other plant GST sequences. Promoter fragments have been used to prepare reporter gene constructs for both transient and stable transformation experiments. Results will be presented indicating that chemically inducible promoter fragments may be used to regulate target genes in trangenic plants.

X1-409 SUSCEPTIBILITY OF TRANSGENE LOCI TO HOMOLOGY-DEPENDENT *TRANS*-INACTIVATION

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Previous work (1) has shown that two unlinked, partially homologous transgene loci can interact, leading to methylation and inactivation of one transgene locus in the presence of the second. To study differences in susceptibility to *trans*-inactivation, we have used four independent tobacco transformants containing the same transgene construct (H) integrated in different chromosomal locations. These four single transformants (STs) were retransformed with a second, partially homologous transgene construct (K). Double transformants (DTs) regenerated from the STs were screened for inactivation of markers (CAT, Hyg^R) on the resident H construct. For two STs, no loss of H marker activity was observed in up to 180 DTs tested, suggesting that these integration sites were resistant to *trans*-inactivation. In contrast, the other two STs appeared to be susceptible to *trans*-inactivation: 3-10% of the DTs were CAT^- and/or Hyg^S. This loss of H marker activity was shown to be due to two factors: (1) Regeneration from stable epigenetic states, in which either one, both or none of the H transgene alleles were active. This reflected an intrinsic instability of the H locus in these two STs. The instability was more pronounced when the locus was homozygous, and was associated with partial methylation and cellular mosaicism. (2) The presence of an ectopic copy of the K construct could weaken Hyg^R in some DTs; this was accompanied by increased methylation in the 35S promoter of the *hptII* gene. These results indicated that susceptibility of a locus to *trans*-inactivation is associated with an inherent instability and a tendency to partial methylation, which is increased when the locus is homozygous and/or when an unlinked homologous transgene construct is present. The implications for somaclonal variation and inbreeding depression in plants are discussed.

(1) Matzke, M. and Matzke, A. (1993) Annu. Rev. Plant Physiol. Plant Mol. Biol. 44: 53-76.

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X1-410 DIFFERENTIAL REGULATION AND EVOLUTION OF DIVERSE *ARABIDOPSIS* ACTIN GENES. J. McDowell, Y.-Q. An, E. McKinney, S. Huang, and R. Meagher. Dept. of Genetics, The University of Georgia, Athens, GA 30602.

Plant actins are required in several essential cellular processes, including cell division plane determination and cell wall morphogenesis. Actin is encoded in higher plants by large, divergent gene families. We have proposed that ancient plant actin gene subclasses have been conserved since the early evolution of vascular plants because they fulfill unique functional and/or regulatory roles. We are utilizing *Arabidopsis* as a model in which to examine plant actin regulation, function, and evolution. 10 *Arabidopsis* actin genes have been isolated and completely sequenced. A quantitative study of the molecular evolution of these and other plant actin gene sequences has confirmed the ancient character of plant actin gene families and has revealed several conserved subclasses.

We are currently analyzing the expression patterns of each *Arabidopsis* actin gene at the RNA level and with promoter-GUS translational fusions. Preliminary data indicates that the *Arabidopsis* actin promoters often direct differential expression with respect to tissue or cell type and developmental stage. For example, expression of the ACT7/*gus* fusion is constitutive in seedlings, but becomes more restricted as development proceeds. In mature leaves, high levels of GUS are detected in wounded tissue and developing trichomes. GUS expression is constitutive in flowers with the exception of anthers, in which GUS is not dectectable until late in development. Further characterization of ACT7 expression will be presented. We will also discuss our progress towards suppressing *Arabidopsis* actin gene expression with gene-specific, inducible, antisense RNA.

X1-411 QUANTITATIVE TRANSIENT GENE EXPRESSION IN MONOCOT CELLS, Kai Schledzewski and Ralf R. Mendel, Department of Botany, Technical University, D-38023 Braunschweig, Germany

The particle gun approach was used for the quantification of promoter efficiency in a test system for transient gene expression. ß-glucuronidase (GUS) was used as reporter gene for determining promoter strength. The variability inherent to this gene transfer system was considerably reduced by calculating a transformation-efficiency factor given by the expression of a cotransferred second reporter gene (firefly luciferase). The calibration of GUS activity by the transformation-efficiency factor caused a lower statistical variance of the values and allowed to obtain reliable results with a smaller set of repetitions. - Four different promoters, the CaMV **35S** promoter as a control and the monocot-specific promoters for **maize polyubiquitin-1, rice actin1** and the **maize-derived Emu** promoter were comparatively characterized with respect to expression strength as tested under identical conditions in suspension cell cultures of **maize, barley and tobacco**. Compared to the 35S promoter, the monocot-specific promoters show high expression in maize and barley but give only weak expression in tobacco. No expression was found for the rice actin-1 promoter in tobacco.

The level of internal cell stress was analysed with respect to the response of two promoters (maize polyubiquitin-1 and CaMV 35S) to changing osmotic potential in the agar medium. For both promoters the calibrated GUS avtivities did not change up to an osmotic value of 8 % sucrose. Further increase of the osmotic potential caused in increase of GUS avtivity reaching a 3 - 4fold higher level at the highest sucrose concentration (32 %) as compared to the 3 % standard sucrose concentration in the medium. This contrasts with the reporter gene activity driven by the 35S promoter that decreased with increasing osmotic values.

X1-412 INTRON RECOGNITION IN PLANT NUCLEI, Mary A. Schuler, Hua Lou, Cesar A. Egoavil, Sara Potthoff, Sherry Xu and Andrew J. McCullough, Departments of Plant Biology and Biochemistry, University of Illinois, Urbana, IL 61801

In contrast to mammalian and yeast systems, the mechanism for intron recognition and splice site selection in plant pre-mRNAs is poorly understood. Differences which exist between plant introns often prevent the excision of heterologous introns and expression of proteins in transgenic plants. Splice site sequences and putative branchpoint sequences are loosely conserved in plant introns compared with other eukaryotes. *In vivo* analyses using an autonomously replicating *Agrobacterium*/ geminivirus vector has allowed us to define the elements critical for 5' and 3' splice site selection in dicot plant nuclei and to develop a model for intron recognition in plant nuclei. This model states that 5' and 3' splice sites in plant introns are selected in part by their position relative to AU-rich elements spread throughout the length of plant introns. Potential splice sites are then selected in a position-dependent manner if they are located upstream (5' splice site) or downstream (3' splice site) from these AU transition points and not if they are embedded within AU-rich intron sequences. Site-directed mutations have identified multiple *cis*-acting elements which are essential for 5' and 3' splice site selection in dicot nuclei. The length and sequence constraints of these intronic elements have been defined in detail. Our analyses demonstrate that these AU-rich elements cooperatively define the boundaries of introns in dicot nuclei and that aberrant recognition of these elements activates cryptic splice sites in the surrounding exons or introns. This mode of recognition relaxes the need for strong splice sites and branchpoint consensus sequences and suggests that plant splicing machineries utilize a variety of novel *trans*-acting factors in intron definition.

X1-413 SYNTHESIS AND TARGETING OF SAPORINS, TYPE 1 RIBOSOME-INACTIVATING PROTEINS FROM *S. OFFICINALIS* L. (SOAPWORT), Lesley J. Sinclair, Raffaella Carzaniga, Nick Harris, Anthony P. Fordham-Skelton and Ronald R. D. Croy, Department of Biological Sciences, University of Durham, South Road, Durham, DH1 3LE, UK.

Ribosome-inactivating proteins (RIPs) have been isolated from the tissues of many plant species. RIPs are base-specific RNA N-glycosidases and cause the inhibition of protein synthesis by removing a single adenine residue from a conserved region of the large ribosomal RNA.

The majority of RIPs are single chain proteins (type 1), but some RIPs are comprised of two disulphide bonded subunits, an active polypeptide A-chain (analogous to the type 1 RIP polypeptide chain) and a lectin-like B-chain (type 2).

Currently, one of the most studied applications of RIPs is the preparation of antibody-RIP conjugates capable of delivering the toxin to specific target cell populations eg. cancer cells. However, relatively little is known about the expression and function of RIPs in planta.

We are investigating the role of saporins, type 1 RIPs, present in the seeds and leaves of soapwort. Immunolocalisation studies and western analyses indicate possible differences in processing/targeting between leaf and seed forms of the proteins. In addition, the leaf form of saporin has been shown to have RIP activity on endogenous and tobacco ribosomes and is presently being purified for sequence analysis to allow comparison with previously characterised seed forms. The current status of this work will be described.

X1-414 THE DEGRADATION OF THE rbcS mRNA, *SRS4*, MAY BEGIN WITH AN ENDONUCLEOLYTIC CLEAVAGE EVENT. Matthew M. Tanzer and Richard B. Meagher. Department of Genetics. University of Georgia. Athens, GA 30602-7223.

The mRNA encoding the soybean rbcS gene, *SRS4*, degrades into a set of discrete lower molecular weight products which are nearly identical in light grown soybean seedlings and in transgenic petunia. The 5' (proximal) products have intact 5' ends, lack poly(A) tails, lack varying amounts of 3' end sequences, and are found at higher concentration in the polysomal fraction. These data suggested that the decay of the *SRS4* mRNA may occur in association with polysomes. Therefore, we developed a cell-free RNA degradation system based on a polysomal fraction isolated from soybean seedlings. In this system endogenous *SRS4* mRNA levels and the proximal products decreased over a six hour time course. In vitro synthesized *SRS4* RNAs were degraded into the expected set of proximal products when added to either soybean or a petunia derived in vitro system. When 3' truncated *SRS4* RNAs were added to the system, they were also degraded into the expected subset of proximal products. By removing the heterogeneous 3' ends of the *SRS4* RNAs by oligonucleotide directed RNase H cleavage, we identified a set of 3' distal fragments containing intact 3' ends, and lacking various portions of the 5' end. Both in vitro systems generated the same set of distal fragments from an exogenous *SRS4* RNA *template*. Both proximal and distal products were generated in essentially a random order. Additionally, vector "tag" sequences on the 5' and 3' ends or truncation of the 3' end of the exogenous *SRS4* RNAs had little effect on the pattern of degradation, contrary to what might be expected if overall secondary structure were important. Therefore, RNA sequences and/or structures in the immediate vicinity of each 3' end point may be important in the degradation machinery. Together these data suggest the *SRS4* mRNA is degraded by a stochastic mechanism and that endonucleolytic cleavage may be the *initial event. To determine what cis factors may be involved in site* selection and if the proximal products and the distal products are generated from the same cleavage events, the ends of these products are being mapped more precisely. Progress toward this goal and an updated model for the degradation of the *SRS4* mRNA will be presented.

X1-415 *Trans*-ACTIVATION OF TARGET TRANSGENES IN TOBACCO SEEDS BY HOMOLOGOUS SEQUENCES, Ulrich Wobus, Winfriede Weschke, Helmut Bäumlein, Angela Stegmann and Renate Manteuffel, Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany

Transgenes can exert inhibitory effects on the expression of related sequences. Our studies on the expression of legumin promoter driven target transgenes revealed that partially homologous modifier transgene constructs can also *stimulate* the expression of target transgenes: First, flowers of tobacco line TA 15/1 homozygous for a faba bean legumin gene were fertilized with pollen from plants expressing RNA from a legumin coding region fragment in sense or antisense orientation under T_R-promoter control. Legumin levels determined in the mature seeds were 4.7-fold (sense construct, mean of 6 plants) and 7.5-fold (antisense construct, mean of 6 plants) higher then in hemizygous TA 15/1 seeds. Second, tobacco line LH22 expressing a legumin promoter/nptII-fusion gene was retransformed with constructs containing the legumin basic promoter/5'-UTR region (-68 to +56) in both orientations driven by the 35S promoter. Both modifier constructs *increased* target gene expression 2.3-fold (sense construct, mean of 16 plants) and 3.8-fold (antisense construct, mean of 11 plants). A number of other combinations of target transgenes and modifier transgene constructs resulted in a decrease of expression in almost all plants. We will provide additional data from transient expression experiments and discuss the results with respect to gene communication within a plant genome.

X1-416 MULTIPLE STABLE ANTISENSE *Bacillus thuringiensis* TRANSCRIPTS ARE PRODUCED BY INTERGENIC SPLICING IN AN ORIENTATION DEPENDENT PROCESSING OF BT GENE SEQUENCES. Richard L. Yenofsky, Miriam Fine and Thirumale S. Rangan, Phytogen, 101 Waverly Drive, Pasadena, CA 91005.

Introduction of *Bacillus thuringiensis* (BT) delta endotoxin genes into transgenic cotton plants has shown the potential utility of such genes in controlling Lepidopteran insects. However, obtaining sufficient expression to confer appropriate levels of resistance has proven problematic. On the one hand, "native" truncated bacterial genes linked with various plant promoters have shown only limited ability to control various Lepidoptera species. On the other hand, substantial alterations in nucleotide composition of BT gene sequences (without subsequent alteration in amino acid coding sequence) have resulted in vastly improved efficacy. This improvement is supposedly a consequence of a stabilization of the bacterial BT transcript emanating from the fact that the transcript appears more "plant-like" to the plant cell machinery regulating mRNA stability and translatability. In the current report, we demonstrate that the "native" truncated BT gene, or a BT gene with minimal modification, can give rise to stable antisense BT transcripts. These are produced through intergenic splicing when the BT gene is placed downstream from, and in opposing orientation to, the plant selectable marker present in the transforming vector. These transcripts are found on polysomes, show up as defined, truncated bands on Northern analysis, are polyadenylated, and are abundant. The transcripts are antisense, as demonstrated by hybridization to radiolabeled sense BT-RNA probes. Further, a cDNA library constructed from polyadenylated polysomal RNA and screened with a BT probe shows that internal portions of the BT gene are linked to other expressed gene sequences. This occurs in such a way that the antisense strand of the BT is linked with the sense strand of the expressed gene. PCR analyses of genomic DNAs indicate that the cDNA structures are not present within the genome. This is consistent with the interpretation that intergenic splicing is occurring.

X1-417 CLONING AND EXPRESSION OF A CYANOBACTERIAL Δ^6-DESATURASE, Richard T. DeRose[1], Avutu S. Reddy[2], Michael L. Nuccio[2], Lisa M. Gross[2], and Terry L.Thomas[2]. [1] Rhône Poulenc Agrochimie, Lyon, France; [2]Dept. of Biology, Texas A&M University, College Station, TX 77843.

Consumption of vegetable oils rich in γ-linolenic acid (GLA; $18{:}3^{\Delta 6,9,12}$) may alleviate hypercholesterolemia and other related clinical disorders which correlate with susceptibility to coronary heart diesease. Most plant seed oils are deficient in GLA, which is the result of desaturation of linoleic acid ($18{:}2^{\Delta 9,12}$) catalyzed by the enzyme Δ^6-desaturase. A cyanobacterial gene encoding Δ^6-desaturase was cloned by expression of a *Synechocystis* genomic cosmid library in *Anabaena*, a cyanobacterium that lacks Δ^6-desaturase but does have linoleic acid, the substrate for this enzyme. Expression of the *Synechocystis* Δ^6-desaturase gene in *Anabaena* resulted in the accumulation of γ-linolenic acid (GLA) and octadecatetraenoic acid (18:4). The predicted 359 amino acid sequence of the *Synechocystis* Δ^6-desaturase shares limited sequence similarity with other reported cyanobacterial and plant desaturases; the hydropathic profiles of these desaturases are even more conserved suggesting phylogenetic conservation of functional protein domains. Expression of *Synechocystis* Δ^6- and Δ^{12}-desaturases in *Synechococcus*, a cyanobacterium deficient in both desaturases, resulted in the production of linoleic acid and γ-linolenic acid. Additional results on the expression of the *Synechocystis* Δ^6-desaturase in transgenic plants will be presented.

X1-418 REGULATION OF PRIMARY STORAGE PRODUCTS OF OIL SEEDS BY MANIPULATING THE LEVEL OF GENES INVOLVED IN LIPID METABOLISM ON PLANT ACETYL COA CARBOXYLASE

Elborough K.M., Farnsworth L., Winz R.A. , Simon J.W., Swinhoe R., Slabas A.R. *Lipid Molecular Biology Group, Biological Sciences Department, University of Durham, South Road, Durham. DH1 3LE U.K.*

The biosynthesis of fatty acids is catalysed by two essential enzymes. The first of these acetyl CoA carboxylase (ACCase) catalyses the biotin dependent carboxylation of acetyl CoA to form malonyl CoA which is the essential substrate for fatty acid biosynthesis. We have cloned partial a cDNA encoding both wheat and rape cDNA. The partial cDNA represents approximately 0.25 of the full length cDNA. The clones were authenticated by ACCase peptide sequencing and immuno cross-reactivity of the overexpressed clone. The derived amino acid sequence showed homology with both Rat/Yeast ACCase sequences.

The cDNA clones have been used to construct antisense vectors in order to down regulate ACCase in *Brassica napus*. Analysis will be made of the appropriate transgenic plants to see the effect on the ratio and absolute level of the principle storage products lipid carbohydrate and protein. We will also look at the effect of down regulation of ACCase on several other FAS genes as pleitropic effects have been observed with similar transgenic studies on other metabolic pathways.

X1-419 GENE EXPRESSION OF THE CORIANDER Δ_4 DESATURASE, David J Fairbairn, Steven Bowra and Denis J Murphy, Department of Brassica & Oilseeds Research, John Innes Centre, Norwich, NR4 7UH, United Kingdom

Plants frequently contain unusual fatty acids in their seed storage oil, which are often characteristic of a family or genus. The seed oils of the family *Umbelliferae* (*Apiaceae*) contain a high proportion of the characteristic fatty acid, petroselinic ($18{:}1^{\Delta 6cis}$) acid. We are interested in petroselinic acid because the position of the double bond allows its oxidative cleavage to potentially provide two important industrial feedstocks, i.e. lauric and adipic acids, which are used in the manufacture of detergents and plastics respectively.

The coriander Δ_4 desaturase message only appeared in seed tissue and not leaf, stem or root tissue and not in carrot or coriander suspension culture cells. This tissue specificity agrees with the distribution of petroselinic acid. The message abundance in seeds peaked around 29 DAF and was not detectable at 65 DAF. Maximum fresh weights for seed and pod were reached after 52 and 29 DAF respectively.

In order to investigate the mechanisms controlling the tissue-specific expression of the Δ_4 desaturase, we have examined cultured somatic and endosperm tissue and compared the results with those from seed tissue. Results of these studies will be presented.

X1-420 MOLECULAR GENETIC STUDIES OF THE EVOLUTIONARY RELATIONSHIP BETWEEN PROKARYOTIC AND PLANT FAS SYSTEMS, Martin M. Kater, Gregory M. Koningstein, H. John J. Nijkamp and Antoine R. Stuitje, Department of Genetics, Institute for Molecular Biological Sciences, BioCentrum Amsterdam, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, The Netherlands

From an evolutionary point of view the fatty acid biosynthetic pathway of plants and prokaryotes are considered to be closely related. In these organisms, *de novo* fatty acid synthesis is catalysed by a so-called type II fatty acid synthetase complex (FAS II), which consists of eight structurally independent and monofunctional components.

In order to investigate whether bacterial FAS genes can be used to manipulate plant lipids, we examined whether components of the bacterial and plant FAS systems are exchangeable

We will present a biochemical and molecular genetic study on the relatedness of the bacterial and plant NADH dependent enoyl-ACP reductases, a key enzyme in the elongation of the acyl-chain during fatty acid biosynthesis. The bacterial complement of the recently isolated *B. napus* (rapeseed) enoyl-ACP reductase clone was shown to be encoded by the *envM* genes of *E. coli* and *S. typhimurium*. Subsequently it was shown that the bacterial, but not the plant, enoyl-ACP reductase was specifically inhibited by a antimicrobial agent called diazaborine.

The use of this specific inhibitor of bacterial enoyl-ACP reductase facilitated gene replacement studies that resulted in the engineering of an *E. coli* derivative in which the coding sequence of the *envM* gene was replaced by that of the rapeseed enoyl-ACP reductase cDNA clone. The potential of such a hybrid plant-bacterial FAS system to study regulation of fatty acid biosynthesis was inferred from the fatty acid composition of the phospholipids found in the recombinant strains, which revealed that, in *E. coli*, enoyl-ACP reductase catalyses a rate-limiting step in the elongation of unsaturated fatty acids.

Our results show for the first time that a component of the plant fatty acid synthetase complex, enoyl-ACP reductase, is fully functional within the bacterial system. Moreover, the gene replacement studies have provided a novel way to analyse rate limiting steps in bacterial fatty acid synthesis.

X1-421 ANALYSIS AND CHARACTERIZATION OF THE OLEOSIN GENE FAMILY IN *HELIANTHUS ANNUUS*
Yolande E Knight, Ian Cummins and Denis J Murphy, Department of Brassica & Oilseeds Research, John Innes Centre, Norwich, NR4 7UH, United Kingdom
Triacylglycerols, the major storage lipids in plants, accumulate as oil bodies in many plant storage tissues. When mature, the oil bodies are surrounded by a protein phospholipid layer. A class of oil body membrane proteins called "oleosins" has been characterized in many oilseeds, including the Crucifers. Oleosins have also been found in the Compositae eg. *Helianthus annuus* (sunflower) and can constitute as much as 20% of the total seed protein. These membrane proteins are thought to be involved in the protection and stabilization of the oil body and they may also act as receptors for lipase binding.

The aim of this investigation is to characterize the oleosin gene family in *Helianthus annuus*. A sunflower cDNA clone (HaI) was obtained by initial screening of the library using antibody raised to oleosin. Further screening of the library used a probe made from the central hydrophobic region of HaI. Previous studies have shown that oleosin genes *in maize and rapeseed* are expressed in both a spatially and temporally regulated manner. Oleosin mRNA is not produced in the leaf and its expression has been seen to increase in the latter stages of embryogenesis and decrease in the dry seed. Different expression patterns of oleosin have been presented by different studies. One study found that in rapeseed embryos, oleosins do not accumulate in significant quantities until most of the storage oil bodies have been formed. Other studies have found that the accumulation of oleosin in maize and rapeseed is concomitant with that of the storage triacylglycerols and similar to that of the seed storage genes. In the light of this anomaly, the expression of the sunflower oleosin gene at different stages of sunflower embryo development has been studied using Western blots, SDS-PAGE and lipid and protein quantification. The results of these studies will be presented.

X1-422 STEAROYL-ACYL CARRIER PROTEIN THIOESTERASE, A NOVEL ENZYME IN PLANT FATTY ACID BIOSYNTHESIS, Dehua Liu and Dusty Post-Beittenmiller, Plant Genetics Section, The Samuel Roberts Noble Foundation, Inc., P. O. Box 2180, Ardmore, OK 73402.
Leek extracts possess at least two acyl-acyl carrier protein (ACP) thioesterase (acyl-ACP hydrolase) activities. The relative activities of the two thioesterases differed between mesophylls and epidermis. In rapidly expanding leaf, the stearoyl-ACP thioesterase was more active in epidermis than in mesophyll, while the oleoyl-ACP thioesterase was more active in mesophyll than in epidermis. These activities, oleoyl-ACP thioesterase and stearoyl-ACP thioesterase, have been separated and chatacterized. The stearoyl-ACP thioesterase was purified 520-fold using ammonium sulfate precipitation, and Q Sepharose, Hydroxyapatite and Mono S column chromatography. Oleoyl-ACP thioesterase was also purified and characterized for comparison with the stearoyl-ACP thioesterase. Substrate-specificity studies showed that the purified stearoyl-ACP thioesterase was very specific for stearoyl-ACP. The oleoyl-ACP thioesterase, however, had a broader specificity, including lauroyl-ACP, myristoyl-ACP, palmitoyl-ACP and stearoyl-ACP. The increased level of stearoyl-ACP thioesterase activities in epidermis suggest that the thioesterases may be involved in the partitioning of fatty acids between glycerolipid biosynthesis and wax biosynthesis.

X1-423 ANALYSIS OF SEED-SPECIFIC GENE PROMOTERS AND THEIR DIFFERENTIAL TEMPORAL REGULATION DURING DEVELOPMENT, Denis J Murphy, David Fairbairn, James S Keddie, Steven Bowra, Stephen P Slocombe, Ian Cummins, Dimitra Milioni and Pietro Piffanelli, Department of Brassica & Oilseeds Research, John Innes Centre, Norwich, NR4 7UH, United Kingdom
The structures of several classes of seed-specific gene promoters from *Brassica napus* will be compared with seed-specific promoters from other plant species in order to identify putative *cis*-regulatory elements. Data from DNAse protection and gel-shift experiments will be presented to confirm the possible regulatory role of several of these *cis*-elements and in particular a G-box-like motif similar to previously identified ABA-responsive elements.

Results of *in situ* hybridisation studies using riboprobes based on oil-related genes and oleosin genes demonstrate that the expression of these two sets of genes is temporally separated in *Brassica napus* embryos. Similarly, the accumulation of their respective gene products, i.e. oil bodies and oleosin proteins, occurs at different times. Differential expression of these two classes of seed-specific gene is supported by their varying response to induction by plant growth regulators, such as ABA or GA, either *in vivo* or *in vitro*. The implications of these findings for the mechanism of storage oil body deposition and maturation in seeds will be discussed. Analysis of the lower DNA strand of the promoters of one of the *Brassica napus* and one of the *Arabidopsis* oleosin genes cloned in this study reveals the presence in each of an open reading frame (ORF-2) encoding a protein similar to the ethylene-inducible E4 protein of tomato. This is the first putative bi-directional gene promoter to be reported in the nuclear genome of a higher plant and studies on the structure and expression ORF-2 product will be reported.

X1-424 ISOLATION AND CHARACTERIZATION OF A cDNA FOR THE ABUNDANT RUBBER PARTICLE PROTEIN FROM *PARTHENIUM*. Z. Pan and R. A. Backhaus. Dept. of Botany, Arizona State University, Tempe, AZ 85287-1601.
Rubber, a polyisoprenoid whose biological function is unknown, occurs in over 2000 species. It accumulates within specialized, 1 μm particles in laticifers of *Hevea* and in the cytosol of non-laticiferous *Parthenium argentatum* parenchyma cells. Rubber synthesis occurs at the particle surface where the biosynthetic enzymes are localized. *Parthenium* possesses an abundant Rubber Particle Protein (RPP), which accounts for ca. 70% of the total protein in rubber. A full-length cDNA for RPP was cloned that encodes a 53,438-dalton protein whose sequence, deduced M_r and isoelectric point (pI 6.15) match authentic RPP. Sequence comparison shows that RPP shares little homology with any known prenyl transferase but does show homology with several cytochrome P450s, suggesting a possible related function. It is not known, however, whether RPP occurs in all rubber producing species.

X1-425 EXPRESSION OF A STEAROYL-ACYL CARRIER PROTEIN DESATURASE FROM *Brassica napus* IN RELATION TO TIME, TISSUE-SPECIFICITY AND HORMONES. Pietro Piffanelli, Steve Slocombe and Denis J. Murphy, Department of Brassica and Oilseeds Research, John Innes Centre, Colney Lane, Norwich NR4 7UH - UK

The soluble plastidic enzyme, stearoyl-ACP desaturase, is responsible for converting stearoyl-ACP (18:0-ACP) to the monounsaturated oleyl-ACP (18:1 *cis* -Δ^9-ACP). Recently, we reported the isolation of a cDNA (Bn9) encoding a stearoyl-acyl carrier protein (ACP) desaturase from *Brassica napus* and its temporal regulation during the embryo development. The transcript level for Δ^9 desaturase were shown to peak at 45 DPA (days post anthesis), in contrast with oleosin transcripts which peaked at 70 DPA. Hormonal regulation of the desaturase message was investigated by culturing immature zygotic embryos in basal media in presence of varying concentrations of different plant hormones. We cloned and sequenced a genomic clone of a Δ^9 desaturase. Expression of the desaturase promoter driving GUS in transgenic tobacco plants was studied and found to be evident in tissues producing TAG such as the embryo cotyledons, endosperm and pollen grains. A role for the promoter in temporal regulation of desaturase expression in the embryo was also established.

X1-426 EXPRESSION OF PLANT ACYL-ACP HYDROLASE IN *E. COLI*: EVIDENCE FOR FEEDBACK REGULATION OF FATTY ACID SYNTHESIS, Dusty Post-Beittenmiller1, Jan G. Jaworski2, Toni Voelker[3] and John B. Ohlrogge[4], 1) The S.R. Noble Foundation, Ardmore, OK 73402, 2) Chemistry Dept., Miami Univ., Oxford, OH 45056, 3) Calgene Inc., Davis, CA 95616, 4) Dept. of Botany and Plant Pathology, Michigan State Univ., E. Lansing, MI 48524

In order to probe regulation of the Type II fatty acid synthases found in plants and bacteria, *E. coli* were transformed with a cDNA encoding a plant medium chain acyl-acyl carrier protein (ACP) hydrolase (MCTE). The cells were grown from early to late log phase and total fatty acids, acyl-ACPs and biotin carboxylase carrier protein (BCCP) were analyzed at various timed intervals. MCTE expression in β-oxidation mutants (*fad D*) resulted in lauric acid accumulation in the growth medium, indicating that MCTE interfered with fatty acid synthesis by hydrolysis of C12 acyl-ACPs. Significantly, in contrast to control cells, fatty acids continued to increase in the medium after the culture approached stationary phase, indicating that fatty acid biosynthesis had become uncoupled from membrane biogenesis. They accumulated 18-fold higher levels of C12, but normal levels of long chain fatty acids. Furthermore, the total fatty acids accumulated were approximately 4-fold higher than the control levels. Immunoblot analyses of these cells revealed higher levels of malonyl-ACP and BCCP, both of which remained elevated until well into stationary phase. These data are consistent with elevated levels of acetyl-CoA carboxylase in the MCTE transformed cells. In addition, the long chain saturated acyl-ACPs (C14-C18) were reduced in these cells compared to the control cells. These data together suggest that a decrease in the long chain acyl-ACP products may relieve a feedback inhibition on FAS resulting in an increase in the rate of fatty acid synthesis. This feedback may be mediated through the acetyl-CoA carboxylase enzyme.

X1-427 MOLECULAR AND BIOCHEMICAL STUDIES OF OLEOSINS FROM SEEDS AND POLLEN OF HIGHER PLANTS, Joanne H.E. Ross, Ceri Batchelder, James S. Keddie, Michael R. Roberts, Rod J. Scott and Denis J. Murphy, Department of Brassica & Oilseeds Research, John Innes Centre, Norwich, NR4 7UH, United Kingdom

Oleosins are oil-body membrane proteins previously believed to be located only in the lipid storing tissues of seeds. It has recently been found that a new class of oleosins occurs in the megagametophytic generation of crucifers, where they are found associated with storage oil bodies of mature pollen grains. Sequence analysis suggests that these pollen-specific oleosins comprise a discrete group from, but share a similar origin to the seed-specific oleosin proteins.

The occurrence of oleosins in both sporophytic and gametophytic generations raises questions about the evolution of seed and pollen in higher plants. We are investigating the presence or absence of oleosin-like proteins in the spores of primitive plants, which have similar roles to both pollen and seeds. The functional similarity of seeds and pollen is reflected in their biochemical and structural similarity in crucifers. Both are organs sequestering storage lipids for mobilisation by the rapidly developing seedling/pollen tube. Also, both are capable of withstanding desiccation. This function has recently been correlated with the presence of oleosin in studies with fruits of olives and avocado. Although these fruits store large amounts of lipid, they do not contain detectable oleosin and neither are they able to withstand desiccation.

The regulation of seed oleosin gene expression has been studied using transformation of tobacco plants with a full length *B.napus* oleosin gene driven by 1kbp of its own promoter. The *Brassica* oleosin was correctly targeted to oil bodies in transgenic tobacco plants, as revealed by immunogold labelling. The gene also exhibited a temporal and tissue-specific expression similar to that observed in normal rapeseed development. In contrast, tobacco plants expressing an antisense oleosin construct exhibited gross abnormalities in oil body morphology but no other phenotypic changes.

X1-428 BIOSYNTHESIS OF SUGAR POLYESTERS, John C. Steffens, Gurdev S. Ghangas, Xiang Li, and Nancy T. Eannetta, Department of Plant Breeding, Cornell University, Ithaca NY 14853-1902

Glandular trichomes of many solanaceous species secrete poly-*O*-acylglucoses and -sucroses esterified with short- to medium- chain length fatty acids (C_4-C_{12}). These acylated sugars act as potent feeding deterrents against a wide range of insect pests. Biosynthesis of these compounds follows a novel route involving activation of free fatty acids via UDPG. UDPG:free fatty acid transglucosylation results in formation of 1-*O*-acyl-β-D-glucose. The 1-*O*-acyl-β-D-glucose molecule provides the group transfer potential necessary for acylation of nonanomeric positions of glucose in a series of transacylation reactions which result in formation of polyacylated glucoses. Progress on the characterization of UDPG:free fatty acid transglucosylase and 1-*O*-acyl-β-D-glucose:glucose transacylase will be discussed.

X1-429 BARLEY LIPOXYGENASE 1: cDNA CLONING AND GENOMIC MAPPING

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In germinating barley two lipoxygenase isoenzymes are present which differ in the product they form from linoleic acid as a substrate. Lipoxygenase 1 solely forms the 9-hydroperoxide as a product, whereas lipoxygenase 2 exclusively produces the 13-hydroperoxide. Our aim was to obtain a lipoxygenase specific cDNA clone from barley and to study the chromosomal location of the corresponding gene.

A bluescript cDNA expression library was made using mRNA from developing barley grains (cv. Triumph). Immunological screening with monoclonal antibodies which crossreact with both lipoxygenase 1 and 2 resulted in the isolation of a cDNA clone with a 2 kb insert (LoxA1).

The loxA1 clone most probably encodes part of barley lipoxygenase 1. Firstly, amino acid sequence data of a peptide derived from lipoxygenase 1 are identical to the amino acid sequence encoded by the loxA1 cDNA clone. Secondly, expression of the loxA1 clone in E. coli, resulted in the production of a protein, which was recognized by specific monoclonal antibodies directed against lipoxygenase 1, but not by antibodies specific for lipoxygenase 2.

The position of the loxA gene on the chromosome has been determined by RFLP analysis. It was shown to be located on chromosome 4.

Analysis of Genome Organization for Breeding and Gene Isolation; Modification of Complex Carbohydrate Composition in Seeds

X1-500 MOLECULAR TAGGING OF TOMATO SPOTTED WILT TOPOSVIRUS (TSWV) RESISTANCE DERIVED FROM *LYCOPERSICON PERUVIANUM*, Sergio H. Brommonschenkel[1], John J. Cho[2], Diana Custer[2] and Steve D. Tanskley[1], [1]Department of Plant Breeding, Cornell University, Ithaca-NY, 14850 and [2]Maui Research Center, University of Hawaii, kula, Hawaii, 96790.

Resistance introgressed into tomato from *L. peruvianum* provides the highest level of protection against TSWV isolates from several geographical locations. RAPD and RFLP techniques were used to identify molecular markers linked with TSWV resistance derived from *L. peruvianum* in hopes of employing these markers to facilitate the breeding process. In addition these markers can be used as starting points for cloning the resistance gene through the map-based cloning approach. DNA from two tomato lines differing in respect to TSWV resistance was screened for polymorphism using random 10 bp primers and PCR. Out of 408 primers tested, one showed a RAPD polymorphism that consistently differentiated resistant and susceptible lines. When mapped in our standard F_2 mapping population (Vendor $Tm2^a$ x *L. pennellii*) by RFLP linkage analysis, this RAPD cosegregated with CT71 on the long arm of the chromosome 9. By probing with CT 71 and flanking markers, we were able to show that the resistant line contained a introgressed segment of *L. peruvianum* DNA which included the distal portion of the long arm of chromosome 9 extending from TG8. To prove that TSWV resistance was indeed associated with this introgressed segment, an F_2 population of 585 plants segregating for TSWV resistance was generated. When scored against an isolate of TSWV, 472 plants were identified as resistant and 113 as susceptible. In this population, the TSWV resistance gene cosegregated with CT220 indicating a map distance of less than 0.01 cM (P=0.99). One TSWV resistant F_2 plant in which a recombination event replaced most of the proximal portion of the introgressed segment with *L. esculentum* DNA was identified. This plant will be useful for future breeding programs aimed to develop a TSWV resistant line with minimal linkage-drag. 'Stevens', a South African fresh market tomato cultivar that contains a single dominant TSWV resistant gene derived from *L. peruvianum* , has the same introgressed *L. peruvianum* DNA on the long arm of chromosome 9 as the TSWV resistant line that we have employed in our mapping studies.

X1-501 USING NEARLY-ISOGENIC LINES OF *AVENA SATIVA* TO IDENTIFY MOLECULAR MARKERS TIGHTLY LINKED TO GENES FOR CROWN RUST RESISTANCE, A. L. Bush[1], P. J. Rayapati[2], M. Lee[2], R. Wise[1,3] 1) Department of Plant Pathology, 2) Department of Agronomy, 3) USDA-ARS, Field Crops Research Unit, Iowa State University, Ames, IA 50011

Crown rust, the most important fungal disease of oat, is caused by *Puccinia coronata*. We examined nearly-isogenic lines of hexaploid oat (*Avena sativa*) to identify markers linked to genes for resistance to crown rust. The four lines of the current study are Y345 and X466 (recurrent parent C237), and D526 and D494 (recurrent parent Lang). The nearly-isogenic lines were created such that a unique resistance gene is present in the two different backgrounds; therefore these four lines encompass at least two different genes. Y345 and D526 are resistant to the ISU isolate of race 345 of *P. coronata*, while X466 and D494 are resistant to the ISU isolate of race 203. The resistant lines were backcrossed to their recurrent parents, and the resultant F_1's were selfed. Using restriction fragment length polymorphisms (RFLP), we have identified markers tightly linked to both resistance genes in F_2 populations segregating for resistance to the appropriate isolate. Particularly useful were those polymorphisms that occur in both lines bearing the resistance gene relative to the respective recurrent parent. Out of approximately 100 F_2 seedlings of each line, Y345 and D526 had respectively 2 and 0 recombinants between the resistance phenotype and RFLP marker, and X466 and D494 had 4 and 1 recombinants, respectively.

X1-502MOLECULAR APPROACHES TO RICE BLAST DISEASE,

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Rice blast is a major disease of rice caused by the fungus Pyricularia oryzae (Magnaporthe grisea). The fungus is known to be highly variable. We have initiated an analysis of the genome organization of this fungus in order to better understand the molecular basis of variability. Several repeat sequences representing different families have been isolated from the local isolates of the fungus. Some of these repeats are being used in indexing genotype and pathotype variability in this pathogen. We have also used RAPD analysis of several monoconidial isolates using random oligomers. Preliminary results indicate variability among monoconidial isolates from the same lesion and DNA rearrangements during passage through culture. One of the repeats shows homology to a retroviral element. Implications of these observations for variability as well as pathogenicity are being investigated.

We have also attempted to tag major genes for blast resistance using RFLP and RAPD analysis. Two populations involving the staminate parent carrying a single dominant gene for blast resistance have been analysed. In one of these populations, a BC_3F1 from a CO39xTongil cross, a total of 235 random primers was used for the RAPD analysis. The techniques of bulked segregant analysis was used. Six BC_3F1 blast resistant and six blast susceptible progenies were analysed. Nineteen distinct polymorphisms were detected in this initial survey. Two of these RAPDs appear to be putatively linked to the resistance genotypes and are currently being mapped on the rice genome.

X1-503 TRANSPOSON MUTAGENESIS IN PETUNIA USING ENDOGENOUS TRANSPOSABLE ELEMENTS

Ronald Koes, Erik Souer, Adele van Houwelingen, Leon Mur, Cornelis Spelt, John Wing, Francesca Quatrocchio, and Joseph Mol, Department of Genetics, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amsterdam, the Netherlands

With the aim of identifying and cloning genes controlling development and pigmentation of the *Petunia* flower we have initiated random transposon-mutagenesis experiments. Using a *Petunia* line in which transposable elements are highly active, we isolated mutants that are disturbed in flower development, flower pigmentation, embryo/seedling development, leaf development, leaf pigmentation, and plant growth (dwarfs).

To characterise the transposable elments responsible for these mutations we first analysed insertions in two flower pigmentation loci that were already cloned via other methods: *An3*, encoding the enzyme flavanone 3ß-hydroxylase (F3H) and *An13*, encoding an anthocyanin biosynthetic enzyme with unknown function. This indicated that the majority of mutations are caused by a single transposable element, *dTph1*. The observations that (1) exisions of *dTph1* from the *f3h* gene leave only rarely typical transposon footprints behind and that (2) somatic reversions of unstable *an3* alleles are prevented if they are made heterozygous with an *an3* deletion allele, suggest that the mechanism of *dTph1* excison may be related to the homologous-gap-repair model described for *P* elements of *Drosophila*.

In studying genes controlling plant development and flower pigmentation the transposon *dTph1* is exploited in two complementary ways: (1) isolation of *dTph1* tagged genes from mutants [the "conventional genetic" approach] and (2) knock-outs of genes with known sequence but unknown biological function ["reverse genetics"]. For the latter approach we have set up an assay based on the polymerase chain reaction (PCR) to identify individual plants carrying a *dTph1* insertion in the gene of interest among large populations of mutagenised plants. Such a plant is generally heterozygous for the *dTph1* insertion allele, but after selfing progeny is obtained in which 1/4 of the plants is expected to be homozygous for the mutant allele and these can be tested for a mutant phenotype.

We established a strategy to construct more permanent banks of insertion mutants, which is also applicable for other plant species with well defined endogenous (maize, snapdragon) or heterologous transposable elements.

X1-504 DELETIONS AND INVERSIONS IN PLANT CHROMOSOMES GENERATED BY CRE-LOX SITE-SPECIFIC RECOMBINATION, Scott L. Medberry, Minmin Qin, Emily C. Dale and David W. Ow, Plant Gene Expression Center, USDA, Albany, CA 94710 and Department of Plant Biology, University of California at Berkeley

Chromosomal deletions and inversions are useful genetic and breeding tools. Deletions offer the potential to identify and clone important genes while inversions can be used to reduce recoverable recombination. Unfortunately, deletions and inversions are often difficult to generate, detect and characterize. We are generating chromosomal deletions and inversions utilizing the bacteriophage P1 Cre-*lox* site-specific recombination system in order to more easily identify and define deletion and inversion events. The system involves 1) introduction of two *lox* sites (one within the non-autonomous transposon *Ds*) into the plant genome at one locus, 2) expression of the *Ac* transposase with subsequent movement of one *lox* site to a new locus, 3) expression of the Cre recombinase to mediate recombination between the two *lox* sites, and 4) generation of a distinguishable phenotype resulting from the rearrangement. Depending on the location and relative orientation of the two *lox* sites, chromosomal translocations, deletions, and inversions can be produced. This system has successfully generated inversions and deletions in plant chromosomes.

X1-505 ANALYSIS OF SITE-SPECIFIC RECOMBINATION AND TRANSPOSITION IN TRANSGENIC TOMATO PLANT AND *IN VITRO* RECOMBINATION ON ITS GENOMIC DNA, H.J.J. Nijkamp, J. Stuurman, J. Hille and M.J.J. van Haaren, Department of Genetics, IMBW, BioCentrum Amsterdam, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam

Transposon tagging is being used successfully now for the isolation of plant genes of which no product is known. The frequency with which a specific gene can be tagged is, however, low. Therefore we are trying to extend the transposon tagging strategy with the possibility to induce chromosomal deletions.

To reach this aim, we combine the use of a well defined transposable element system (*Ac/Ds* of maize) and the site-specific recombination system of bacteriophage P1 (Cre/*lox*). In our model plant tomato (*Lycopersicon esculentum*) the *lox* recombination sites are introduced via *Agrobacterium tumefaciens* mediated transformation. The transposable element system *Ac/Ds* is subsequently used as a tool to translocate recombination sites within the plant genome. Therefore one of the *lox* sites is located within a Ds element present on the T-DNA, while a second *lox* site is located on the same T-DNA outside the *Ds*-element. After trans-activation of the transposable element (*Ds-lox*) to different locations in the plant genome, recombination between the *lox* sites can be induced by introduction of the *cre*-gene, encoding the site-specific recombinase. Depending on the relative orientation of the *lox* sites compared to each other and the location of the two sites in the genome either a deletion, inversion or chromosomal translocation can be induced.

Here, we present the analysis of deletion events in the tomato genome and the work we did on the development of an *in vitro* cloning procedure for the deleted genomic DNA.

X1-506 MOLECULAR MARKERS FOR APOMICTIC REPRODUCTION: RFLPs, RAPDs, AND STSs, Peggy Ozias-Akins, Edward L. Lubbers and Wayne W. Hanna, Department of Horticulture and USDA/ARS, University of Georgia Coastal Plain Experiment Station, Tifton, GA 31793

Apomixis is asexual reproduction through seed, i.e., the seed-derived progeny of an apomictic plant are genetically identical to the maternal parent; thus, apomictic reproduction would preserve heterosis and uniformity in selfed hybrids. Several species in the genus *Pennisetum* reproduce through a form of apomixis termed pseudogamous apospory in which aposporous embryo sacs develop from nucellar cells. One aposporous species, *P. squamulatum* has been successfully crossed with cultivated pearl millet (*P. glaucum*) in an effort to transfer apomixis. Molecular markers tightly linked with apomixis potentially could accelerate the introgression of this trait into pearl millet, as well as provide a foundation for possible map-based cloning of the gene(s). An apomictic backcross plant (BC_3) has been used for genomic DNA library *preparation*. *Anonymous* probes from the BC_3 library have been screened for RFLPs against DNA from all genotypes present in the pedigree of BC_3. Restriction fragments common only to BC_3 and its apomictic parent, *P. squamulatum*, were considered informative. Some of the informative RFLP probes have been converted to sequence tagged sites which can be amplified in the polymerase chain reaction. Informative random amplified polymorphic DNAs also were found. Segregation of STSs and RAPDs in a BC_4 population indicated that at least two linkage groups from *P. squamulatum* were present in BC_3. Only one of the linkage groups was required to transmit apomixis and two of the molecular markers. One apomixis-linked marker also has been observed in a variety of apomictic *Pennisetum* species but no sexual species, which suggests a common origin for certain apomixis-associated chromosomal segments. Strategies proposed for mapping apomixis will be presented.

X1-507 CHARACTERIZATION OF TWO PUTATIVELY *Ac* TAGGED *ARABIDOPSIS THALIANA* MUTANTS , Pascual PEREZ, Gaelle BAUDOT and Denise GERENTES, BIOCEM / Groupe LIMAGRAIN, 24, avenue des Landais, 63170 Aubiere (FRANCE).

A major limitation to the use of the maize *Activator* element in the plant *Arabidopsis thaliana* has been the low transposition frequency of the unmodified element ; also we have attempted gene tagging by using modified *Ac* elements : Δ*Ac* and 35S*Ac* (Jean Finnegan *et al*, Plant Mol Biol 22 : 625-633, 1993). Somatic and germinal excisions were evaluated on 800 independent primary transformants (T_1 plants) and 120 corresponding T_2 progeny. 35S*Ac* is the most efficient with a germinal transposition frequency ranging from 0,2% to as high as 45%. 279 T_1 lines showing good transposition activity have been multiplied to generate T_2 and T_3 seed lots which have been screened for mutants affected in plant development. Among 23 well segregating mutants (color, embryo lethal and fertility) only two (3-A and 41-A) have a transposed 35S*Ac* tightly linked to the mutation ; other two are potentially tagged by the T-DNA.The first one, 3-A, a mendelian recessive mutation conferring a chlorina phenotype (lethal in soil), is 16,4 cM linked to the T-DNA where the 35S*Ac* element has excised. By southern analysis we have checked 49 F1 plants (36 +/m and 13+/+) and only all the heterozygotes presented a same transposed 35SAc element. Sequencing of IPCR flanking fragments demonstrates that the transposed element is deleted at its 5' and 3' ends, leading to a stabilized Ac which is able to transactivate a Ds element (kindly provided by G.Coupland).The analysis of different genomic and cDNA clones reveals that the transposon has inserted in the 5' leader of a developmentaly regulated 1Kb mRNA. As it is undetectable in leaves mRNA obtained from in vitro grown chlorina plants we believe that it is the gene responsible for the mutation. The second one, 41-A, exhibits a recessive male sterile phenotype where the microspore development is completely impaired just after their release from the tetrads ; although plant vegetative development seems completely normal. On one F2 population we have not observed any recombination between a specific transposed 35SAc and 45 male sterile plants. This element is still active somatically. Currently we are characterizing the corresponding wild type genomic region by standart procedures and we are studying more precisely its heritability because preliminary results suggest a transmission defect.

X1-508 GENETIC DIVERSITY IN SWEETPOTATO GERMPLASM ANALYZED BY DNA AMPLIFICATION FINGERPRINTING, **C. S. Prakash[1], Guohao He[1], and Robert L. Jarret[2] , [1]Tuskegee University, School of Agriculture, Milbank Hall, Tuskegee, AL 36088; [2]USDA/ARS, Regional Plant Introduction Station, 1109 Experiment St. Griffin, GA 30223.**

DNA Amplification Fingerprinting (DAF) is a rapid and powerful approach for genetic resource characterization that has numerous uses in the study and management of plant germplasm including analysis of genetic diversity and population structure, identification of duplicate accessions in germplasm collections, biosystematic studies, and determination of outcrossing rates. In this study, 28 octamer primers were tested for their ability to provide informative DAF profiles using genomic DNA of sweetpotato (*Ipomoea batatas* (L.) Lam.). Eight of the 28 primers tested were found to be suitable for DAF analysis of sweetpotato germplasm and were used to generate profiles for 100 accessions of sweetpotato including U. S. cultivars and New and Old World landraces. Great genetic variability was evident among the sweetpotato landraces. In contrast, most of the U. S. cultivars were relatively monomorphic for their PCR products, confirming the previously expressed concerns for the narrow genetic base of U. S. sweetpotato cultivars. Several primers resulted in the generation of genotype-specific DAF profiles. DAF profiles were highly reproducible with little variation in banding patterns between duplicate runs.

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X1-509 RECOMBINATION AND HIGH RESOLUTION MAPPING OF THE *Mla* POWDERY MILDEW RESISTANCE LOCUS IN BARLEY, Roger P. Wise[1,2,3], Richard A. DeScenzo[1,2], and Mamatha Mahadevappa[2,3]. [1]Field Crops Research, USDA-ARS, [2]Department of Plant Pathology, and [3]Interdepartmental Genetics Program, Iowa State University, Ames, IA 50011

In barley (*Hordeum vulgare* L.), the *Mla* locus conditions reaction to the powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei*. Two-hundred seventy individual recombinant isolines, each representing an independent recombination event between the flanking *Hor1* and *Hor2* loci, were used to construct a high density RFLP map of the *Hor1/Mla/Hor2* region. The population was screened with 35 cDNA and genomic clones, that had been shown to map near *Mla* on barley chromosome 5 or on wheat group 1. RFLP mapping data placed 10 of the 35 markers within the *Hor1/Hor2* interval, establishing a high density map of the *Mla* region. Eight of the remaining markers were placed outside of the interval, proximal to *Hor1*. Six of the recombinant isolines had regions of DNA which were heterozygous at one or more RFLP loci within the *Hor1/Hor2* interval. In addition, five of the probes used in this study hybridized to multiple sites. The barley cDNA clone, BCD249, hybridized to two loci 0.4 cM apart (bcd249.1 and bcd249.2), indicating a sequence duplication event within the *Hor1/Hor2* interval. Data from the high resolution RFLP map of the *Hor1/Hor2* region was integrated with previous recombination data which positioned the *Mla6*, *Mla13*, and *Mla14* resistance alleles, in relation to *Hor1* and *Hor2*. Recombination among *Mla* alleles was analyzed by inoculating the recombinant isolines with three isolates of *E. graminis*; A27, MK24-76, and CR3, which recognize specific *Mla* alleles. Recombination within the *Mla* complex produced *mla6-mla13* susceptible recombinants accompanied by reciprocal non-parental flanking marker exchange. An unequal crossover model is presented to account for the two types of flanking marker exchange.

X1-510 STARCH BIOSYNTHESIS IN POTATO TUBERS. Michael M. Burrell[1], Stephen A. Coates[1], Lee J. Sweetlove[2], David O'Reilly[1] and Tom ap Rees[2]. [1]Advanced Technologies (Cambridge) Ltd.,Science Park,Cambridge CB4 4WA,UK. [2] Department of Plant Sciences, Downing Street, Cambridge, CB2 3EA, UK.

The pathway of starch biosynthesis involves the synthesis of adenine diphosphoglucose (ADPG) from glucose-1-phosphate in the amyloplast. It is believed that the glucose-1-phosphate is imported directly from the cytosol. The ADPG is then used by starch synthases to form the α -(1->4) glucan polymer which is the major component of starch. How the synthesis of starch in a potato tuber is regulated is not understood. One potential step for regulation is the enzyme ADPG pyrophosphorylase (EC 2.7.7.27) which synthesises ADPG. Several metabolites allosterically regulate the enzyme from both plant and bacterial sources. To study the importance of this enzyme in the regulation of the pathway we have introduced into potato two forms of the enzyme from E. coli which differ in their response to metabolic intermediates. The effect of different amounts of these different forms of the enzyme on starch biosynthesis will be presented.

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X1-511 FRUCTAN ACCUMULATION IN TRANSGENIC PLANTS, Michel J.M. Ebskamp, Ingrid M. van der Meer, Elizabeth A.H. Pilon-Smits, Peter J. Weisbeek and Sjef C.M. Smeekens, Department of Molecular Cell Biology, University of Utrecht, Padualaan 8, 3584 CH UTRECHT, The Netherlands

Plants can use alternative carbohydrate storage forms. Most plants employ starch in plastids and sucrose in the vacuole as non structural carbohydrates. Some plant species can also use fructan (polyfructose molecules) in the vacuole for storage. Fructan can be used as a source for fructose, for the production of low calory sweeteners and in many non-food applications (1). But most of these plants produce only low molecular weight fructan and have other unfavourable properties.

To obtain transgenic plants which produce high molecular weight fructan, we used bacterial fructosyltransferases; one of this fructosyltransferases makes fructan of the levan type (2-6 linkage), the other of the inuline type (2-1 linkage). Both fructosyltransferases make high molecular weight fructan. We have modified the genes of these fructosyltransferases and fused them to targeting sequences for different cellular compartments: to the vacuole, the apoplast and the cytosol. Expression of the constructs is directed by plant regulatory sequences. We have introduced these fructosyltransferase constructs in tobacco, potato and other plants.

We found stable fructan accumulation in plants transformed with either of both genes and with all different localization signals. Analyses of the fructan revealed that it is indeed high molecular weight fructan. Analyses of the potato plants transformed with the vacuolar constructs revealed an increase in non structural carbohydrates from 7% in untransformed to 35% in transgenic plants. With these plants we want to study the source-sink relations and mechanisms which govern the interaction between sinks.

(1) A. Fuchs, Ed., (1993) Elsevier, Amsterdam.

X1-512 EXPRESSION OF THE MAIZE BRANCHING ISOZYMES IN *Escherichia coli*, Han Ping Guan and Jack Preiss,Department of Biochemistry, Michigan State University, East Lansing, MI 48824

Starch branching enzyme (SBE) plays a major role in the synthesis of amylopectin, therefore, alternation of the proportion and/or properties of SBE and/or starch synthase could modify the structure and properties of starch. In order to facilitate the study of their specific roles in starch synthesis, the genes coding for the mature SBEI and SBEII of maize endosperm have been separately expressed in *Escherichia coli* using the T_7 promoter. Each of the expressed SBE was purified and characterized. The expressed SBE showed similar properties to the enzyme purified from developing maize endosperm. The expressed SBE activity was efficiently neutralized by antibody prepared against maize SBE, but not by the antibody prepared against the *E. coli* glycogen branching enzyme. Preliminary results indicate that maize SBEI is mainly involved in synthesis of the B chains of amylopectin while SBEII plays a major role in the synthesis of the A chains. At present, we are using the bacterial expression system as a tool to study the structure-function relationships of the maize branching isozymes with respect to their roles in starch synthesis.

X1-513 ISOLATION OF BARLEY ENZYMES THAT SHOWS STARCH BRANCHING ENZYME ACTIVITY *IN- VITRO*

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Starch is composite of amylose and amylopectin. Amylose is an essentially linear α-1,4-glucan with few α-1,6 branch points whereas amylopectin is highly branched. Starch branching enzyme (SBE or Q-enzyme), is responsible for the synthesis of amylopectin by catalyzing the hydrolysis of α-1,4 bonds and the transfer and rebinding of the released glucan chain at an α-1,6 position. SBE has been isolated from a variety of plants and tissues and varies in size from 40 -114 kDa. SBE from barley seeds were purified by FPLC and four active fractions were detected. The fractions separated into several forms upon further purification. Two forms of 51 kDa and 50 kDa from one fraction and another form of 80 kDa from another fraction were studied in some detail. The branching enzyme activity of the low molecular weight SBE was demonstrated in several ways: 1. two different activity assays, 2. Spectrophotometric scanning of iodine-starch or -amylose complexes showed the decrease in iodine staining observed in the amylose-branching assay was indeed caused by the production of amylopectin, 3. SBE gel assays and 4. TLC analysis confirmed that the assayed activity was due to α-1,6 linkage formation. The N-terminals for all three forms were blocked. V8, Lys C and CnBr cleavage of the lower molecular weight forms were performed and N-terminal sequences obtained. Microsequencing of the Lys C cleaved fragments of the 80 kDa fraction was also performed. The sequences showed little homology to any known SBE sequences or other formerly sequenced barley peptides. Peptide mapping and amino acid sequence analysis indicated that the 51 kDa and 50 kDa forms are related. The nature of the relationship is not yet established. Assays for phosphorylation, glycosylation and ribosylation proved negative but other forms of possible post-translational modification remains to be investigated.

X1-514 MODIFICATION OF STARCH METABOLISM IN TRANSGENIC POTATO PLANTS, Jens Kossmann, Gernot Abel, Volker Büttcher, Ruth Lorberth, Ivar Virgin and Lothar Willmitzer, Institut für Genbiologische Forschung GmbH Berlin, Ihnestr. 63, 14195 Berlin, Germany

Starch is the major storage compound in potato tubers. It is composed of linear (Amylose) and branched (Amylopectin) glucans. The synthesis of amylose is abolished in mutants defective for granule bound starch synthase I. The formation of amylopectin however is not as well understood. We are undertaking experiments to elucidate the synthesis of the branched glucans with respect to factors determining the degree of branching and side chain length distribution. To this end cDNAs encoding enzymes which are possibly involved in starch metabolism are cloned and expressed in antisense orientation in transgenic potato plants. This strategy gives rise to the possibility to analyse starch synthesis in the absence of the respective gene and the resulting effects on starch structure. Furthermore we are expressing the cDNAs in glycogen forming microorganisms in order to analyse the influence of the activity on the structure of the reserve carbohydrate, which is a complementary approach to analyse enzymes of potato starch metabolism in a *in vivo* system.

X1-515 MANIPULATION OF THE STARCH BIOSYNTHETIC ENZYMES TO ALTER STARCH QUANTITY AND QUALITY IN PLANTS, J. Preiss*, H.P. Guan*, Y. Libal-Weksler*, M.N. Sivak*, Y.-y. Charng*, L.N. Bloksberg* and T.W. Okita#, *Dept of Biochemistry, Michigan State University, East Lansing, MI 48824, USA, #Inst of Biological Chemistry, Washington State University, Pullman, WA 99164 USA.

Synthesis of starch occurs by three enzymatic reactions. First, ADPglucose (ADPGlc) synthesis is catalyzed by ADPGlc pyrophosphorylase (ADPGlc PPase). Second, the glucosyl portion of ADPglucose is transferred to an α-1,4 glucan primer for synthesis and elongation of the α-1,4 glucosyl chain via starch synthase catalysis. Finally, the branching enzyme (BE) transfers a portion of the elongated α-1,4 glucosyl chain to form the α-1,6 branch points present in amylopectin (and to small extent, in amylose). Regulation of starch synthesis in photosynthetic as well as in non- photosynthetic tissues, occurs mainly at the ADPGlc PPase step where 3-phosphoglycerate allosterically activates ADPGlc synthesis and orthophosphate is an allosteric inhibitor. The amount of and structure of starch in a plant are dependent on the catalytic properties of the three enzymes mentioned above. Thus, alteration of enzyme amounts and their properties will in turn, affect the properties of the starch synthesized. Transformation of certain plants, eg.,potato, with a bacterial ADPGlc PPase allosteric mutant gene has dramatically increased their starch content (Science (1992) 258, 287-292). The increase in starch amount can also be affected by modifying the regulatory properties of the ADPGlc PPase either making it insensitive to Pi inhibition or active in the absence of the activator, 3PGA. Alteration of the proportion or amounts of branching enzyme and/or starch synthase may affect the structure and physical properties of the starch synthesized. Thus, the branching isoenzymes and soluble starch synthase isozymes of maize endosperm have been purified free of amylolytic activity, so their properties and roles in the synthesis of amylopectin and amylose with respect to chain elongation and the nature of branch chain transfer can be determined. Studies suggest that maize BE I isoenzyme is mainly involved in synthesis of the B chains of amylopectin while BE IIa and IIb are involved in the synthesis of the A chains. We have isolated the complete or almost complete cDNA clones encoding maize endosperm BE I and BE II and have expressed their activity in *E. coli*.. Moreover, the cDNA clones encoding the large and small subunits of the potato tuber ADPGlc PPase and the genomic DNA representing the *Anabaena* ADPGlc PPase have been expressed in *E. coli*.. Site-directed mutagenesis of these genes have enabled us to characterize their glucose-1-phosphate and allosteric activator binding sites.

X1-516 ROLE AND FUNCTION OF SUCROSE TRANSPORTERS FROM HIGHER PLANTS

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In higher plants sucrose is the major form of photoassimilates produced in mature leaves, which is used for transport *via* the phloem to sink organs such as tubers and roots. In order to assess how carbohydrates are distributed within the plant, we try to create mutants by overexpression or antisense repression of genes involved in transport processes.

To this end a yeast complementation system was used to isolate sucrose transporters. A yeast strain was designed, which is invertase-deficient and expressing the potato sucrose synthase in the cytoplasm. This strain was transformed with plant cDNA libraries. Selection on sucrose as the carbon source led to the isolation of cDNA clones from potato, sugar beet and spinach, which were further analysed. The transporter showed a saturable sucrose uptake activity. The uptake is sensitive to protonophores and to sulfhydryl group modifying agents. Analysis of the sequence support the hypothesis that it encodes a membrane transport protein. The expression of the gene is confined mainly to source leaves. *In situ* hybridization experiments showed the expression of the gene in minor veins of mature leaves. Antisense repression of the protein in transgenic potato plants leads to reduced growth and decreased root and tuber formation accompainied by an accumulation of carbohydrates within the leaf. Transgenic plants overexpressing the protein in different tissues are under investigation.

X1-517 INHIBITION OF THE STARCH-BRANCHING ENZYME GENE IN BARLEY CELLS BY ANTISENSE CONSTRUCTS, P. Sathish , Sun Chuanxin, Anna Deiber and Christer Jansson, Department of Biochemistry, Arrhenius Laboratories for Natural Sciences, Stockholm University, Stockholm 106 91, Sweden

Starch is the major reserve carbohydrate found in the endosperm of barley. Like other starch, barley starch is composed of essentially a linear α-1,4 glucan molecule called amylose and a branched amylopectin. Starch-branching enzyme (SBE) introduces the α-1,6 branch points in the linear α-1,4 glucan chain. Often, more than one gene for SBE has been cloned from a variety of plants. We have cloned a sbe gene from barley using the Rapid Amplification of cDNA Ends (RACE) technique. mRNA from 10 day post anthesis barley endosperms were isolated by the phenol:chloroform procedure. A part of the mRNA was reverse transcribed using poly $d(T)_{12-18}$ primers and MoMLV reverse transcriptase. The first strand cDNA was then amplified as four small fragments using with or without degenerate primers in conjecture with specific primers. The gene specific primers for this cloning technique was prepared using the rice sbe-I sequences found in genbank. The cloned sbe gene from barley had high homology to rice, maize and potato sbe genes. The clone was later constructed in full in the phagemid pBluescript SK-. The activity of the cloned gene was further confirmed by performing functional complementation studies in glycogen branching enzyme mutant *E. coli* and yeast cells (gifts from Drs. Vos-Scheperkeuter and John Cannon respectively). Full and partial length antisense sbe vectors were constructed in the vector pACT-1F (gift from Dr. R. Wu) containing a rice actin promoter, a ß-glucouronidase coding reporter region, and a *nos* terminator sequence. Barley cell suspensions, calli and immature embryos were transformed with the antisense sbe constructs using a particle bombardment delivery system. Transient expression was monitored by *Gus* assays.

X1-518 THE GENE FAMILY OF ADPGLUCOSE PYROPHOSPHORYLASE SUBUNITS. Brian J. Smith-White and Jack Preiss; The Genetics Program and the Department of Biochemistry, Michigan State University, E. Lansing.

The cDNAs corresponding to the tissue-specific isoforms of ADPglucose pyrophosphorylase have been isolated from libraries originating from different tissues of various plants. These have been have been characterized by nucleotide sequence determination. The encoded proteins show grouping into 4 classes on the basis of primary structure. All C3 angiosperm plants examined seem to possess all classes in the genome. For most plants, all classes appear to be present as a single copy in the genome. The gene for one of the classes has been isolated from *Arabidopsis thaliana*.

Late Abstracts

RAPD-PCR ANALYSIS OF THE GENOME OF TRANSGENIC RICE (ORYZA SATIVA L.) AND OF ITS PROGENY

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We have produced transgenic rice plants (*Oryza sativa* L., cv Chinsurah Boro II, an Indica-type variety) by treating protoplasts with a plasmid carrying the hygromycin phosphotransferase (hph) gene and grown progeny of fertile plants under strictly controlled self-pollination conditions for three generations.

The following questions were asked. (a) Do the in vitro manipulations produce unwanted and deleterious genomic changes? (b) What is the fate of the foreign gene in the progenies of transgenic plants?

The transgenic nature of surviving plants was controlled by PCR analysis and Southern blotting. Analysis of progeny from many independently derived transgenic rice families showed that the foreign gene is inherited for at least three generations through meiosis. In some cases the gene was lost, possibly due to Mendelian segregation.

A RAPD-PCR analysis with 10 different random oligonucleotide primers was performed on the DNA of 3 different transgenic plants and on their progenies, up to the third generation. The amplified DNA sequences were analysed by agarose gel electrophoresis. Relationships among the tested DNA samples was tested by band sharing analysis and expressed in principle coordinate plots. Control DNA preparations from embryogenic cell suspension cultures were also assayed.

The results evidenced limited but evident sequence and/or structural changes in the genome of transgenic plants. These are inherited in the progeny and may justify the production of morphologically modified and/or sterile plants in connection with the in vitro culture of transgenic protoplasts.

ASSEMBLY AND PROCESSING OF 11S GLOBULINS,

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The 11S globulins are abundant storage proteins found in seeds of higher plants. They are synthesized and assembled during a complex process that involves both post-translational modification events and transport of precursors through several intracellular compartments. As an aid in dissecting this process, an *in vitro* synthesis and assembly system was developed that results in the formation of trimers equivalent in size to oligomers found in endoplasmic reticulum. Assembly of 11S proglobulin trimers is dependent upon ATP. This observation, together with other evidence, is consistent with the hypothesis that molecular chaperones are involved in the assembly of proglobulin trimers.

The proglobulin subunits in trimers are cleaved post-translationally at an Asn-Gly bond that has been conserved during evolution. This cleavage is required for assembly of trimers of proglobulin subunits into hexamers of mature subunits. Proteolytic activity has been detected in developing soy bean seeds that is capable of cleaving this bond. The preponderance of this activity is due to glycosylated proteins, although a small amount of the total activity appears associated with a non-glycosylated peptide. To study the specificity of the proteases that are glycosylated, mutant proglobulin subunits or peptides were constructed in which amino acids around the conserved Asn-Gly bond were modified, and then modified subunits were assembled into trimers and subjected to proteolysis. Deletion of the P1 asparagine, or substitution of aspartate or glutamine for the asparagine, eliminated the ability of the protease to cleave the proglobulins. Conservative changes of the amino acids on the COOH-terminal side of the cleavage site (P1'-P7' positions) seemed not to hinder the proglobulin processing into acidic and basic subunits. In contrast to the normal 11S propeptides found in trimers, unmodified monomers, unfolded or malfolded normal 11S proglobulins, fusions with CAT, and several high methionine mutants, are cleaved into small peptide fragments by the purified, glycosylated protease. Our result demonstrate that cleavage sites other than the conserved Asn-Gly bond found in unmodified proglobulin subunits are inaccessible to the protease when they are contained in trimers.

ENGINEERING OF BACILLUS THURINGIENSIS TOXINS AND PROTEINASE INHIBITORS FOR DURABLE PLANT RESISTANCE TO CROP PESTS, Ruud A. de Maagd, Dirk Bosch, Maarten Jongsma, Bert Schipper, Hilde van der Klei, Petra Bakker, Jeroen Peters and Willem Stiekema. Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, NL-6700 AA Wageningen, The Netherlands.

Expression of B.t. toxins or proteinase inhibitors in transgenic plants and an integrated application of these can confer resistance to insect pests of economically important crop plants. Our research has concentrated on the mode of action of these agents and on developing strategies for their improvement. We have produced chimeric B.t. toxins consisting of the N-terminal part of cryIC and the C-terminal part of cryIE, and vice-versa, by in vivo recombination. The majority of cross-overs occured in the C-terminal part, i.e. domain III. Analysis of the toxicity of the resulting chimeric toxins to *Manduca sexta* (Tobacco Hornworm), *Mamestra brassicae* (Cabbage looper), and *Spodoptera exigua* (Florida moth) suggested that in cryIC domain III is responsible for its extended specificity range (toxic to all three insects) compared to cryIE (toxic to *M. sexta* only). The main gut proteinases of three important crop pests, *Liriomyza trifolii* (leaf miner), *Frankliniella occidentalis* (thrips) and *Spodoptera exigua* were characterized and were shown to belong to three different mechanistic classes. These proteinases are currently purified for further study and use in selection of efficient inhibitors. For the development of a procedure for such a selection, Potato inhibitor II (PI-2) was genetically fused to the N-terminus of the phage f1 coat protein gp3 for use in a phage display system. Phages expressing this fusion protein showed specific binding to chymotrypsin, a natural ligand for PI-2. Following mutagenesis of the binding site of PI-2, these phages will be used for biopanning to select for mutant proteinase inhibitors with increased specificities for the aforementioned insect gut proteinases.