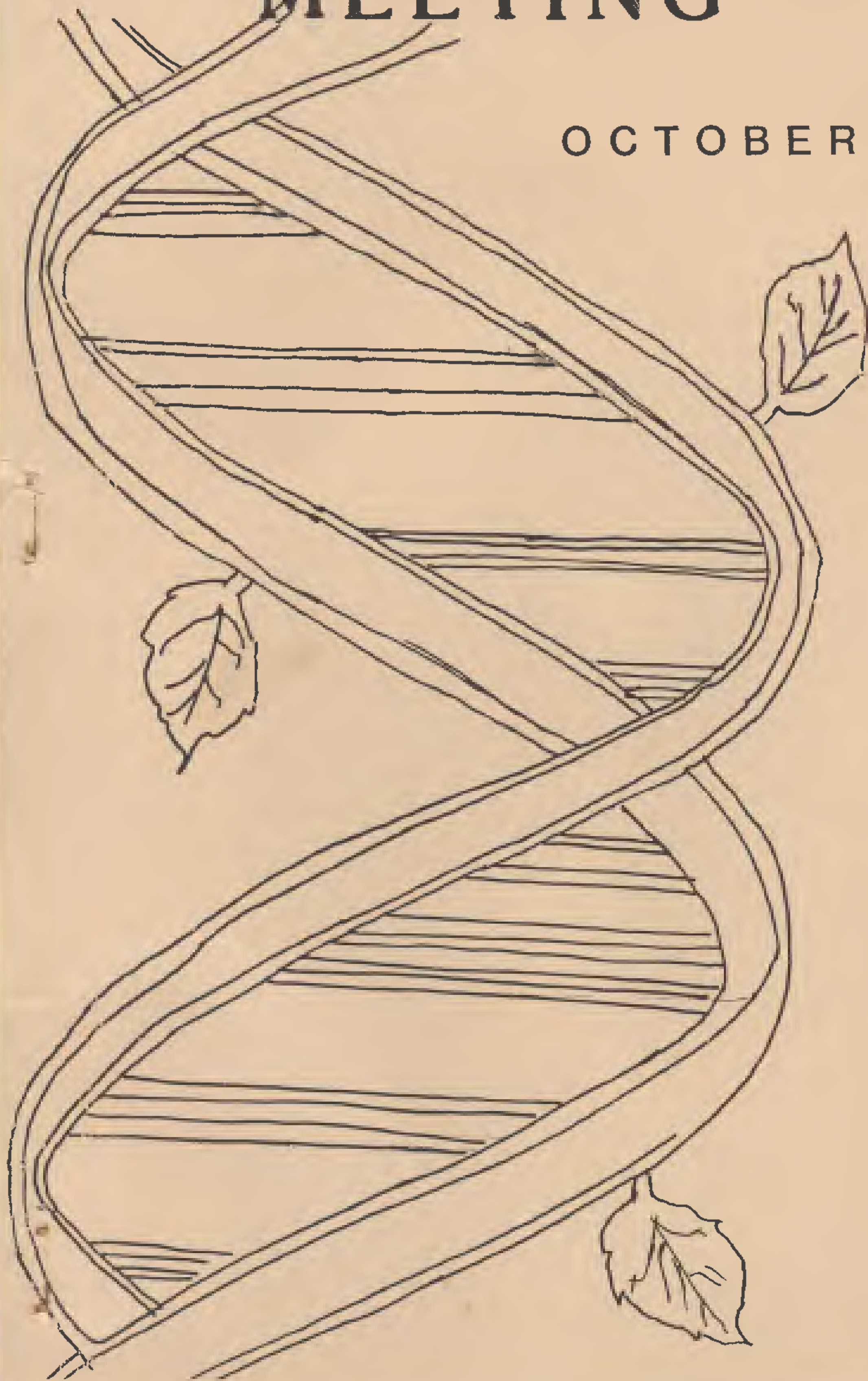
MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY MEETING

OCTOBER 16 & 17, 1986



THIRD ANNUAL MEETING

MID-ATLANTIC PLANT MOLECULAR BIOLOGY SOCIETY

OCTOBER 16 & 17, 1986

Beltsville Agricultural Research Center
Beltsville, MD

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INTRODUCTION

Welcome to the annual meeting of the Mid-Atlantic Plant Molecular Biology Society. This Society was formed to provide a forum devoted to the exchange of ideas and information concerning plant molecular biology. Hopefully, the Society will ensure scientists in the Mid-Atlantic region of a high quality, accessible and affordable plant molecular biology meeting each year. The Society especially wishes to encourage graduate student and postdoctoral participation in presenting oral reports, posters, and contributing to discussions.

Since its inception two years ago, the MAPMBS has grown to have over 200 members. It is now on a firm financial foundation with over $3800 in the bank and it has been recognized by the IRS as a non-profit institution. Next year's meeting will be held at the University of Maryland Baltimore County (UMBC), Catonsville, MD and will be organized by Drs. Lynn Zimmerman and Madeline Wu. Continued support and participation by all of its members is needed and I urge you to help Drs. Zimmerman and Wu with the 1987 meeting. There is much to be done.

On Friday at 12:00 noon, there will be a brief business meeting, which the entire membership is urged to attend. Changes and suggestions for program, format and location of the 1988 meeting are invited.

The MAPMBS meeting this year would not be possible without the efforts of many people. I especially thank Joel Chandlee and Jon Lindstrom (Registration); Candice Collmer, John Hammond, Janet Slovin and Sue Mischke (Program); Lynn Zimmerman and Lorin DeBonte (Funding); Larry Wenko, Sally McCammon and Jim White (Abstracts); Rosemarie Hammond and Andy Kalinski (Publicity). Their efforts are appreciated very much.

BENJAMIN F. MATTHEWS
Chairman
Organizing Committee

GENERAL INFORMATION

All sessions will be held in building 003 of the USDA at Beltsville, MD. Oral presentations will be held in the auditorium, while posters and exhibits will be held in the lower lobby outside the auditorium and will extend down the hallways. Refreshments will be available in the lobby during breaks. There were too few members who desired box lunches, so it was impractical for us to provide lunch services. However, there are many restaurants and fast food places near the USDA along Route 1.

EXHIBITORS

We wish to thank the exhibitors for their displays and financial support, which helped to make this meeting possible. Please visit with the technical representatives to find out how they can help you with your research.

FISHER SCIENTIFIC CO.	Paula Blanchard
INTERNATIONAL BIOTECHNOLOGIES, INC.	Dick Vento
QUEUE SYSTEMS, INC.	Kim Glover
TM ANALYTIC, INC.	Robert Waite
WATERS	Laura Thompson

PROGRAM SCHEDULE

THURSDAY, OCTOBER 16

Time	Speaker / Event	Title	Page
8:00	Assemble Posters		
8:30 - 8:45	Introduction		

SESSION I - Convenor - Janet Slovin

Time	Speaker / Event	Title	Page
8:45 - 9:40	Roger Beachy	DNA sequences that regulate expression of embryo specific genes in transgenic plants.	1
9:40 - 10:00	Jon Lindstrom	Expression of the soybean (*Glycine max*) seed lectin gene in transformed plants of *Nicotiana plumbaginifolia* and *N. tabacum*.	2
10:00 - 10:20	Larry Wenko	The isolation of a gene encoding diaminopimelate dehydrogenase from *Oryza sativa*.	3
10:20 - 10:40	Exhibitors		
10:40 - 11:00	Break*		
11:00 - 11:55	Prem Das	Differential gene expression in maize endosperm.	4
11:55 - 12:15	Kamaruzaman bin Mohammad	The time-course of zein degradation in the endosperm of germinating maize seeds.	5

SESSION II - Convenor - Lorin DeBonte

Time	Speaker / Event	Title	Page
12:15 - 1:15	Lunch		
1:15 - 2:10	David Lonsdale	Cytoplasmic male sterility and the mitochondrial genome.	6
2:10 - 2:30	Frank Turano	The cytochrome oxidase subunit II g from *Daucus carota* contains an intron.	7
2:30 - 2:50	Adina Brieman	Organization of nuclear and mitochondrial genes in progenies of wheat and barley plants regenerated from tissue culture.	8

			Page
2:50 - 3:30		Posters / Exhibits / Break*	
3:30 - 4:25	Autar Mattoo	Inta-membrane translocation and palmitoylation of specific chloroplast proteins.	9
4:25 - 4:45	Peter Straub	SDS soluble protein patterns in salt (NaCl) treated suspension cultures of three halophytes.	10

FRIDAY, OCTOBER 17

SESSION III - Convenor - John Hammond

8:30 - 9:25	Roger Beachy	Resistance to virus infection in transgenic plants.	11
9:25 - 9:45	Paul Miller	Electroporation of potato protoplasts.	12
9:45 - 10:05	Mohammed Aly	A simple system for plant cell microinjection and culture.	13
10:05 - 10:25	Break*		
10:25 - 11:20	Sondra Lazarowitz	Molecular characterization of geminiviruses.	14
11:20 - 11:40	John Hammond	Similarities between the capsid protein amino acid sequences of bean yellow mosaic virus and other potyviruses.	15
11:40 - 12:00	Ramon Jordan	Analysis of antigenic specificity of monoclonal antibodies to several potyviruses.	16
12:00 - 12:15	Business Meeting		
12:15 - 1:15	Lunch		

SESSION IV - Convenor - Sue Mischke

1:15 - 2:10	Dean Gabriel	Specifificity in host-parasite interactions.	17
2:20 - 2:30	Joyce Loper	Role of a fluorescent siderophore in biocontrol of *Pythium ultimum* by *Pseudomonas fluorescens 3551*.	18

			Page
2:30 - 2:50	Break		
2:50 - 3:10	Ann Callahan	Molecular investigation of ripening.	
3:30 - 3:50	Ann Smigocki	The cytokinin ipt gene under transcriptional control of the CaMV 35S promoter induces shoots on tobacco and cucumber galls.	19
3:30 - 3:50	Andy Kalinski	DNA Topoisomerase activity of transcriptionally active/ inactive barley aleurones.	20
3:50 - 4:10	B. D. Kim	Four-stranded DNA and inter-coiled DNA:Intermediates of recombination.	21

DNA sequences that regulate expression of embryo-specific genes in transgenic plants.

R. N. Beachy, Z. L. Chen, S. Nieto, Department of Plant Biology, Washington University, St. Louis, Missouri.

β-conglycinin is a major seed storage protein of soybeans and is comprised of three types of subunits referred to as: the α'-, α-, and β-subunits. The expression of the genes encoding these subunits is tightly regulated through plant development and during the development of the soybean embryo. Genes encoding the α'-subunit and β-subunit have been isolated and characterized. Each has been expressed singly in transgenic tobacco and petunia plants. The level of expression of the two genes, however, is markedly different as is the timing of the expression of the two genes during development of tobacco embryos, and in a manner similar to the expression of the genes during soybean seed development. A series of promoter deletion mutations were produced and each of the mutant genes was introduced into petunia plants. The results of these experiments demonstrated that sequences between -150 and -250 5' of the structural α' gene were required for expression of this gene. Within that sequence elements have been identified that are suspected of conferring high levels of tissue specific gene expression. When the α' and β gene are introduced together into petunia plants, the proximity of the α' and the β gene promoters governed the level of expression of the two genes. When the promoters were separated by approximately 4 kb, expression of the genes was similar to the expression when the genes were introduced individually. However, when the promoters were approximately 1.5 kb apart, the level of expression of the β-subunit gene was increased by 10-15 fold. However, the timing of the expression of the β gene was not altered. These results indicate that the level of expression of these genes is separable from the temporal regulation of the expression of these genes.

EXPRESSION OF THE SOYBEAN (Glycine max) SEED LECTIN GENE IN TRANSFORMED PLANTS OF *Nicotiana plumbaginifolia* AND *N. tabacum*.

Lindstrom, J.T., R. Harding, R. Goeken and L.O. Vodkin. USDA-ARS, Plant Molecular Genetics Lab, Bldg. 006 Room 105, Beltsville, MD 20705

Two species of *Nicotiana*, *N. plumbaginifolia* and *N. tabacum* cv. 'Xanthi' were transformed by an *Agrobacterium tumefaciens* Ti plasmid construct containing a 5.5 kb BglII fragment of soybean DNA which includes the seed lectin gene, *Le1*. Leaf disks were infected with *Agrobacterium* and shoots regenerated from transformed disks were selected for their ability to root in the presence of kanamycin and to produce nopaline. Transformed plants were established in the greenhouse and allowed to seed. Soybean lectin mRNA was detected in immature seeds beginning nine days after anthesis and continuing to seed maturity (18-21 days after flowering in *N. tabacum*). Soybean lectin protein was detected in immature seeds twelve days after flowering. No soybean lectin mRNA or protein was detected in non-seed tissue of tobacco. In four of five *N. tabacum* plants assayed, *Le1* was inherited as a single dominant gene and in the fifth as a multiple insertion. Developmental and organ specific control of *Le1* was maintained in transformed tobacco plants.

THE ISOLATION OF A GENE ENCODING DIAMINOPIMELATE DEHYDROGENASE FROM *Oryza sativa*. L. K. Wenko and G. W. Schaeffer. USDA, ARS, Plant Molecular Genetics Laboratory, Rm. 116, Bldg. 011-A BARC-W, Beltsville, MD 20705.

Plaque hydridization of the previously isolated soybean diaminopimelate dehydrogenase gene fragment to a rice EMBL-3 genomic library yielded three clones. Upon restriction endonuclease digestion the three clones were found to be identical containing a 12.38 kb rice DNA insert. Further hybridization resulted in localization of the rice diaminopimelate dehydrogenase gene on a 2.1 kb Hind III-Hind III fragment. Subcloning of this 2.1 kb gene containing fragment into the pGEM-3 vector has been completed. Sequence analysis and examination of *in vitro* transcription products from the rice gene fragment are currently in progress. Transformation of the plasmid containing the rice diaminopimelate dehydrogenase gene yielded positive complementation in the *E. coli* auxotroph DP50. Diaminopimelate dehydrogenase enzyme activity was also demonstrated in fifteen day post anthesis embryos from *Oryza sativa*. Demonstration of enzyme activity and isolation of the rice gene has allowed postulation of the existence of the diaminopimelate dehydrogenase shunt for lysine biosynthesis in rice.

Prem Das and Joachim Messing, Waksman Institute of Microbiology, Rutgers University, P.O. Box 759, Piscataway, NJ 08854

We have studied the structure and developmental expression of the genes coding for the 27kD maize storage protein. Analysis of cosmid clones derived from the inbred line W22 shows that there are only two tandemly linked genes that code for this zein. Each gene is part of a 12kb duplication unit, the two copies of which are separated by a 2.5kb spacer. Sequence analysis shows that 2.8kb of the 5' regions of the 2 genes are 99.95% homologous, the coding sequence is 99% homologous and the 3' region has 97% homology. Further, the downstream gene has a unique 1.7kb element inserted 1kb from the stop codon. In spite of the extensive homology, the two genes are expressed at different levels during endosperm development. Allelic variation exists at this locus, with the lines A188, W22 and W23 sharing the structure described while W64A, B37 and BSSS53 have only one copy of the gene. Studies are under way to elucidate the mechanism for differential expression of these genes.

The Time-Course of Zein Degradation in the Endosperm of Germinating Maize Seeds

Kamaruzaman bin Mohammad and Asim Esen
Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA

Zein, the prolamin of maize, is a group of alcohol soluble proteins and is the major storage protein in maize endosperm. The time-course of zein degradation in the endosperm was monitored in greenhouse-germinated seeds up to 26 days after germination (DAG) by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Electrophoretic analysis showed both qualitative and quantitative changes in zein profiles starting on the second DAG. Of the three major zein classes (alpha-, beta- and gamma-zeins), gamma-zein (27 kD zein) was the first to be degraded, and the degradation was complete by the third DAG. The second component to be degraded was beta-zein (17 and 18 kD zeins), beginning on the third DAG and continuing gradually thereafter. A minor zein component (10 kD zein) began to be degraded on the fourth DAG. The last component to be degraded was alpha-zein (22 and 24 kD zeins) which began to be degraded on the fifth DAG. Some of the alpha-zein components appeared to be stable and were still present even up to the 26th DAG. The degradation of zein polypeptides was closely associated with the appearance of numerous new low molecular weight fragments on the SDS-PAGE.

THE MITOCHONDRIAL GENOME AND CYTOPLASMIC MALE STERILITY IN PLANTS

DAVID LONSDALE & WILLIAM ROTTMANN

Molecular Genetics Unit, Plant Breeding Institute, Cambridge, U.K.

Cytoplasmic male sterility (CMS) is common to many higher plant species (1). It is maintained in natural breeding populations by nuclear genotypes which supress the effects of the cytoplasm and allow normal pollen development and pollen shed to occur. Such genes are known as Restorer genes (Rf). Cytoplasmic male sterility is one of the common genetic defects associated with intergeneric/interspecific crosses (2,3) or cell fusion regenerants (4,5). In maize the Rf genes of the 3 male sterile cytoplasms are non-complementary. This suggests that the CMS phenotype results directly from an incompatibility between mitochondrial and nuclear gene products, perhaps involving one of the inner membrane complexes involved in electron transport and ATP synthesis.

By analysing the fertile cytoplasmic revertants of the T male sterile cytoplasm of maize a region of the mitochondrial genome involved in fertility reversion has been identified by restriction endonuclease mapping and sequencing.

The mitochondria of the male sterile T-cytoplasm of maize synthesise a unique polypeptide of 13 KD. The synthesis of this polypeptide is reduced by the Rf genes (6) and cannot be detected in the fertile revertants (6). Restriction endonuclease analysis of the mitochondrial genome of the male sterile and fertile revertants identified a region present in the male sterile genome which was deleted from the fertile revertants. Sequencing and transcript analysis of this deleted region revealed a highly transcribed ORF. It seems likely that this ORF may code for the unique 13 KD polypeptide.

The role of additional polypeptides which characterise CMS mitochondrial polypeptide profiles, for example the 13 KD polypeptide of CMS-T, will be discussed.

1 Edwardson, J.R.(1970).Bot.Rev.36:341.
2 Connolly, V. & Wright-Turner, R.(1984).Theor. Appl. Gen.68:449.
3 Burnham et al.(1981).Crop Sci.21:659.
4 Galun et al.(1982).Mol. Gen. Genet.186:50.
5 Medgysey et al.(1985).Theor. Appl. Genet.70:590.
6 Dixon et al.(1982).Theor. Appl. Genet.63:75.

THE CYTOCHROME OXIDASE SUBUNIT II GENE FROM *DAUCUS CAROTA* CONTAINS AN INTRON

Frank J. Turano (1,2), Lorin R. DeBonte (1), Kenneth G. Wilson (2), and Benjamin F. Matthews (1). (1) USDA-ARS Plant Molecular Genetics Laboratory, Beltsville, MD 20705 and (2) Department of Botany, Miami University, Oxford OH, 45056.

Introns in the cytochrome oxidase subunit II (COXII) gene in plant mitochondrial DNA (mtDNA) have been observed only in monocots. The COXII gene in dicots investigated to date do not contain an intron. This is the first report of an intron in the COXII gene of a dicot.

The carrot COXII intron was verified by restriction mapping and hybridization using specific maize and wheat COXII probes. A comparison of the restriction map of the carrot COXII gene with the restriction maps of the COXII genes from pea, *Oenothera*, maize, wheat, and rice revealed that the carrot and wheat restriction maps are similar. Hybridization data show that regions of the carrot COXII intron are homologous to the maize COXII intron and homologous to the wheat COXII intron.

ORGANIZATION OF NUCLEAR AND MITOCHONDRIAL GENES IN PROGENIES OF WHEAT AND BARLEY PLANTS REGENERATED FROM TISSUE CULTURE: Adina Breiman, Department of Botany, Tel Aviv University, Israel.

Variability among plants which were regenerated from tissue culture was termed somaclonal variation and was vastly documented on the cytogenetic, morphological and biochemical level. The occurrence and frequency of the event is a debated issue ranging from reports on uniformity to 40% variation.

Progenies of plants regenerated from scutellar callus of Triticum aestivum and Hordeum spontaneum were analyzed for the organization of nuclear and mitochondrial genes. The nuclear marker chosen was the intergenic spacer of the rRNA gene located at the site of the nucleolar organiser region (NOR loci). Cosmids representing contiguous 40 kb wheat mtDNA fragments and clones of mitochondrial DNA coding sequences were used as specific probes for detecting variation in mtDNA organization. Wheat and barley DNA were digested with the restriction endonuclease Taq 1 and probed with two plasmids pTA71 and pHV293 which are cloned fragments of the intergenic spacer and rRNA genes of wheat and barley. Thirty eight progeny plants of two wheat cultivars and twenty progeny plants of four barley cultivars were found to be stable in the organization of the NOR loci, whereas three progenies of one wheat cultivar and three progenies of one barley accession showed reduction in the number of repeats in the integenic spacer. Since no variation in the NOR loci could be revealed in the control wheat and barley plants, the reduction in the spacer length is a result of in vitro culture. The presented results demonstrate that tissue culture induces heritable changes in repeated sequences (intergenic spacer) during a short period of time, a comparable event occurring through several generations in evolution.

Analysis of mitochondrial DNA organizatrion in the wheat and barley progenies revealed a lack of variation. Although the occurrence of repeated sequences and recombination among them was documented in wheat, it appears that the passage through tissue culture does not cause any changes in the organization of mtDNA in the sexually produced seeds. Possible causes for these results will be discussed.

INTRA-MEMBRANE TRANSLOCATION AND PALMITOYLATION OF SPECIFIC CHLOROPLAST PROTEINS

Autar K. Mattoo, Franklin E. Callahan and Marvin Edelman
Plant Hormone Laboratory, USDA/ARS, Beltsville, Md. 20705, and Department of Plant Genetics, The Weizmann Institute of Science, Rehovot, Israel

Dynamic membrane events in the metabolic history of the 32kDa herbicide binding protein, a regulatory component of photosystem II, have been elucidated. The protein is synthesized as a membrane-associated 33.5kDa precursor within the chloroplast. We show that membrane attachment of the precursor and processing to the 32kDa form occur in the unstacked stromal lamellae. Once processed, the 32kDa protein translocates, within the thylakoids, to the topologically-distinct stacked granal lamellae. Another photosystem II component, the 30kDa protein (probably D-2), is similarly translocated from stromal to granal lamellae.

An additional new feature is posttranslational palmitoylation of the processed forms of several chloroplast proteins, viz., the 32kDa protein, light harvesting chlorophyll a/b protein, acyl carrier protein and the large subunit of ribulose-1, 5-bisphosphate carboxylase/oxygenase. Protein acylation is light stimulated. For the 32kDa PSII protein, this modification takes place in a membrane-protected domain and is mainly confined to the protein assembled in the granal lamellae where functional photosystem II centers are concentrated. Potential role(s) for protein acylation in targetting of chloroplast proteins will be discussed.

Peter F. Straub and John L. Gallagher. College of Marine Studies, University of Delaware, Lewes, De 19958. SDS SOLUBLE PROTEIN PATTERNS IN SALT (NaCl) TREATED SUSPENSION CULTURES OF THREE HALOPHYTES.

A unique 26 Kd protein and numerous quantitative changes in protein patterns have been detected in tobacco cell cultures in response to salt (NaCl) treatment. This study looked for analogous proteins in native halophytes. Callus cultures were initated in Distichlis spicata, Sporobolus virginicus and Kosteletskya virginicus from seed, rhizome, and seedling hypocotyl respectively on a Murashige and Skoog medium with 1 mg/l of each 2,4-D and IAA. Callus cultures started on solidified media were transfered to a liquid formulation of the same medium and agitated at 100 rpms on a gyratory shaker. Cell suspensions were maintained by serial transfer (2:5) to fresh medium at 7-10 day intervals. Cells were harvested by vacumn filtration on #2 paper filters, washed, homogenized with 0.05M tris-HCl pH-6.8 and 1% SDS. Protein concentration was measured with the Bradford assay after TCA precipitation. Proteins were run on a 12% SDS discontiuous polyacrylamide system and stained with coomassie blue. Protein bands were recorded and quantitated by densitometry. No unique protein in the 26 Kd region was detected although there were some quantitative differences in protein patterns noted in each of the species.

Resistance to virus infection in transgenic plants.

R. N. Beachy, P. Powell-Abel, R. E. Nelson, S. McCormick, and R. T. Fraley. Department of Biology, Washington University, St. Louis, Missouri; and Monsanto Company, St. Louis, Missouri.

Tobacco and tomato plants were transformed with a disarmed strain of *Agrobacterium tumefaciens* carrying a chimeric gene containing the coat protein gene of the common strain of tobacco mosaic virus (TMV) driven by the promoter for the 35S RNA transcript from cauliflower mosaic virus. Transgenic plants were examined for the numbers of chimeric genes introduced and for levels of RNA and protein that accumulated therein. R_1 seedlings derived from self-pollinated transgenic plants were subsequently inoculated with TMV and the response of the seedlings to infection was noted. Transgenic plants exhibit a degree of resistance to TMV as measured by several features: 1) there was a reduction in the number of sites of infection in plants expressing the coat protein gene compared to those that did not express this gene; 2) spread of virus from the site of infection to non-inoculated leaves was significantly delayed in plants that expressed the coat protein gene compared to those that did not; 3) resistance was effected against several strains of TMV as well as several strains of tomato mosaic virus; 4) the level of resistance was closely correlated to the level of expression of the chimeric gene. These and other results will be discussed and compared with resistance afforded by the phenomenon of cross-protection which has been used for many years by plant pathologists to provide a degree of disease resistance.

EFFECTS OF PROTOPLAST SOURCE AND BUFFER ON ELECTROPORATION EFFICIENCY. Paul D. Miller and Stephen L. Sinden, GH 13, Bldg. 011, BARC-W, USDA, ARS, Vegetable Laboratory, Beltsville, MD 20705

Mesophyll protoplasts from potato (Solanum, six varieties), chinese cabbage (Brassica campestris), and protoplasts from carrot (Daucus carota) cell suspension cultures have been electroporated with plasmid coding for chloramphenicol acetyltransferase (CAT). Transient gene expression has been detected in all genotypes (potato >> carrot >> chinese cabbage) with levels as high as 0.1 unit/10^5 protoplasts, relative to purified E. coli CAT activity. In an attempt to optimize plasmid uptake, we found that the replacement of Na^+ by Li^+ in the electroporation buffer gives significant enhancement of electroporation efficiency. In order to estimate the number of protoplasts taking-up plasmid, potato protoplasts were electroporated with plasmid coding for potato spindle tuber viroid (PSTV). Of 177 calli derived from these protoplasts 40 were shown to be infected as determined by hybridization probing of dot blots of calli extracts. None of 65 calli were infected when protoplasts were incubated with plasmid but not electroporated. These data indicate that at least 23% of the protoplasts were infused with at least one copy of the plasmid.

A SIMPLE SYSTEM FOR PLANT CELL MICROINJECTION AND CULTULRE.

Mohammed A. M. Aly and Lowell D. Owens, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Maryland, 21228, and U.S. Department of Agriculture, Agricultural Research Service, Tissue Culture and Molecular Biology Laboratory, Beltsville, Maryland, 20705.

Microinjection of plant protoplasts and cells has been recently reported, however, a system that combines simplicity of design, harmless immobilization, high resolution visibility and ability to monitor individual target cells is lacking. This report describes a system which combines all these features. It consists of a microinjection-microculture dish containing immobilized protoplasts and a simple chamber that maintains sterility and humidity during injection. Highly purified protoplast preparations are plated at low population density as a thin monolayer of widely separated cells embedded in agarose layered over a thicker (0.2 mm at center to 1 mm at edge) layer of agarose-solidified medium. This physical arrangement allows for rapid location, mapping and injection of the immobilized protoplasts and also their subsequent location for growth monitoring. The double layers of agarose provide adequate nutrition for culturing injected cells to the microcalli stage. In addition to protoplast injection this system was also used to inject three- to four-day old nonspherical cells derived from protoplasts. Colony formation rates from injected protoplasts and from injected cells with regenerated walls were equivalent to those of uninjected controls. Furthermore, tobacco protoplasts stored at 4°C in liquid medium for up to two weeks remained fully competent for plating and injection. These cold-stored protoplasts, when injected, formed colonies at rates similar to those from fresh preparations. The ability to store protoplasts without loss of viability considerably increases the ease and convenience of cell injection experiments.

Cells were injected with plasmid pMON200 DNA carrying a chimeric gene for kanamycin resistance and the nopaline synthase gene. Colonies regenerated from injected cells did not exhibit resistance to kanamycin or nopaline synthesis. Currently we are investigating whether injected DNA integrated into the plant genome.

THE MOLECULAR CHARACTERIZATION OF GEMINIVIRUSES. Sondra G. Lazarowitz and Inara B. Lazdins, Department of Embryology, The Carnegie Institution of Washington, Baltimore, MD 21210.

The geminiviruses are unique eukaryotic viruses characterized by paired icosahedral virions and a genome of circular single-stranded DNA. Members of this family are transmitted either by whiteflies or by leafhoppers, each subgroup having distinct biological properties. The whitefly-transmitted geminiviruses, with characteristically restricted host ranges, have bipartite genomes consisting of two distinct ~2.8 kb DNA components. Members in this subgroup have been reported only in dicotyledenous plants. The leafhopper-transmitted geminiviruses have surprisingly broad host ranges and genomes consisting of only a single ~2.7 kb DNA component. Members of this latter subgroup have been found in monocotyledenous and dicotyledenous host plants. As an approach to understanding viral determinants of pathogenesis and host range, we have been analyzing the geminiviruses causing squash leaf curl disease and maize streak disease.

In squash leaf curl disease we have identified two different, but highly homolgous, Squash Leaf Curl Viruses which have the bipartite genomic organization typical for whitefly-transmitted geminiviruses. SqLCV-NR and SqLCV-BR can be distinguished by the structure of their cloned genomic components and their host range properties, the former having a characteristically narrow host range and the latter unexpectedly having a broader host range. Sequence analysis of the Homology Regions of these two viruses, a region thought to contain important cis-acting sequence elements, and the flanking potential gene coding sequences demonstrates that these two viruses are very closely related to each other evolutionarily and/or functionally. The Homology Regions are almost identical to each other except for two short sequences. The few sequence differences in the Homology Regions of SqLCV-NR and SqLCV-BR may be important in their different host range phenotypes. Our results are consistent with the Homology Region playing an important role in the determination of host range properties in geminiviruses.

For the leafhopper-transmitted Maize Streak Virus (MSV) we have identified a single genomic DNA component of ~2.7 kb consistent with the results of other groups analyzing different strains of MSV. Sequence analysis has identified four potential gene coding regions and a region equivalent to the Homology Regions of the whitefly-transmitted viruses. Based on the molecular analysis of these and other whitefly- and leafhopper-transmitted geminiviruses a picture is emerging of the evolution of these plant pathogens and their potential as vectors for the introduction and expression of exogenous genes in plants.

SIMILARITIES BETWEEN THE CAPSID PROTEIN AMINO ACID SEQUENCES OF BEAN YELLOW MOSAIC VIRUS AND OTHER POTYVIRUSES John Hammond [1], Ramon L. Jordan [1] and Rosemarie W. Hammond [2], USDA-ARS, [1] Florist and Nursery Crops Laboratory, and [2] Microbiology and Plant Pathology Laboratory, Beltsville Agricultural Research Center, Beltsville MD 20705.

A cDNA clone obtained from the 3' end of bean yellow mosaic virus (BYMV) RNA contains the complete capsid protein gene, which is expressed as a fusion protein from the plasmid vector, pUC9. The fusion protein is reactive with polyclonal rabbit antisera and some mouse monoclonal antibodies raised against purified BYMV.

Nucleotide sequencing of the clone expressing the fusion protein, and an overlapping clone containing a part of the capsid protein gene, have allowed comparison of the deduced amino acid sequence with those obtained in other laboratories for the capsid proteins of related potyviruses. There are extensive amino acid sequence homologies with the capsid proteins of pepper mottle virus, potato virus Y, tobacco etch virus, and tobacco vein mottling virus. The conservation of long stretches of the amino acid sequence is presumed to be necessary to preserve the three-dimensional structure of the capsid protein, which must interact with the viral RNA and other capsid protein subunits to form the virion. The differences between the capsid proteins of the different viruses probably reflects the evolutionary distance between the viruses; many of the substitutions are conservative. The major differences are at the amino terminus of the capsid protein, which probably protrudes from the external surface of the virion and hence would be less critical in preservation of the subunit structure.

Comparison of the amino acid differences and cross-reactivities of monoclonal antibodies with different potyviruses are being examined in order to help determine the epitopes recognized by the monoclonal antibodies. Deletions from the expressing clone will be used to further this analysis.

ANALYSIS OF ANTIGENIC SPECIFICITY OF MONOCLONAL ANTIBODIES TO SEVERAL POTYVIRUSES. Ramon Jordan and John Hammond. USDA-ARS, Florist and Nursery Crops Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705.

Using as immunogen a mixture of potyviruses [including six strains of bean yellow mosaic virus (BYMV) isolated from various bulb crops (gladiolus, iris, tulip), iris mild mosaic virus, iris severe mosaic virus, and potato virus Y], forty-three potyvirus specific monoclonal antibody secreting hybridoma cell lines were generated. All 43 monoclonal antibodies (McAbs) react with at least one BYMV isolate. Twenty of the McAbs react only with one or more of the BYMV isolates, whereas the remaining 23 McAbs react with a BYMV isolate and with at least one of the other 20 potyvirus isolates tested.

Most of the McAbs gave higher reactivity values in indirect ELISA with dissociated antigens than with intact virions, indicating that these McAbs are specific to antigenic sites not found on the surface of intact virions. Approximately 27 different coat protein epitopes can be defined with the 43 McAbs based on antigen specificity using the 26 different potyvirus isolates. The reactivities of these McAbs indicates the presence of conserved antigenic determinants common to members of the potyvirus group previously characterized as serologically distinct or unrelated. One of the McAbs recognizes an epitope apparently conserved on all potyviruses tested to date. This McAb has been shown to be useful in the detection of potyviruses in infected plants, as well as for the detection and characterization of fusion proteins expressed in bacteria containing cloned inserts of the viral coat protein gene.

SPECIFICITY IN HOST PARASITE INTERACTIONS

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Phenotypic analyses of induced and naturally-occurring variation in plant pathogens reveals at least four functionally distinct classes of genes: parasitism, pathogenicity, host-range specificity and race specificity (ASM News 52:19-25). Work in our lab has focused on the genetics of both host-range specificity and race specificity in the bacterium Xanthomonas campestris. This species exhibits a large amount of relatively stable variation in both types of specificity. For example, there are over 125 listed pathovars of the species listed in Bergey's Manual, each exhibiting a unique host range. In one of the pathovars, malvacearum (Xcm), there are 16 different known avirulence (A) genes, giving a possible 2^{16} = 65,536 races of this pathovar alone.

We cloned ten A genes from Xcm, and demonstrated that five of them specifically interact, gene-for-gene, with individual R genes in congenic cotton lines (PNAS 83:6415-9). Some A / R gene interactions appeared qualitatively different from others, suggesting that the physiological mechanism(s) of gene-for-gene specified incompatibility may be unique to the interactive gene pair. All A genes appeared to be chromosomally determined, and several of them appear to have the equivalent of virulence (a) alleles. The genetics of race specificity in this phytopathogenic bacterium appeared in all respects to be identical to that found in phytopathogenic fungi. Recently, three of the A genes were found to be tightly linked in a 5.7 kb cluster, which has been physically mapped. Two open reading frames have been identified on this cluster by making lacZ gene translational fusions.

Different pathovars of X. campestris are indistinguishable by common microbial tests, and the number of genes involved in host range selection is unknown. We screened genomic Southern blots of total DNA from over 76 strains of 23 pathovars for restriction fragment length polymorphisms (RFLPs), using cosmid sized clones (~35 kb inserts) from various pathovars as probes. The probes revealed RFLPs that were pathovar-specific genetic markers for nearly all pathovars tested. Pv. citri proved to be exceptional in that it appears to be comprised of at least four distinctive RFLP groups. Furthermore, a group of isolates responsible for the recent citrus canker outbreak in Florida comprised a separate RFLP group which more closely resembled pv. alfalfae than any of the other three known pv. citri groups. In greenhouse tests, the Florida canker isolates (but not other pv. citri RFLP groups) were pathogenic on both bean and alfalfa (the host range of pv. alfalfae). This unexpected relationship revealed by RFLP analyses, and other evidence, suggests that host range specificity may be mutable, unstable, and not based upon race specificity (avirulence) gene interactions.

ROLE OF A FLUORESCENT SIDEROPHORE IN BIOCONTROL OF *PYTHIUM ULTIMUM* BY *PSEUDOMONAS FLUORESCENS* 3551. J. E. LOPER, SOILBORNE DISEASES LABORATORY, AGRICULTURAL RESEARCH SERVICE, U.S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND 20705, U.S.A.

Fluorescent siderophores produced by rhizosphere pseudomonads are reported to play a role in the suppression of several fungal plant pathogens. *Pseudomonas fluorescens* strain 3551, which was isolated from the rhizosphere, produces a fluorescent siderophore, protects cotton seed from colonization by *Pythium ultimum* and controls pre-emergence damping-off caused by *P. ultimum*. The role of fluorescent siderophore production by strain 3551 in its antagonism against *P. ultimum* was investigated. Fourteen non-fluorescent (Flu^-) *Tn5* insertion mutants of *P. fluorescens* 3551 were obtained following matings with *E. coli* SM10 (pSUP1011). Strain 3551 grew on an iron-deficient medium whereas the 14 Flu^- derivatives did not. Southern analysis of *EcoR1* digested genomic DNA from these mutants probed with nick-translated λ::Tn*5* confirmed the presence of single insertions of Tn*5* in the chromosomal DNA of each derivative strain. The insertions fell into five size classes with *EcoR1* fragments of 17.5, 12.5, 11.1, 9.6 and 8.6 kb. Cotton seeds treated with a fungicide standard, strain 3551, or derivative Flu^- strains were evaluated for colonization by *P. ultimum* 24 hr following planting into infested soil or for subsequent seedling emergence. While the parental strain 3551 decreased cotton seed colonization by *P. ultimum* and increased emergence to a level statistically equivalent to a standard chemical seed treatment, Flu^- derivative strains did not. Differences between 3551 and Flu^- derivatives were greater with respect to emergence than *P. ultimum* colonization. No consistent differences were observed between strain 3551 and Flu^- derivatives with respect to their growth rates or population sizes in the cotton spermosphere. These results indicate that fluorescent siderophore production by *P. fluorescens* 3551 contributes to its antagonistic activity against *P. ultimum*.

THE CYTOKININ ipt GENE UNDER TRANSCRIPTIONAL CONTROL OF THE CaMV 35S PROMOTER INDUCES SHOOTS ON TOBACCO AND CUCUMBER GALLS.

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The 5' regulatory region was removed from the isopentenyltransferase (ipt) gene cloned from pTiB6S3. The truncated gene was then inserted downstream from the CaMV 35S promoter in the binary plasmid vector Bin 19. This construct and the unmodified ipt gene, also in a binary vector, were transferred to *A. tumefaciens* strain LBA4404 (pAL4404) and used to infect three species of tobacco plants and cucumber hypocotyls. After infections with the 35S promoter-ipt gene construct, galls and shoots were observed within 7 to 10 days on cucumber plants and within 21 to 33 days on tobacco plants, respectively. No shoots were observed on cucumber plants following infection with the binary vector carrying the unmodified ipt gene or with tms328::Tn5, the shooty mutant strain carrying ipt on the Ti plasmid. However, shoots were observed on *N. tabacum* galls induced by tms328::Tn5 some 63 days following infection. Cucumber galls and shoots displayed traits introduced by the binary vector, i.e., resistance to G418 (10 ug/ml) and growth on medium without added cytokinins. It appears that the morphogenic potential of transformed plant cells can be altered by increasing the promoter strength of a gene directing synthesis of a morphogenic phytohormone.

DNA TOPOISOMERASE ACTIVITY OF TRANSCRIPTIONALLY ACTIVE/INACTIVE BARLEY ALEURONES: A. Kalinski and G.R. Chandra, Plant Molecular Genetics Laboratory, USDA-ARS, Beltsville, Md. 20705

We have been studying the changes in structural organization of barley chromatin accompanying hormonal activation of specific genes in aleurone cells. We have recently investigated the endogenous nucleases of aleurone nuclei and the integrity of active/inactive α-amylase genes (A. Kalinski et al., 1986). This report is the continuation of our research on DNA enzymes that may play a regulatory role in hormonal regulation of genes expression in aleurone cells.

We have found that the level of DNA topoisomerase activity (enzymes controlling the supercoiled state of DNA) in aleurone tissue is related to the activation of transcription process regulating gene(s) expression. The lowest level of DNA topoisomerase activity was found in dry and 0.5 mM cordycepin treated aleurones when total RNA synthesis is low or blocked. In water imbibed aleurones the level of topoisomerase activty increases 25 fold and in the tissue treated with gibberellic acid 195 fold as compared to aleurones with RNA transcription blocked by cordycepin. Since after the treatment of aleurones with 1 μM gibberellic acid many genes including α-amylase are actively transcribed, it suggests the involvement of DNA topoisomerase in regulation of genes expression in aleurone cells.

Approximately 50% of DNA topoisomerase activty was detected in mononucleosomes (7-8% of total chromatin) released from aleurone nuclei during the process of autodigestion. Aleurone DNA topoisomerase is a fraction of a nonhistone high mobility group proteins found in the 0.35 M NaCl extract of purified mononucleosomes.

Kalinski, A., Chandra, G.R., and Muthukrishnan, S. 1986. J. Biol. Chem. (in press).

FOUR-STRANDED DNA AND INTERCOILED DNA: INTERMEDIATES OF RECOMBINATION: B.D. Kim, Department of Plant Sciences, University of Rhode Island, Kingston, RI 02881

Direct and inverted repeat sequences are known to play an important role in transposition and other celluar functions. What is not clearly understood is how DNA molecules actually recognize and align themselves at the homologous sequences and undergo recombination.

The possibility that native DNA can accomodate intramolecular intercoiling in the major grooves and subsequent four-stranded base pairing of the homologous sequences is suggested from a collection of EM data of secondary structures of DNA. Novel DNA configurations, namely; "lasso", rigid linear, loop, stem, stem-and-loop, and rolling circles were found in the native mtDNA preparations of pearl millet. Stem and key ring structures of pBR322 were also found when incubated with pearl millet mtDNA preparations. Some of these molecules were considered *in vivo* intermediates of recombination and replication. It is indicated that native DNA is flexible enough to make sharp 90° bending and 180° turnabout for intramolecular intercoiling. This secondary structure of DNA has novel features for protein recognition and recombination.

To examine the feasibility and the consequences of four-stranded DNA in recombination, a space-filling model (Academic Press/ Molecular Design) has been constructed using atomic bond angles of B-DNA (Drew *et al*., 1981). When the physical support of the helix axis was removed, the B-DNA model was found to be flexible to bending and twisting with its original B-DNA atomic bond angles unaltered. A stem-and-loop and a loop structures were constructed by intraduplex intercoiling in the major grooves and by McGavin (1971) type four-stranded base pairing of the inverted and direct repeats, respectively. The four-stranded DNA maintains 10.5 base pairs per turn, 34 A pitch and 22 A diameter. The four-stranded DNA further facilitated recombinations of the stem-and-loop and the loop models to result in inversion and deletion, respectively, as predicted for transposable elements. Three steps of intramolecular recombination: 1) four-stranded base pairing, 2) base pair exchange, and 3) strand excnange, are described. Use of the stem-and-loop structure in intermolecular recombination is also described.

TRANSCRIPTION IN THE REPLICATION ORIGIN (ORIA) OF CHLAMYDOMONAS REINHARDTII CHLOROPLAST DNA: Chung-Hwa Chang and Madeline Wu, Department of Biological Sciences, University of Maryland Baltimore County, Catonsville, Md 21228

In studies of many DNA replication systems, e.g. E. coli oriC and lambda DNA, it is found that transcription is involoved in the regulation of the initiation process. Transcription proceeds into ori region may alter DNA configuration and favor protein binding. RNA also may serve as primer for DNA replication. We demonstrate here one replication origin region of Chlamydomonas reinhardtii chloroplast DNA shows transcription activity. A 1.05 kb restriction fragment containing one chloroplast DNA replication origin (oriA) of C. reinhardtii was cloned and sequenced. One open reading frame (ORF) of 136 amino acids overlaps the D-loop region. Northern blot hybridization of oriA containing fragment to total RNA isolated from asynchronized vegetative cells showed that the ORF region undergoes transcription. The coding strand of ORF has been used to hybridize total RNA followed by S1 nuclease mapping of the 5′ end and 3′ end of stable transcripts. Total of eight 5′ ends and five 3′ ends all with different abundancy are localized. A small portion of RNAs coincide with the boundaries of ORF whereas the majority of RNAs have their 5′ and 3′ ends inside ORF. The smallest possible species is about 167 nucleotides in length. We do not know yet what roles these RNAs play during the cell cycle of algal growth. Whether the short RNAs are involved in the initiation of DNA replication will be investigated.

ISOLATION AND CHARACTERIZATION OF B-GLUCOSIDASE FROM MAIZE

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B-GLUCOSIDASE (B-D-GLUCOSIDE GLUCOHYDROLASE, EC 3.2.1.21, B-GLU) CATALYSES THE HYDROLYSIS OF THE LAST DEGRADATION PRODUCT OF CELLULOSE, CELLOBIOSE, AS WELL AS ARYL AND ALKYL-B-D-GLUCOSIDES. MAIZE B-GLUCOSIDASE WAS ISOLATED FROM COLEOPTILES BY USING ION EXCHANGE AND GEL PERMEATION CHROMATOGRAPHY. IT WAS PARTIALLY CHARACTERIZED WITH RESPECT TO ISOZYMIC COMPOSITION, SIZE, ISO-ELECTRIC POINT (pI), Km, AND pH AND TEMPERATURE OPTIMA. THE ACTIVITY OF MAIZE B-GLUCOSIDASE FROM DIFFERENT TISSUES AND DEVELOPMENTAL STAGES WAS ASSAYED. TWO DISTINCT GROUPS OF B-GLUCOSIDASE WERE RESOLVED FROM MOST OF THE INBREDS BY ELECTROPHORESIS AT ALKALINE pH. MAIZE INBREDS KNOWN TO POSSESS NULL ALLELES AT THE Glu1 LOCUS WERE ASSAYED FOR REACTIVITY WITH ANTIBODIES TO B-GLUCOSIDASE AND YIELDED POSITIVE REACTIONS. ACTIVE MULTIMERIC FORMS OF B-GLUCOSIDASE WERE RESOLVED IN THESE "NULL" INBRED LINES BY ELECTROPHORESIS. THE ISOLATION AND CHARACTERIZATION OF MAIZE B-GLUCOSIDASE mRNA(s) AND GENE(s) ARE NOW UNDERWAY.

EXPRESSION OF A TISSUE SPECIFIC LECTIN GENE FAMILY IN THE TREE LEGUME *SOPHORA JAPONICA*: Eliot Herman, Charles Hankins*, and Leland Shannon*, Plant Molecular Genetics Laboratory, USDA/ARS, Beltsville, MD 20705, and *Department of Biochemistry, University of California, Riverside, CA 92521.

The leguminous tree *Sophora japonica* contains a family of closely related but distinct lectin genes. Different members of this gene family are independently expressed in seed, leaf, and bark tissues. The lectins of each of these tissues has been purified to homogeneity. Specific antisera raised against each of these lectins crossreact with each of the other lectins. SDS/PAGE-immunoblot analysis indicates that the seed contains a single lectin gene product, the leaf contains two lectins (I and II), and the bark contains a single lectin. Double diffusion Ouchterlony gels demonstrated that these lectins are closely related but not identical. Amino terminal sequencing of each of the lectins indicates that each is a distinct tissue specific gene product. The leaf and bark lectins have been localized by electron microscope immunocytochemistry. The lectins were found to be exclusively localized in protein rich vacuoles of the leaf and bark tissues. These protein filled vacuoles are apparently very similar to the protein bodies of legume seeds. The presence of lectins closely related to the seed lectin in a similar subcellular compartment of nonseed tissues has implications for the development of new protein rich legume crops.

COMPLETE NUCLEOTIDE SEQUENCE OF THE BROAD HOST RANGE SQUASH LEAF CURL VIRUS: POTENTIAL CODING REGIONS AND REGULATORY SEQUENCES. I.B. Lazdins and S.G. Lazarowitz, Carnegie Institute of Washington, Baltimore, MD 21210.

Squash Leaf Curl Virus is a member of the whitefly-transmitted geminivirus group, characterized by paired icosahedral virions, which separately encapsidate a bipartite genome of circular single-stranded DNAs. Two different, yet highly homologous Squash Leaf Curl Viruses have been isolated, which can be distinguished by the structure of their cloned genomic components and their host ranges. As a fundamental step towards understanding the viral determinants of pathogenesis in geminiviruses, we report here the complete nucleotide sequences of the infectious cloned DNA components of the broad host range Squash Leaf Curl Virus. Potential coding regions and regulatory sequences have been identified and compared to those of the narrow host range Squash Leaf Curl Virus, as well as other whitefly-transmitted geminiviruses.

PRODUCTION OF MUTANT LINES OF *LEMNA GIBBA* G_3 BY CELL CULTURE AND WHOLE PLANT MUTAGENESIS.
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The primary interest of our laboratory is the metabolism of the plant hormone indole-3-acetic acid (IAA). Hormones are responsible for plant growth and development. We want to understand how a plant decides how, how much, where, and when to increase or decrease the level(s) of free IAA, which we believe to be the active form of the hormone. To this end we have developed a new system, using the aquatic monocot *Lemna gibba* G_3, with which we can easily do rapid kinetic labeling *in sterile* conditions, and which is now shown to be amenable to genetic manipulations.

We have obtained many different kinds of mutants of *Lemna* by various techniques, including tissue culture and *whole plant* mutagenesis. By whole plant mutagenesis we have obtained a high rate of chloroplast pigment abnormality. One such variant has no apparent chlorophyll b and very low or no steady state levels of the light harvesting chlorophyll a/b binding protein. Another type of variant exhibits a developmental anomaly: the pollen tube grows while the pollen is still on the anther, so that the anther becomes hairy in appearance.

We have obtained several mutant lines by tissue culture of *Lemna* on B_5 medium containing 2,4-D and 2iP. One of these lines (R-1) is approximately 1.5 times the size of the parental line (PL), and by microfluorodensitometry has the same amount of DNA per nucleus as the PL. At a specific time of the culture growth cycle, R-1 contains up to 100 times the amount of free IAA as PL, and, unlike PL, contains no detectable IAA-conjugates at these times. We have initiated experiments using this mutant to determine the pathway of IAA biosynthesis in plants and to study the processes by which plants regulate the formation of IAA-conjugates, and the hydrolysis of such conjugates to yield free IAA.

Supported, in part, by US-Israel BARD grant No. US-842-84.

IN VIVO BREAKDOWN PRODUCTS OF THE 32kDa THYLAKOID HERBICIDE BINDING PROTEIN

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The 32kDa herbicide binding protein of PSII is degraded rapidly in the light. This phenomenon was investigated in *Spirodela oligorrhiza*. When fronds were radiolabeled in the presence of cycloheximide only the chloroplast-encoded proteins were labeled. Under these conditions, a breakdown product of 23.5kDa was observed. This polypeptide was further degraded with kinetics similar to that of the 32kDa protein. The 23.5kDa polypeptide cross-reacted with an antibody specific to the 32kDa protein. By protease digestion it was determined that the 23.5kDa polypeptide is the intact N-terminal piece of the 32kDa protein. Thus, this product corresponds to the membrane anchor of the 32kDa protein. Using the same antibody, breakdown products of 16kDa, 14kDa and 12kDa were observed. These products have also been observed in *Zea mays* and *Solanum nigrum*. Using DCMU during pulse-chase experiments at different light intensities, evidence was obtained that the 23.5kDa breakdown product is generated *in vivo*.

CONTROL OF DEGRADATION OF THE 32kDa THYLAKOID HERBICIDE BINDING PROTEIN BY FAR RED LIGHT

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White light (400-700nm) and far red light (>700nm) stimulate degradation of the chloroplast-encoded 32kDa herbicide binding protein. Degradation is minimal in darkness. The far red light causes less than 1% of the PSII activity of the white light, measured by photo-acoustic techniques, although PSI is still active. Therefore, rapid degradation of the 32kDa protein, a component of PSII, can occur in the absence of photosynthetic electron flow through the reducing side of PSII (the DCMU-sensitive site). Hypotheses considered to explain far red light effect on 32kDa degradation include: PSI-activated protease activity, damage to the protein due to PSI activity, phytochrome action, and an alternative pathway of electron flow. Surprisingly, degradation of this herbicide binding protein is blocked by DCMU under all light conditions tested. This suggests that herbicide binding changes the protein conformation so that the protein is less susceptible to degradation.

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