PLANT GENOME

Panel Manager-Dr. Tom Osborn, University of Wisconsin
Division Director-Dr. Ed Kaleikau

The NRI Plant Genome Program is part of the interagency National Plant Genome Initiative (NPGI) coordinated by the National Science and Technology Council (NSTC) whose long-term goal is to understand the structure and function of genes in plants important to agriculture, environmental management, energy and health. Areas of research supported by the NRI include: (1) generation of molecular markers and mapping data, and map-based cloning of agriculturally important genes; (2) development of new technologies for genome mapping, genome manipulation, gene isolation or gene transfer; (3) use of genome technologies to create new germplasm or varieties that solve problems important to U.S. agriculture or that will create new products for the U.S. agricultural industry and; (4) development of bioinformatic tools to support comparative mapping, QTL analysis and pedigree analysis.

2000-01389 Molecular Evaluation of Rice Germplasm Using Microsatellite Markers

Tai, T.H.; McCouch, S.R.; Cartinhour, S.
USDA Agricultural Research Service; Dale Bumpers National Rice Research Center; Stuttgart, AR 72160
Grant 00-35300-9216; $140,000; 2 years

Genetic diversity is a key factor in sustaining agricultural productivity. In order to preserve and make this diversity available for crop improvement, tremendous efforts have been made in the collection, maintenance, and classical characterization of germplasm. The ability of researchers to assess genetic variation by examining differences at the DNA level has enhanced the evaluation of these genetic resources and increased their utility. Rice is an example of a major crop species for which several germplasm collections exist and for which an abundance of molecular marker data is available. As is the case in many other species, microsatellites are widely distributed throughout the rice genome and exhibit a high level of allelic diversity, making them ideal markers for assessing genetic variation. In this study, a group of 576 rice accessions (representing various geographical origins and cultural types) will be examined using a set of 150 microsatellites. Fluorescent-labeled markers and an automated genotyping system will be used to facilitate this analysis. This project will contribute to the sustainability of the U.S. rice industry by providing valuable information for the isolation of important genes and promoting the use of molecular marker-assisted selection in U.S. rice breeding programs. All the data and analyses generated will be publicly available via the GRIN and RiceGenes databases (www.ars-grin.gov; http://ars-genome.cornell.edu/rice).

2000-01678 Positional Cloning of Wheat and Barley Vernalization Genes

Dubcovsky, J.
University of California, Davis; Department of Agronomy & Range Science; Davis, CA 95616
Grant 00-35300-9565; $290,000; 3 years

The adaptability of wheat and barley to a large range of environments is partially due to genetic variation in sensitivity to vernalization (acceleration of the ability to flower by a chilling treatment). The difference in vernalization requirement divides wheat and barley cultivars into winter and spring classes, and is mainly determined by vernalization genes Vrn-1 and Vrn-2. The objective of this proposal is to clone these two genes using a positional cloning approach. We will first delimit the minimum regions encompassing Vrn-1 and Vrn-2 using large insert clones (BAC clones) from wheat, barley, and rice and then we will sequence them. We will identify the genes present in these regions using gene-finding computer programs; comparative sequencing of wheat, barley and rice; and hybridization of the DNA from this region with available cDNA libraries (expressed genes). To identify Vrn-1 and Vrn-2 among the candidate genes we will analyze the expression patterns of the candidate genes, their genetic variation in induced and natural mutants, and their complementation ability in transformation experiments. Cloned vernalization genes will allow a fast transformation of winter to spring genotypes and vice versa,

accelerate the development of spring genotypes resistant to frost damage, and make possible the development of molecular markers for the different vernalization alleles to study their agronomic value. Besides their practical applications, the Vrn clones will facilitate the elucidation of the basic mechanisms of vernalization in temperate cereals.

2000-01972 Evolution of the Arcelin-Phytohemagglutinin-alpha Amylase Region in Phaseolus

Paul Gepts
University of California, Davis; Department of Agronomy and Range Science; Davis, CA 95616
Grant 00-35300-9243; $290,000; 3 years

Plants are engaged in an evolutionary arms race with insect pests and pathogens. Plants put up new defense mechanisms whereas pests and pathogens overcome these mechanisms. Understanding how plants generate new genetic defense mechanisms toward insect pests is the major focus of this project. In turn, information from this project may assist us in developing more durable resistance to pests and pathogens, thus reducing the need for pesticides. To investigate this question, the project will focus on the APA family of insect resistance genes in common bean. Previous research has shown that this family has become larger over time and in the process has acquired resistance to different insects, particularly seed weevils. In this project, we will develop a map of the chromosome region that carries this gene family in four bean lines that have been carefully chosen to contain an increasingly complex organization of the APA family. This map will be established by isolating and ordering large segments of DNA (into bacterial artificial chromosome (BAC) libraries) and subsequently determining the location of the different APA genes on overlapping DNA segments. The BAC libraries and the maps generated in this project will be made publicly available and represent a significant future resource for bean improvement. Beans represent an important crop in many states, their consumption has increased 50% over the last 15 years, and the U.S. is a major exporter.

2000-01683 Conifer Comparative Genomics Project

Neale, D.B.
USDA Forest Service; Institute of Forest Genetics; Department of Environmental Horticulture; University of California, Davis, CA 95616
Grant 00-35300-9316; $220,000; 3 years

The primary goal of the Conifer Comparative Genomics Project (CCGP) is to create a biological and informatics infrastructure for conifer geneticists to compare and contrast genome information gathered from a variety of species. The conifer genome research community is small and is not focused on a single model species. Thus, it is critical to have an infrastructure for comparative genomics. The essential elements of the comparative mapping collaboration of the CCGP is shared use of genetic markers and mapping populations. A common set of genetic markers is mapped to genetic maps from a number of pine and other conifer species. These markers serve as anchor points to identify the homologous chromosomes among species. Once comparative maps between species are constructed, it then becomes possible to identify the chromosomal location a gene in one species based on its map position in another species. In addition to the biological framework, there must also be an informatics framework. This will be accomplished through the Dendrome Database project and development of software tools designed specifically for performing comparative genomic analyses in conifer.

2000-01387 Fine Mapping of Disease Resistance QTL and Linkage Drag Loci using Tomato NILs

St.Clair, Dina A.; West, Marilyn A.L.
University of California, Davis; Department of Vegetable Crops; Davis, CA 95616-8746
Grant 00-35300-9413; $200,000; 3 years

The fungus Phytophthora infestans causes late blight disease on tomato plants and fruit, and is a major cause of crop loss. Alternaria alternata, a ripe fruit fungus, causes extensive harvest losses in processing tomatoes. Fungicides do not adequately control late blight and are being eliminated for control

of both diseases due to environmental and consumer safety concerns. No adequate late blight or blackmold resistance exists in cultivated tomato (Lycopersicon esculentum). Previously, genes (quantitative trait loci or QTL) governing resistance to late blight and blackmold from wild tomato species were mapped to tomato chromosomes using DNA markers. Unfortunately, these resistance QTL were also associated with negative horticultural traits, suggesting that undesired genes are linked to the desired resistance QTL, making the use of these genes problematic unless such associations can be broken. To address this issue, the markers linked to the resistance QTL will be used to select plants that contain different and smaller segments of the wild parent donor chromosome to create lines (i.e., near-isogenic lines or NILs) to fine map the location of the resistance loci and loci associated with negative horticultural traits. The NILs exhibiting resistance and no or few negative horticultural traits will be tested for their ability to express resistance in hybrid combinations (i.e., combining ability). The best NILs, and the DNA markers associated with the resistance QTL, will be released publicly for further breeding efforts.

2000-01426 Comparative Genomic Studies of Mi-1 Related Sequences in Solanaceous Plants

Williamson, V.M.; Yaghoobi, J.
University of California, Davis; Department of Nematology; Davis, CA 95616
Grant 00-35300-9263; $200,000; 3 years

The tomato gene Mi-1, which was isolated in our laboratory, confers effective resistance against several species of root-knot nematodes as well as against some isolates of the potato aphid. It was the first gene found to confer isolate specific resistance against an insect as well as the first to confer resistance to plant pests from two distinct phylogenetic groups. Both of these pests are significant detriments to the cultivation of many important crops worldwide. With the impending phasing out of methyl bromide and decreased availability of chemical insecticides and nematicides, alternative control strategies such as host plant resistance are becoming more urgent. There are about six highly similar genes to Mi-1 of so far unknown function in susceptible and resistant tomato as well as in wild tomato relatives and in potato. We propose to isolate these genes and to determine their genomic positions within each plant species. Most investigations conducted to date are associated with plant resistance genes effective against fungal and bacterial diseases in plants. We propose to examine a small and defined subset of genes that resemble Mi-1. The DNA sequences that we isolate and characterize will enrich genetic maps and provide markers for plant breeding. The current project also lays the ground work for development of novel resistance by molecular shuffling of the cloned genes. This strategy should ultimately lead to the development of host resistance with improved specificity and durability under broader environmental conditions against nematodes, potato aphids, and, perhaps, other pests.

2000-01716 Bayesian Analysis of Quantitative Traits under the Plant Model

Xu, S.
University of California, Riverside; Department of Botany and Plant Sciences; Riverside, CA 92521
Grant 00-35300-9245; $150,000; 3 years

The long-term goal of this project is to develop statistical models for studying the genetic architecture of quantitative traits in plants using molecular markers. The statistical technique is called the plant model. Specifically, we will accomplish the following objectives: (1) establish the infrastructure for the plant model quantitative genetic analysis and QTL (quantitative trait loci) mapping using a Bayesian statistic, (2) develop a general algorithm for QTL analysis in arbitrarily complex pedigrees under arbitrary mating designs, (3) extend the general algorithm to QTL mapping with epistatic effects and pleiotropic effects for multiple traits, and (4) develop an algorithm for QTL mapping in polyploid plants.

Most QTL mapping techniques require designed experiments of line crosses. These crosses do not exist in natural populations and are rarely used in plant breeding. The proposed plant model has shed new light on quantitative genetics because it can handle models with almost arbitrary complexity. Results of this research will greatly increasing the power of genetic studies and thus enhance our understanding of the genetic architecture of quantitative traits. The proposed research is relevant to the area specified by the

USDA Plant Genome Program: development of new technologies for genome mapping. The result of this project will benefit US agriculture.

2000-01682 Down-Regulation of Thermostable Pectin Methylesterase in Citrus

Burns, Jacqueline K.; Grosser, Jude W.
University of Florida, Gainesville; Citrus Research and Education Center; Lake Alfred, FL 33850
Grant 00-35300-9391; $120,000; 2 years

Striking advances have been made in plant molecular biology. It is important that new technologies of this field be translated into new or improved products. One such product that could be improved by relatively small genetic changes using recombinant DNA technologies is citrus. One problem limiting marketability of citrus that could be improved with recombinant DNA technology is stability of citrus juice. Stability of citrus juices are decreased by the enzyme pectin methylesterase. The enzyme is responsible for separation of juice into a clear serum and particulate phase. High temperature pasteurization, above what is necessary for microbial control, is used commercially to reduce activity of the enzyme. Despite this treatment, residual thermostable activity persists, and flavor can be negatively affected. Eliminating thermostable pectin methylesterase activity would allow juice processors to lower pasteurization temperatures, saving energy, reducing costs and improving juice flavor. We propose to identify the thermostable pectin methylesterase enzyme(s) in orange juice. Amino acid sequences will be determined for each enzyme and the information used in a reverse genetics approach to identify the thermostable pectin methylesterase gene(s) in Valencia orange. To verify that each gene encodes a thermostable pectin methylesterase protein, we will overexpress the citrus protein in tobacco using pectin methylesterase cDNA constructs with our tobacco mosaic virus expression vector. The citrus protein will be collected and thermostable enzyme activity tested using thermal assay techniques. cDNAs that encode thermostable pectin methylesterase(s) will be saved for future work to down-regulate this quality-reducing enzyme in citrus.

2000-01662 High Through-put Transposon Mutagenesis and Cloning of Maize Endosperm Mutants

Settles, A. M.
University of Florida; Horticultural Sciences Department; Gainesville, FL 32611-0690
Postdoctoral Fellowship; Grant 00-35300-9390; $90,000; 2 years

Corn is a major food source throughout the world, and understanding which genes are necessary for the seed to develop allows directed research towards crop improvement. We are developing technologies for the rapid identification of genes required for seed development in corn. We are using natural mutagens known as transposons or jumping genes. These are small DNA sequences that disrupt the normal functions of genes by inserting their sequence into a gene. We have bred a variety of corn that only varies in the number and locations of their transposons. Every plant in this variety has a unique spectrum of transposon insertions. Thus, plants that show differences in the way they look or how much grain they yield will have a transposon inserted into a gene required for that characteristic. By discovering the genes disrupted in seed mutant plants, we will better understand of seed development. We developed a method to fingerprint the gene disruptions by using PCR to extract the DNA sequences surrounding the spectrum of transposon insertions in any plant. We will sort through these fingerprints using the information from the public plant genome projects to find the genes required for seed development. Specifically, we will use the fingerprint to identify insertions in genes that are normally active in the seed, but their role in making the seed is unknown. These techniques should greatly increase the speed that specific mutations are associated with particular genes and are applicable to other crops or commercially important plant characteristics.

2000-01719 Dissecting the Genetic Control of Floral Induction, Plant Height, and Apomixis

Paterson Andrew H.; Burow Gloria B.; and Hussey Mark A.
University of Georgia; Plant Genome Mapping Laboratory; Athens, GA 30602

Grant 00-35300-9215; $290,000; 3 years

This proposal focuses on two closely-linked genes that have profound consequences for plant reproductive biology, and correspond to closely-linked loci (albeit on different genomes). The timing of the vegetative-reproductive transition plays a central role in determining how plants allocate photosynthate to economically-useful organs. The possibility of vegetative propagation through seed offers the means to permanently capture the benefits of hybrid vigor at only nominal cost. The small chromosomal region that contains the Ma1 and PApo1 loci also plays a disproportionately large role in the genetic control of plant stature, leaf length and width, seed size, tillering, rhizomatousness, and regrowth after overwintering. The association among these traits is reminiscent of other associations such as disease resistance and insecticide tolerance, that have been shown to reflect the presence of 'clusters' of structurally and functionally related genes (cited above). Further dissection of this chromosomal region will ultimately enable us to clone the genes underlying these important traits. Due to its enormous economic ramifications of bringing the benefits of hybrid vigor to all crops at tiny cost, the transfer of apomixis to crop plants has often been listed to be one of the most important technological and commercial challenges facing agriculture. Similarly, better understanding of the vegetative-reproductive transition offers many applications in genetic manipulation of plant productivity, such as redirection of resource allocation to the plant parts sought by humans, rather than those needed for survival in the wild. Achievement of these goals will also add new basic information to current understanding of flowering and reproduction, processes that are central to the life cycles of all plants.

2000-01493 Genetic Studies of Cereal Low Phytic Acid Mutants

Raboy, V.
USDA Agricultural Research Service; National Small Grains Germplasm Research Facility; Aberdeen, ID 83210

Grant 00-35300-9201; $220,000; 3 Years

Most phosphorus in seeds is found as a single compound called phytic acid. Animals such as chickens, pigs and fish, as well as human beings, excrete nearly all the phytic acid they consume. Phytic acid excretion can contribute to water pollution, and to mineral deficiency such as iron and zinc deficiency in humans as well. *Low phytic acid* (*lpa*) mutants in maize and rice have been isolated. Grains produced by *lpa* mutants have normal levels of total phosphorus but greatly reduced levels of phytic acid. As a result the amount of “available phosphorus” (phosphorus that animals and humans can absorb and use) is greatly increased. Breeding “low phytic acid” crops using selected *lpa* mutants is straightforward. However, the optimization of this approach to the “phytic acid problem”, and possibly its ultimate acceptance and use, requires the genetic mapping and identification of genes represented by these *lpa* mutants. This project will focus on the genetics of one class of *lpa* mutants, referred to as *low phytic acid 1* (or *lpa1)* mutants. These are currently being used in breeding new types of corn and rice. Selected *lpa1* genes and mutants will be mapped and sequenced. This work will identify genetic markers useful in breeding, and will identify new genes useful for the optimal engineering of the “low phytic acid/high available P” trait. This work will help meet new environmental standards for agricultural production, and yield more nutritious human foods as well, thus strengthening the efficiency and competitiveness of U.S. agriculture.

2000-01665 Genetic Analysis of Sunflower Domestication

Burke, J.M.
Indiana University, Bloomington; Department of Biology; Bloomington, IN 47405

Grant 00-35300-9244; Postdoctoral Fellowship $89,710; 2 years

The domestication of plants from their wild progenitors has led to the production of a wide variety of seed crops that share a number of traits in common. Results of recent research suggest that, at least in the major cereals (i.e., maize, millet, rice, sorghum and wheat), phenotypic changes involved in crop domestication are determined by a relatively small number of major genes, each contributing to domestication in multiple independent lineages. To date, however, this research has left several issues

unresolved. One such issue is the extent to which these findings apply to other, less closely related seed crops. A second issue involves the extent to which individual genes influence one or more apparently unrelated traits. The proposed research will investigate the genetic basis of sunflower domestication along two main lines of inquiry. First, genetic mapping of loci contributing to phenotypic differences between cultivated and wild sunflower will be employed to explore the number of loci involved in sunflower domestication, the magnitude of their effects and their chromosomal locations. These results will allow a distinction to be made between traits that have arisen as a direct result of artificial selection and those that are an indirect byproduct of such selection. Second, by implementing the results of recent research on the domestication of maize, the proposed research will allow a test of the hypothesis that sunflower domestication has involved changes at loci homologous to those involved in maize domestication. Taken together, the results of this research will provide a better understanding of the process of domestication and will ultimately assist in the utilization of wild germplasm for crop improvement.

2000-01409 Transposon Tagging of Nuclear Genes that Control Mitochondrial Gene Expression

Gabay-Laughnan, S. J.
University of Illinois at Urbana-Champaign; Department of Plant Biology; Urbana, IL 61801-3707
Grant 00-35300-9409; $290,000; 3 years

The objective of this study is to identify and clone restorer-of-fertility (*Rf*) genes specific for S-type cytoplasmic male sterility (CMS-S) in maize (corn). Maize plants with CMS-S are male sterile, i.e., they shed no pollen. CMS-S results from a mutation in the DNA of the mitochondria, cytoplasmic organelles required for plant respiration. *Rf* genes in the nucleus of a cell over-rule the male-sterile effect of the cytoplasm. If CMS-S plants carry *Rf* genes they produce pollen even though they carry S-type cytoplasm.

We have recently found that some *Rf* genes not only disrupt expression of the mitochondrial CMS-S gene but they also disrupt expression of essential mitochondrial genes. We can exploit this fact to identify and clone nuclear genes that function in mitochondrial gene expression. We will use transposable elements (TEs) to create mutations in nuclear genes that control the expression of mitochondrial genes. These mutations are created when a TE inserts into a particular gene. We will select for *Rf* gene mutations by their restoring properties. The fact that the new *Rf* genes were caused by TEs inserting into their DNA will allow us to recover the new *Rf* genes. Since the sequence of the TE is already known, we use its sequence to find the new *Rf* gene and then we sequence the DNA flanking the TE. By comparing the sequence of a new *Rf* gene with the sequences of known genes we hope to determine the nature of the new *Rf* gene. Understanding the interaction of nuclear and mitochondrial genes has potential long-term application for the genetic improvement of crop plants. *Rf* genes regulate pollen fertility and influence seed development and seed size. Only when we understand the genetic regulation of mitochondrial function in detail can we hope to manipulate this critical organelle for the improvement of plant performance.

2000-01672 Comparative Functional Genomics in adh Regions of Maize, Sorghum and Rice

Bennetzen, J.
Purdue University; Department of Biological Sciences; West Lafayette, IN 47907
Grant 00-35300-9412; $230,000; 2 years

Three important crop species, maize, sorghum and rice, also serve as well characterized model systems for the study of plant genome structure, function and evolution. These three grasses have received extensive molecular and genetic to investigate the evolution of gene function and gene regulation in colinear segments of these three genomes. We have already sequenced the DNA from two homologous regions in the maize, rice and sorghum genomes, showing that the genes are highly conserved in sequence two more homologous regions from maize, rice and sorghum genomes. Then, we will use high throughput microarray technologies to measure the transcription of genes in these regions, to see if they are expressed in similar tissues, at similar times in development, and in response to similar signals. Through these studies, we hope to determine the mechanisms, rate and degree to which genes evolve new

functions within this important segment of the plant kingdom. Understanding these processes will help us determine how we might modify crops for improved agricultural productivity.

2000-01676 Structural and Functional Genomics of Mla-Mediated, Resistance Signaling in Barley
Wise, R. P.
USDA Agricultural Research Service; Corn Insects and Crop Genetics Research; Iowa State University; Ames, IA 50011-1020
Grant 00-35300-9213; $220,000; 3 years

Powdery mildew of grasses (barley, wheat, rye, and oats) is an excellent model system for investigating disease resistance mechanisms among fungal pathogens and their cereal hosts. Resistance to this disease in barley is conferred by genes designated Ml. Over thirty variants have been identified at the Mla resistance-gene cluster in different cultivars. Each of these variants enables the barley plant to elicit a unique reaction in response to infection by Erysiphe graminis f. sp. hordei, the causal agent of powdery mildew disease. In the previous funding period (grant 98-35300-6170), we have cloned the complete Mla cluster from the cultivar Morex, a powdery mildew susceptible, malting barley. In the current funding period, we will: 1) Complete the 240-kilobase sequence of the Mla complex. This Morex reference sequence will provide the composition of the different genes in this important region of the barley genome. 2) Determine the functional identity of the Mla1 and Mla6 alleles. Since Mla1 and Mla6 function via different resistance pathways, comparison of different alleles at Mla will enable the simultaneous determination of resistance specificity and also signaling specificity. 3) Identify additional genes that are required for Mla6 and Mla13 resistance. Completion of this project will provide a significant contribution towards understanding the complexity of host resistance to obligate fungal pathogens. Thus, the proposed research is expected to have a positive effect on plant agriculture by increasing the availability and sustainability of genetic resistance; the most cost effective way of reducing the impact of disease in crop production.

2000-01113 Introgressing Alleles from the Wild Species *G. soja* into Soybean
Pfeiffer, T.W.
University of Kentucky; Department of Agronomy; Lexington, KY 40546
Strengthening Award; Grant 00-35311-9384; $73,123; 2 years

As a soybean breeder my continuing goal is to produce soybean varieties with increased productivity. U.S. soybean varieties, however, have a narrow genetic base, and continued increases in productivity derived from breeding efforts might benefit from an infusion of new genes that broaden that base. One logical place to begin searching for different genes is in *Glycine soja*, the most closely related ancestral species of soybean. All of the favorable genes present in the ancestral species were probably not carried over to soybean during its domestication. In the past we have been unable to identify specific favorable genes that should be transferred from the wild species because all of the unfavorable genes that it also contains obscures the identification of the useful progeny. Now, molecular markers can be used to identify favorable genes that are found mixed among the unfavorable genes in the wild species and to transfer only the favorable genes to soybean. Molecular markers chosen from the current soybean molecular marker genetic map will be used in conjunction with yield testing an advanced *G. max* x *G. soja* interspecific population to identify those genetic regions of *G. soja* that contain favorable genes which should be transferred to soybean. This research will be significant to U.S. agriculture when it enhances the genetic composition of our soybean breeding populations and improves the genetic yield potential of our important soybean crop.

2000-01308 Conference Entitled: "Genetic Engineering to Improve Attributes of Crop Plants"
Rajasekaran, K.; Finley, J.; Jacks, T.J.
UDSA Agricultural Research Service; New Orleans, LA 70124
Conference Grant 00-35300-8852; $2,500; 1year

Recent revolution in U.S. agriculture is largely due to the introduction of genetically modified crops. By 2010 the value of the transgenic seed market is expected to reach $20 billion. In the U.S., about one third of the annual corn crop and one half of the soybean and cotton crops were grown from genetically modified seed in 1999. Genetically modified crops offer several advantages over conventional crops such as improved agronomic or horticultural traits, improved nutritional values, ability to produce pharmaceuticals and biochemicals using transgenic plants and improved food and feed safety through control of toxin producing microbes and production of edible vaccines. Genetically modified crops are also environment-friendly because of less dependence on harmful herbicides and pesticides which otherwise would contaminate air and ground water quality. All these achievements have been possible due to the recent and dynamic developments in plant molecular biology, genomics and plant cell culture. Advancing the science of genetic engineering through free exchanges of ideas and information is the main theme of this conference. The National Research Initiative (NRI) Competitive Grants program Award from the Plant Genome Program will provide partial expenses for three participants to attend the conference when otherwise they could not. Although political aspects of genetically modified crops are important and will be included, communications on scientific aspects among hands-on scientists is needed and will be featured in this conference through auspices of the American Chemical Society's 219th National meeting in San Francisco, March 26-30, 2000.

2000-01714 Comparative Physical Mapping Between Rice, Wild Rice and Maize using Fiber-FISH

Jackson, Scott A.
University of Minnesota, St. Paul; Department of Agronomy and Plant Genetics; St. Paul, MN 55108
Postdoctural Fellowship; Grant 00-35300-9214; $89,863; 2 years

Rice has become the rosetta stone by which all other cereal crops (i.e. maize, oat, barley, wheat, sorghum and wild rice) will be interpreted. Rice has the smallest and least complex genome of the cultivated cereals and will soon be sequenced by an international consortium, including the United States. In order to more effectively utilize the rice DNA sequence data, we propose to examine the physical basis of genomic conservation between several cereal crops. It is already known that genetic loci can be conserved in linear order between these cereal species, but the physical basis of this conservation is unknown. It is important to have a grasp on the physical relationship of these related cereal genomes relative to rice in order that the rice genome data can be more effectively used to improve US agriculture. Using a technique, fiber-FISH, whereby it is possible to analyze genomes with a very high resolution, we will use the rice genome to physically analyze the related genomes of maize and wild rice. Fiber-FISH is a fluorescence-based mapping technique that approaches the resolution of DNA sequencing without the associated expense. With the data generated from this project it should be possible to more efficiently use data generated from the rice genome project for the improvement of related cereal crops important to US agriculture.

2000-01413 The Mechanism of Transgene Integration Locus Formation in Plants

Somers, D.A.
University of Minnesota; Dept. of Agronomy and Plant Genetics; St. Paul, MN 55108
Grant 00-35300-9197; $200,000; 2 years

Microprojectile bombardment is currently being used for genetically engineering plants both for basic research and crop improvement. Transgene integration loci produced from DNA introduced using microprojectile bombardment exhibit variations in copy number, transgene rearrangements and interspersion of genomic DNA between tightly linked clustered transgenes. Increased transgene locus complexity likely is, in part, responsible for increased problems with transgene expression and aberrant transmission to progeny of transgenic plants produced using microprojectile bombardment. Our previous results on characterization of transgene integration loci in oat provide strong evidence for a model of transgene integration based on chromosome and plasmid breakage during DNA delivery on an accelerated microprojectile. For this renewal we are proposing to investigate features of transgene integration loci predicted by this model. Characterization of transgene loci will provide insights into strategies to produce

simplified transgene integration loci that exhibit higher fidelity of expression and transmission. Improving the fidelity of DNA integration following microprojectile bombardment will provide researchers with greater flexibility in choice of transformation strategies. Understanding the transgene integration process and mechanisms leading to the formation of the transgene integration locus will provide basic knowledge about the process of DNA integration in plant genomes, which is emerging as an important component of genome evolution, and as a framework for investigating the development of targeted integration in plants.

2000-01400 The Genetic Basis of Agronomic Traits Controlled by Chromosome 3A in Wheat

Baenziger, P.S.; Gill, K; Nettleton, D.; Eskridge, K.
University of Nebraska-Lincoln; Departments of Agronomy, Mathematics and Statistics, and Biometry; Lincoln, NE 68583-0915
Grant 00-35300-9266; $250,000; 3 years

One of the unanswered questions in wheat improvement is the genetic basis of agronomic performance (specifically yield and yield components). In our previous research using reciprocal chromosome substitution lines between Cheyenne (CNN) and Wichita (WI), we identified chromosome 3A as containing genes that affect agronomic performance (anthesis date, height, yield, yield components, and grain volume weight). In the proposed research, we will evaluate reciprocal recombinant inbred chromosome lines (RICLs) for chromosome 3A in two backgrounds (CNN or WI) in multiple environments and use chromosome 3A molecular markers and mapping software to identify quantitative trait loci (QTLs). Reciprocal recombinant inbred chromosome lines of wheat are lines in which 20 chromosome pairs come from a single parent (in this case CNN or WI) and the remaining chromosome pair is the recombinant inbred products for the chromosome of interest (in this case from the recombination of chromosomes 3A from WI and 3A from CNN). Our hypotheses are:

1. Factors (QTLs) controlling much of the yield and yield component variation within an environment (E) due to genes on chromosome 3A can be localized and quantified,
2. QTLs are expressed in both the CNN and WI backgrounds, and
3. QTL x E interaction is an important effect in populations using adapted cultivars and is sufficiently large that it hinders the identification of QTLs that are expressed across environments.

We further hope to understand the well documented compensatory relationships among yield component QTLs and their effect on grain yield QTLs. These analyses should provide new insights on agronomic performance, QTL epistasis, QTL linkage, and QTL x E interactions.

2000-01407 Investigating the Molecular Basis of Tomato Size and Shape: A Model for Understanding Quantitative Trait Variation and the Evolution of Fruit

Tanksley, S. D.
Cornell University; Department of Plant Breeding and Department of Plant Biology; Ithaca, NY 14853
Grant 00-35300-9264; $350,000; 3 years

Most traits in nature and of importance to agriculture are quantitatively inherited. This includes many of the traits that are the most biologically interesting and economically important, including yield, stress tolerance and quality. Quantitative inheritance not only makes the genetic study of such traits difficult, but it has virtually blocked the cloning of the genes responsible for these traits. Until we can learn how to systematically clone the genes responsible for quantitative traits, it is unlikely that we will ever gain a full understanding of the processes controlling plant development or be able to engineer the traits most critical for crop production. This project will allow the molecular and developmental characterization of the two quantitative trait -- fruit size and shape. Both are key factors affecting the yield and consumer acceptance in tomatoes and other fruit bearing crop plants. This project also provides a starting point for understanding a critical, but largely unexplored aspect of plant development: how ovaries are transformed from small reproductive organs into the large, conspicuous fruit that display the array of shapes and sizes that we associate with modern agriculture. Finally, this research may help reconstruct events involved in the domestication of tomato and other fruit bearing crop species. By

cloning these genes underlying these traits, we can ask whether the same genes were involved in the domestication of other fruit-bearing species.

2000-01416 Resistance to Barley Stripe Rust: A Model for Understanding Genetically Complex Traits

Hayes, P.; Mundt, C.
Oregon State University, Corvallis; Dept. of Crop and Soil Science, Dept. of Botany and Plant Pathology; Corvallis OR 97331
Grant 00-35300-9263; $140,000; 2 years

Stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) is an important disease of barley in the western United States. Genetic resistance is the most economical and sustainable method for controlling stripe rust and many other plant diseases. Plants have two principal types of disease resistance – qualitative and quantitative. Theoretical considerations, and experience, suggest that the latter will be more durable than the former. However, it is difficult to breed for quantitative resistance because of the continuous spectrum of disease reactions, "resistant" plants allow some symptom development, and the trait shows complex inheritance. Quantitative Trait Locus (QTL) analysis is a tool for understanding and manipulating quantitatively inherited traits. We have used QTL techniques to map and use quantitative stripe rust resistance genes in barley. However, important questions relating to estimates of gene location, effect and interaction remain unanswered. We propose to address these questions using a large population of homozygous lines. We obtained this population by crossing an experimental selection possessing three mapped quantitative resistance genes (BCD47) with the most widely grown, but stripe rust susceptible, variety in the Pacific Northwest (Baronesse). We will determine the genotype of each line in the population using molecular markers and we will determine the level of resistance to stripe rust in field tests. The genotype and phenotype data will be integrated to map and determine the effects of genes segregating in the test population. Our goal is to use this information to increase the profitability and sustainability of barley production for Pacific Northwest farmers.

2000-01974 Positional Cloning of Ripening and Ethylene Response Loci of Tomato

Giovannoni, J.; Barry, C.
USDA-Agricultrual Research Service Plant Soil and Nutrition Laboratory; Ithaca, NY 14853
Grant 00-35300-9356; $200,000; 3 years

Fruit is a major component of the human diet contributing a large portion of vitamins, minerals and fiber intake once fruit have reached the "ripe" stage of maturity. While specific attributes of ripening fruits vary from species to species, many common themes under similar regulatory control have been demonstrated among a wide range of diverse fruit bearing species and include changes in color, texture, cell wall metabolism, aromatic and flavor volatiles, and susceptibility to opportunistic pathogens. Greater understanding of the genetic and molecular basis of the ripening process will promote both our collective understanding of plant development and additionally yield tools useful for sustaining and improving 1) agricultural productivity, 2) resulting fresh and processed fruit quality, and 3) human health and nutrition while 4) minimizing impact on the environment necessary for production. This project involves implementation of a strategy toward isolation of a tomato gene known as *high-pigment-1* which when naturally mutated impacts the accumulation of fruit pigments with antioxidant activity, and thus ultimately ripe fruit color, quality and nutritional composition. Once isolated, the *high-pigment-1* gene will represent a molecular tool for modification of fruit quality and nutritional content via either traditional breeding or biotechnology.

2000-01389 Molecular Evaluation of Rice Germplasm Using Microsatellite Markers

Tai, T.H.; McCouch, S.R.; Cartinhour, S.
USDA Agricultural Research Service; Dale Bumpers National Rice Research Center; Stuttgart, AR 72160
Grant 00-35300-9216; $140,000; 2 years

Genetic diversity is a key factor in sustaining agricultural productivity. In order to preserve and make this diversity available for crop improvement, tremendous efforts have been made in the collection, maintenance, and classical characterization of germplasm. The ability of researchers to assess genetic variation by examining differences at the DNA level has enhanced the evaluation of these genetic resources and increased their utility. Rice is an example of a major crop species for which several germplasm collections exist and for which an abundance of molecular marker data is available. As is the case in many other species, microsatellites are widely distributed throughout the rice genome and exhibit a high level of allelic diversity, making them ideal markers for assessing genetic variation. In this study, a group of 576 rice accessions (representing various geographical origins and cultural types) will be examined using a set of 150 microsatellites. Fluorescent-labeled markers and an automated genotyping system will be used to facilitate this analysis. This project will contribute to the sustainability of the U.S. rice industry by providing valuable information for the isolation of important genes and promoting the use of molecular marker-assisted selection in U.S. rice breeding programs. All the data and analyses generated will be publicly available via the GRIN and RiceGenes databases (www.ars-grin.gov; http://ars-genome.cornell.edu/rice).

2000-01279 Sugar Maple DNA Fingerprinting

Baribault, T.J.
University of Vermont; Department of Botany and Agricultural Biochemistry; Proctor Maple Research Center; Underhill Center, VT 05489
Strengthening Award; Grant 00-35300-9217; $50,000; 2 years

The sugar maple industry is a vital part of sustainable agriculture and forestry in the Northeast, with sap sweetness dominating the economics of production efficiency. The current maple forest decline problem and the reforestation of abandoned cropland add further impetus to the establishment of methods for breeding genetically superior trees. The heritability of sap sweetness varied considerably among sugar maple populations studied previously. Environmental factors exert a considerable influence on sap characters from year to year. Because the uncertainty in trait assessment is compounded by a long generation interval, emphasis in the proposed research will be to further define the correlation between randomly amplified polymorphic DNA (RAPD) fragments and sap sweetness. This knowledge will be required for identifying appropriate parents of a suitable mapping population that will subsequently segregate for DNA markers and the important phenotypes of sap sucrose content and maximum stress tolerance. Provenances from both Ohio and the Northeast will be included in this assessment of RAPD fingerprints and phenotypic variation. Existing clonal lines or F_1 plants with the most informative combinations of physical traits and DNA marker genotypes will be used to derive F_2 populations for later analysis. New F_1 progeny will be established from parents with maximal trait divergence as necessary. RAPD fragment analysis will also afford the opportunity to monitor genetic diversity in existing plantations and in open pollinated progeny from elite, “sweet-tree” genotypes.

2000-01162 Equipment to Enhance Research Capability in Plant Gene Transfer

Murray, E.
Marshall University: Integrated Science and Technology Department; Huntington, WV 25755
Strengthening Award; Grant 00-35300-9262; $17,000; 1 years

Stripe rust (caused by *Puccinia striiformis* f.sp. *hordei*) is an important disease of barley in the western United States. Genetic resistance is the most economical and sustainable method for controlling stripe rust and many other plant diseases. Plants have two principal types of disease resistance – qualitative and quantitative. Theoretical considerations, and experience, suggest that the latter will be more durable than the former. However, it is difficult to breed for quantitative resistance because of the continuous spectrum of disease reactions, “resistant” plants allow some symptom development, and the trait shows complex inheritance. Quantitative Trait Locus (QTL) analysis is a tool for understanding and manipulating quantitatively inherited traits. We have used QTL techniques to map and use quantitative

stripe rust resistance genes in barley. However, important questions relating to estimates of gene location, effect and interaction remain unanswered. We propose to address these questions using a large population of homozygous lines. We obtained this population by crossing an experimental selection possessing three mapped quantitative resistance genes (BCD47) with the most widely grown, but stripe rust susceptible, variety in the Pacific Northwest (Baronesse). We will determine the genotype of each line in the population using molecular markers and we will determine the level of resistance to stripe rust in field tests. The genotype and phenotype data will be integrated to map and determine the effects of genes segregating in the test population. Our goal is to use this information to increase the profitability and sustainability of barley production for Pacific Northwest farmers.

2000-01385 New Roots for Agriculture: Genetics of Phosphorus Efficiency and Root Traits in Maize

Kaeppler, S.M.; Lynch, J.; Coors, J.G.
University of Wisconsin-Madison, Madison, WI 53706
Grant 00-35300-9246; $200,000; 3 years

Efficient use of mineral resources is critical to the long-term sustainability of U.S. and world agriculture. This research is focused on phosphorus nutrition in maize and will lead to the development of maize cultivars which can maintain a high yield potential with reduced applied phosphorus. Phosphorus is important to U.S. and world agriculture for several reasons. First, phosphorus is the most limiting nutrient for plant growth on the planet, especially in weathered soils such as those found in the tropics. Second, mineral resources such as phosphorus are finite and non-renewable. Finally, conservative use of mineral nutrients in agricultural production will protect surface and ground water quality by reducing nutrient run-off and leaching. Plant cultivars which maintain yield under reduced-input conditions would greatly facilitate the dual goals of productivity and responsible utilization of inputs. This research will define root traits such as root branching or root biomass which are most important for enhancing phosphorus efficiency in maize. Maize genotypes will be grown in controlled environments with specific nutrient inputs to assess root systems and plant responses to varying nutrient levels. These same genotypes will be analyzed in the field for grain production and root characteristics to determine which root traits are most important for phosphorus efficiency. Genes controlling root traits and grain yield under reduced phosphorus levels will be genetically mapped. Genetic mapping of these genes will facilitate their manipulation in breeding programs and will allow more detailed analysis in subsequent research.

2000-01394 A Clade-Based Search for Genome Rearrangements and Useful Diversity in *Solanum*

Spooner, D.; Hanneman, R.; Bonierbale, M.
USDA, Agricultural Research Service, Vegetable Crops Research Unit, University of Wisconsin, Department of Horticulture, Madison, Wisconsin, 53706-1590
Grant 00-35300-9411; $220,000; 3 years

The wild relatives of potato present an exceedingly rich resource for potato improvement. The cultivated potato has over 200 wild species relatives, many that possess valuable disease resistances and quality traits that can be incorporated into commercial potato cultivars through plant breeding. Luckily, there are about 5000 living collections of these wild species available for potato breeders. Because it is a time-consuming and expensive task to use any individual wild species in a breeding program, many of these 5000 collections have not yet been evaluated for their breeding potential. This research uses insights from recent DNA-based studies of potato relationships to choose wild species breeding stock from a relatively unexplored group of potatoes technically known as the "*Solanum Piurana* Group". We propose a systematic approach to capturing useful resistances to one of the most important diseases of potato called late blight, the very disease responsible for the Irish potato famine. In addition, we will search for the region of the potato chromosomes containing this resistance. The long-range goals of the research are the breeding of potato cultivars that harbor diverse resistances and other valuable genes, and knowledge of chromosome structure for breeding purposes. Unique technical strategies (called comparative sibship analysis and chromosome number manipulation) are proposed, to exploit the unusual opportunities and

challenges for the use of these wild potato species. This collaborative research will draw on complimentary expertise in basic and applied disciplines of taxonomy, genetics, and potato breeding, to locate and use valuable genetic diversity for crop improvement.