Plant Biological and Molecular Processes
FY 2001 National Program Annual Report

Introduction

This program includes much of the Agricultural Research Service's fundamental research that is necessary for practical advances, but is too far upstream to provide direct solutions for practical problems. The research focuses on mechanistic understanding of specific plant processes and properties. The knowledge and tools developed can be used to improve functions and properties of plants. The program is divided into three components: Analysis and Modification of Plant Genomes (functional genomics, focusing on the molecular end of the spectrum; technology for modifying plant genomes); Biological Processes that Determine Plant Productivity and Quality (mechanisms of plant growth and development, photosynthesis, productivity, and environmental responses that relate processes and attributes of the whole organism to their genetic and metabolic underpinnings and provide the context for molecular manipulations); and Mechanisms of Plant Interactions with Other Organisms (plant defensive reactions to pests and pathogens, emphasizing those that reduce the need for applied pesticides; interactions with beneficial organisms; secondary metabolism and products). Together, the results of the research approaches under this National Program provide a continuum of understanding of plant function from genes to phenotype (plant attributes and performance).

The Program was reviewed by independent panels of peer experts in November, 2000. Following this review, ARS scientists responded to comments, if any, and modified their project plans accordingly. Most plans were officially approved on June 1, 2001, after one round of review, whereas a few others awaited a second round of peer scrutiny. The advances reported here thus occurred partly under the temporary project plans that preceded final implementation, and partly under the official project plans approved following completion of the review process.

During 2001, there were many important discoveries and advances, some of which are described below. By no means do these selected accomplishments capture the important achievements of the entire research program. Instead, they highlight the type of activities carried out under this program and the type of benefits that result. The advances are grouped by program components.

Component I. Analysis and Modification of Plant Genomes

New methods for identifying important genes for plant improvement. Biotechnology does not involve solely genetic engineering, or moving genes between unrelated organisms. It also encompasses numerous methods to improve the utilization of genes already present in the crop breeding pool, thereby enhancing the efficiency and impact of conventional plant breeding. In 2001, ARS scientists showed that single nucleotide polymorphisms (SNPs) in soybean DNA occur relatively frequently in regions of the DNA coding for genes, indicating for the first time that about 40 percent of all soybean genes show polymorphism (variability). The SNPs represent a new and abundant source of molecular markers for generating a detailed map of the soybean genome.

Some important soybean varieties are "iron-inefficient" at high soil pH, becoming yellowed because they cannot take up iron from alkaline soil easily. In 2001, ARS scientists identified the region of the genome that controls this phenomenon and developed molecular markers that are associated with it. The use of these markers for breeding iron efficiency will greatly speed the development of soybean varieties that are productive on calcareous (alkaline) soils.

ARS scientists also created a microarray ("gene chip") that contains 2,800 genes associated with nutrient quality and health-promoting compounds of tomato fruits. This chip is a key development in learning how and when nutritionally important genes become active or inactive. It will provide a powerful "road map" to researchers for identifying how to genetically improve nutritional properties of foods and will greatly speed the day that such enhancement is routine.

Still another type of biotechnology was used to identify important genes. In 2001, ARS scientists found that selection for anthracnose resistance in tissue cultures derived from strawberry germplasm works well. This gives geneticists a rapid, year-round method for more rapidly evaluating disease resistance of strawberry plants, and possibly many other species.

Transgenic plants to solve very difficult problems. In many cases, crops face problems that conventional breeding cannot address because there are no suitable genes in the breeding pool. Some diseases or pest problems fall into this category. Even more importantly, there are some properties of plants that severely limit their use as foods or feeds, or that can be dangerous to sensitive individuals. For example, the ubiquitous soybean causes serious allergic reactions in up to 5 percent of infants (less in adults). In 2001, ARS scientists, in cooperation with the University of Arkansas, finished evaluation of hypoallergenic soybeans created by "knocking out" expression of the genes for the allergens. As predicted, the engineered soybeans showed virtually no allergenic potential, making them a much safer product as a base for infant formula.

In 2001, ARS scientists for the first time successfully transformed a variety of hard red winter wheat that is grown in the United States. This advance means that genetic traits can be put into agronomically acceptable wheat lines directly, eliminating the costly and slow step of extensive backcrossing. ARS scientists also transformed wheat with a variety of anti-fungal genes to combat head scab. The gene that worked best confers the ability to detoxify mycotoxins from the Fusarium fungus that causes the disease. This new finding, in collaboration with the University of Minnesota, will alter the focus of efforts to make wheat more resistant to head scab. Barley has also been transformed with similar genes, and preliminary results in 2001 indicate a substantial reduction in head scab in some of the lines.

ARS scientists also reported advances in viral disease resistance in *Gladiolus*, an important species to the floral industry. Antiviral genes to bean yellow mosaic virus were expressed in *Gladiolus*, and all transgenic lines were shown to be more resistant to the virus. This demonstration is a first in overcoming virus diseases, which may be the most difficult problem faced by flower producers.

<u>Improving the specificity of genetic engineering</u>. In 2001, ARS reported for the first time a method for moving a gene that had been randomly inserted into the genome to a specific site in the genome. A patent has been filed for this invention jointly with BASF, the co-inventor. In addition, the same laboratory found that another site-specific transformation method is effective in a wide variety of types of organisms. One practical effect of site-specific transformation will be to reduce unknown or unwanted genetic rearrangements that might occur during transformation. More importantly, in the future this technology will create "linkage" of introduced genes so that multiple genes will be inherited almost like single genes. This is necessary to begin to manage multi-gene traits more efficiently.

Component II. Biological Processes that Determine Plant Productivity and Quality

<u>Improving plant responses to environmental stress</u>. An important and previously unrecognized component of plant adaptation to freezing stress has been described. In the crown of winter wheat plants, freezing adaptation involves water (but not solutes) moving from inside the cell into the cell wall, where it can freeze. This accomplishes two purposes: it concentrates the solutes left inside the cell, lowering the freezing point; and ice formation in the wall releases some latent energy as heat. ARS research in 2001 has identified and characterized genes for "water channels" in the cell membrane that allow the water movement to occur. The genes are expressed only in the crown tissue and only when the crowns are subjected to freezing stress. Manipulation of these genes is expected to be very useful for directly improving frost tolerance of plant tissues.

ARS scientists in 2001 identified molecular markers in rice for chilling tolerance at the seedling and reproductive stages. Chilling at these stages of growth can seriously affect yield. The markers are currently in use to transfer cold tolerance into high-yielding tropical rice, which performs poorly in the United States because of its lack of cold tolerance. An efficient screening tool was also developed to rapidly identify cold tolerance in germinating rice seedlings, and this will be used in selecting germplasm for crosses. Molecular markers were also identified for submergence tolerance in rice and have been used to breed submergence tolerance into a widely-used California cultivar. These improved lines were extensively tested in field trials in 2001.

Irrigating cotton during heavy flowering can cause substantial shedding of flowers. ARS scientists demonstrated during 2001 that when sprinkler irrigation sprays water droplets into open flowers, the pollen is rapidly hydrated and ruptured, which reduces yield potential. Minor alterations in irrigation practices were shown to increase yield significantly. Use of pure water to rupture pollen also has promise as a tool for geneticists to eliminate self-pollination and facilitate cross-breeding or hybrid seed production.

More effective use of herbicides. In 2001, ARS scientists at two locations reported strong temperature effects on herbicide efficacy. Glyphosate, a widely used herbicide especially in herbicide-tolerant crops, is slow-acting, and this creates problems in knowing whether weed control is effective. High temperatures greatly speed its action, leading to a temperature-based strategy for timing its use to improve efficacy in the field. Other herbicides were more effective if applied during the warmer times of the day, when air temperatures are generally optimum for good herbicide efficacy. Recognition of the time-of-day (temperature) influence on herbicide efficacy is a simple means to improve weed control while reducing the amount of herbicide used.

Cryopreservation of difficult seeds. One of ARS's prime responsibilities is to preserve germplasm in seedbanks. Many species are "recalcitrant," however, or fatally injured by standard preservation techniques. In cooperation with scientists from South Africa, a device was built that cools seeds very rapidly at rates from hundreds to thousands of degrees (Centigrade) per second. In 2001, specific combinations of seed hydration, cooling rate, and seed mass were shown to preserve seeds of some recalcitrant species that had never been preserved before. This breakthrough is expected to be especially valuable for seeds of many tropical fruit and temperate forest tree species.

Component III. Mechanisms of Plant Interactions with Other Organisms

Improved Host Plant Resistance to Pests and Pathogens. In many cases, the most efficient means to reducing damage from pests and diseases is through breeding to improve the resistance of the crop. This is often the method of choice when available germplasm contains genes that enhance resistance. Breeding is made easier and more successful by knowing the nature of the genes or, if the trait is complex, the location of the genes in the genome. In 2001, ARS scientists identified genetic lines of wheat with complete resistance to Cereal Yellow Dwarf Virus (CYDV) and moderate resistance to Barley Yellow Dwarf Virus (BYDV). DNA markers are being developed and one of the lines will be made available to wheat breeders around the world. ARS scientists also identified 24 genetic strains of durum wheat with excellent resistance to Hessian fly (even to the diverse biotypes, or races, which exist in the eastern United States). Seeds are now being made available to wheat breeders for incorporation into locally adapted soft winter wheat varieties.