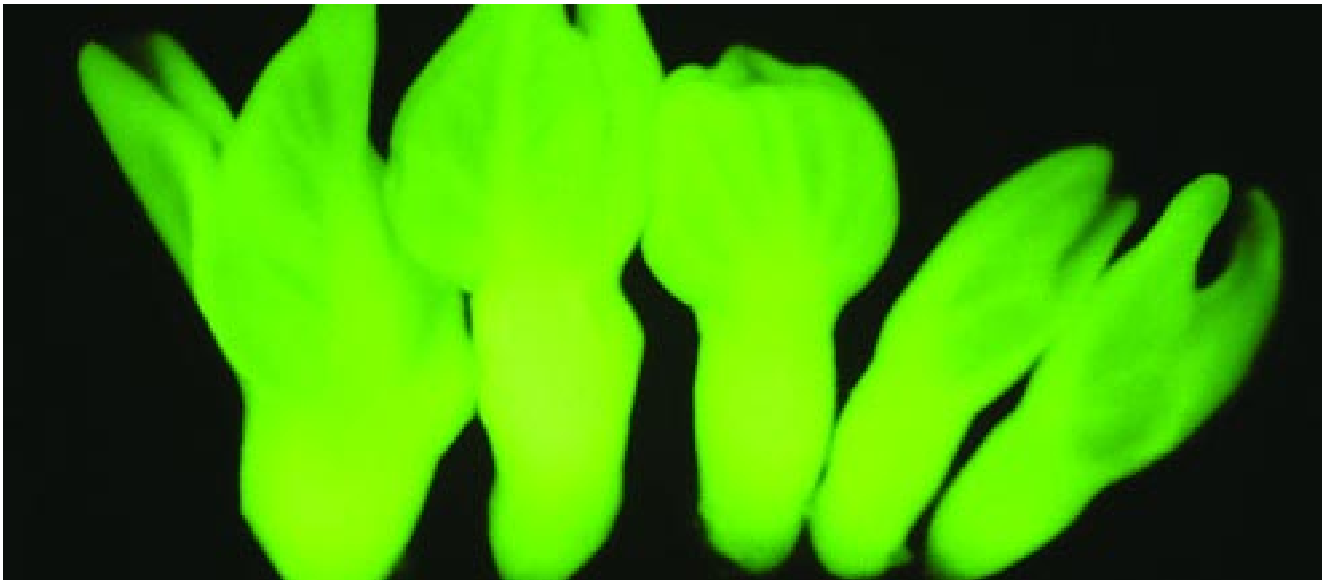
Molecular Genetics for Enhanced Plant Breeding



Florescent marker gene in grape

In recent years, FAES breeders have joined forces with FAES molecular geneticists. Molecular genetics can make breeding programs more efficient, accelerate progress in breeding, and enable the introduction of novel traits. Molecular genetics seeks to improve plants by three methods: 1) Functional Genomics: a series of steps to determine the location, sequence, function and interrelationships of the genes in an organism; 2) Marker-Assisted Selection for Breeding: using DNA-based markers to identify the presence of certain genes and to select for commercial traits; and 3) Transformation: the insertion of genes into a plant to develop a new genotype for commercial use or to improve understanding of traits or physiological mechanisms. (Transformation uses biochemical methods to transfer genes between organisms whereas conventional breeding uses the organism's natural processes to transfer genes between organisms.) After these laboratory steps, new plants undergo conventional breeding techniques to develop commercially viable varieties that carry the new trait.

Some examples of molecular genetic successes and contributions to plant breeding at FAES include:

New Disease-Resistant Varieties of Grapes

Currently bunch grape used in wine making cannot be produced in Florida, due primarily to Pierce's disease caused by *Xylella fastidiosa*, a microbe that occurs in the grape plant's xylem sap. Through breeding and use of molecular genetic tools, disease-resistant varieties can be developed. Dennis Gray's laboratory has identified a lytic peptide gene that may kill the microbe. He has inserted the gene, tagged with a green fluorescent protein marker gene from jellyfish, into Thompson Seedless grape plants. Plants with green fluorescent veins were selected; their xylem sap was tested and shown to have the antimicrobial lytic peptide. These plants are being tested in the greenhouse by inoculation with Pierce's disease (*Xylella*). In 2001, a U.S. patent was issued to UF and the USDA for the use of lytic peptide genes to control Pierce's disease. Marker-assisted selection and gene transformation are moving us closer to the production of new varieties of Pierce's disease-resistant grapes for the southeastern U.S.

Flowers That Will Last Longer

In many flower crops, there are physiological problems during production and postharvest handling that lead to loss of quality. Dave Clark is studying ways to solve these problems by using molecular genetics tools. He is identifying the genes involved in flower development and senescence in petunia, a model plant. By understanding gene expression – where and when a gene is

Study of gene expression in petunia

used in a plant – the basic physiological processes in plant development will be elucidated. He will then clone the genes and employ them in transgenic plants. The goal is to produce flowers that last longer and have leaves that stay green longer, making them more hardy for shipping, more valuable for commercial use, and longer lasting.

Rye for Better Bread-Making

Rye is second in popularity to wheat for bread making worldwide. Yet, rye dough has less elasticity and poorer rising properties that result in denser bread. Compared to wheat, rye is better adapted to adverse environmental conditions such as drought and cold temperatures, and it needs less fertilizer, herbicides and fungicides. An international team of scientists (Juan Carlos Popelka, I.P.K. Gatersleben, Herbert Wieser, and Rolf Kiefer from the Kurt Hess Institute, Germany) coordinated by Fredy Altpeter has developed a highly reproducible genetic transformation protocol for rye that has improved its bread-making quality. In addition to the successful gene transfer, the new cells could be regenerated in fertile rye plants. Many experiments have resulted in the production of a large number of the new transgenic plants and identification of the best genotypes. The high molecular-weight glutenin subunit genes from wheat that were introduced into rye changed the protein composition in the rye grain, and the bread-making quality of the transgenic rye was improved significantly.

Genetic Markers Help to Select the Best Tomatoes

Disease-resistant varieties have been one of the main achievements of tomato breeding, and since the 1980s, genetic markers have been instrumental in advancing the selection process in Jay Scott's laboratory. A marker linked to a resistance gene (*I-3*) for Fusarium wilt (*race 3*) was discovered and the resistant breeding lines are now incorporated into commercial hybrid varieties in Florida. For spotted-wilt virus, another devastating tomato disease, scientists used a SCAR marker linked to resistance gene *Sw-5* to select for more resistant varieties. RAPD markers have been used to determine the location of geminivirus resistance genes. The industry continues to search for greater disease resistance in their tomato crops, and marker-assisted breeding will continue to speed up new selections.

In collaboration with USDA and industry, FAES scientists have also initiated a program to identify genes controlling tomato flavor. The program is an integrated project involving biochemical and molecular characterization of diverse germplasm. Work is currently being performed in the laboratory of Harry Klee, in collaboration with Jay Scott and Elizabeth Baldwin (USDA, Winter Haven). Molecular markers that will track synthesis of various chemicals contributing to flavor are being isolated. In the near term, these molecular markers can be used to select lines in the existing breeding program. In the longer term, genes encoding synthesis of these chemicals can be manipulated via molecular techniques. In this way, we expect to be able to significantly improve overall flavor quality.

Increased Yield for Rice and Wheat

Curt Hannah's laboratory has transformed rice and wheat with a maize gene that increases the yield of both rice and wheat. Rice and wheat produce some seeds that do not mature and that have excess photosynthetic capacity, indicating that more resources may be available for growth, representing potential for increased yield. An enzyme in corn, ADP-glucose pyrophosphorylase (AGP), plays a key role in starch production in seed. When this corn gene was inserted into rice and wheat, there was an increase in AGP activity in the

Harvesting disease resistant tomatoes

seed. As a result, both rice and wheat plants had increased seed weight and total plant weight. Wheat plants, for example, had 38% more seed weight and 31% more plant weight.

Vegetables: New Sources of Disease Resistance

Begomoviruses, DNA plant viruses transmitted by whiteflies (*Bemisia tabaci*), infect many important crops and are often impossible to manage even with a heavy reliance on insecticides. The begomovirus, Tomato yellow leaf curl virus (TYLCV) has been a big problem in tomato production since the 1990s in the United States, the Caribbean and Western Mediterranean. Ernest Hiebert and Jane Polston have developed immunity in tomato to TYLCV by inserting parts of the *Rep* gene from TYLCV into tomato chromosomes. Preliminary evidence suggests that the TYLCV *Rep* gene constructs elicit a natural host defense mechanism. This pathogen-derived resistance may be better than known plant-derived resistances because it could completely prevent virus replication.

Mapping Citrus

Gloria Moore has been a pioneer in the development of routine protocols for citrus tissue culture and genetic transformation. She was the first to publish on the use of *Agrobacterium* for the genetic transformation of citrus. Her lab, along with Fred Gmitter Jr., published the first genetic maps of the citrus genome, and it remains active in marker development and mapping. They have mapped many important genes in citrus, particularly those involved in cold hardiness and salt tolerance. They are cloning several genes of interest to citrus variety improvement, including genes for cold tolerance, fruit color development, and disease resistance. Much of this work is now accomplished in the Core Citrus Transformation Facility, managed by Vladimir Orbovic, which was established in 2002 to integrate molecular genetics research with other related disciplines such as food science, post harvest physiology and plant pathology.