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Application of Agricultural Biotechnology

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APPLICATION OF AGRICULTURAL BIOTECHNOLOGY

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The term biotechnology encompasses a wide range of different disciplines and techniques. Some of these techniques are becoming standard practice for breeding efforts at seed companies and public institutions where the crop economics govern low monetary expenditures. There are other techniques which require a greater level of expense which are being carried out by companies or public institutions with large research budgets or funding from the end users of the crop.

Techniques applicable to breeding efforts are used to speed the production of homozygous lines by haploidy, to decrease plant generation time, to introduce genes from related non-crop species which are difficult to cross sexually and to screen for diseases. These techniques include another or microspore culture, and embryo rescue. Anther and microspore culture are becoming routine tools in the development of inbred lines in several crop species, including rapeseed, corn and rice. Embryo rescue is used for the production of wide cross hybrids from related wild species to the crop species where embryos from crosses do not survive in vitro. Embryo rescue can also be used to speed generation time in crops, such as tomatoes where fruit and seed mature slowly.

Immunological techniques using monoclonal antibodies are being developed for use in viral and bacterial disease detection. This technology is being used routinely in potatoes to screen seed pieces.

Other techniques being developed and utilized in biotechnology are selection from somaclonal variation and embryo encapsulation. Somaclonal variation is similar to mutation breeding, only it takes advantage of the natural increase in mutation rates that occurs during cell culture. The potential of somaclonal variation breeding can be realized when a selection pressure for the desired trait can be applied in culture. Under these conditions selection can be made from millions of individual mutated cells and the chances of finding the proper genotypes are greatly increased over classical mutation breeding. This process is limited to species which are amenable to regeneration from protoplasts, plant cells with the cell wall enzymatically removed. Another problem with the technique is that traits

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observed in culture do not always carry through to whole plants or to further generations. This type of effect is termed epigenetic. Salt tolerant genotypes in tobacco and peppers, reportedly, have been derived from somaclonal variation. Herbicide tolerances in tobacco and corn have also been reported, however, the corn cultures have not been regenerated.

Embryo encapsulation is being developed as a technique to asexually propagate crops from somatic embryos, embryos derived from vegetative tissues through culture. These embryos can be produced in large numbers in culture and then the embryos are encased in a gel coat of calcium alginate. The technique could be of use in crops where seed is either difficult or expensive to produce, such as certain hybrid crops, or where asexual propagation is currently used. this technique is not presently at a level of development where commercial use is possible. Problems exist in obtaining uniform high quality embryo production, soil germination and refinement of storage, transport and handling of the capsules.

Libraries of restriction fragment length polymorphism (RFLP) are being developed for a large number of species. These are pieces of DNA which can be used as genetic markers for genome mapping. They can be utilized by breeders for varietal identification and protection, parentage determination and as a tool to identify and monitor multigenic quantitative traits.

Genetic engineering, the manipulation of an organism using recombinant DNA to change it's genetic make-up, is perhaps the most exciting of the biotechnology procedures, because it allows unusual changes or solutions to problems by utilizing DNA from any source. In order to give a clear picture of where genetic engineering of plants stands in terms of commercialization of genetically engineered varieties, it is appropriate to describe the steps involved in genetic engineering and where problems can arise within those steps.

The first step in genetic engineering is choice of the trait to be engineered and the approach to expressing the desired trait. This often takes a multidiscipline collaboration between molecular biologists, biochemists and physiologists to evaluate the feasibility of an approach. Often some preliminary experimentation to evaluate the feasibility is carried out by biochemists. The next step is identifying the gene or protein product to be used, finding a source for the gene and cloning it into a bacterial vector. Then the gene is modified or engineered as necessary, for example it is made so that it will only express in a desired tissue type. Then it is introduced into the chosen crop.

One advantage of genetic engineering is that one trait, for example herbicide tolerance, can be put into several crops once the gene has been optimized. The limitations of the technique are that an

optimized transformation system for the target crops must be in place and the traits are limited to those which can be affected by transfer or a small number of genes. Limits in the number of species which can be transformed efficiently and problems in isolating the protein products necessary for gene isolation are the two rate limiting steps at the present time.

Table 1 is a list of plants which reportedly have been transformed. Of these only a few can be transformed effectively enough to produce commercial products. The most common technique and the only one at present with commercial application is transformation by **Agrobacterium tumefaciens(At)**. At causes a crown gall disease in plants. It is known to transfer DNA to the host plant as part of the infection process. Molecular biologists can "disarm" At so disease symptoms do not appear and insert the genes wanted in the plant in place of the normally inserted DNA. By growing the disarmed At with regenerable tissue from the desired crop (cocultivation), then killing the At with an antibiotic and regenerating plants, transformed plants can be produced. However, not all species are amenable to this system. Some are not hosts for At. Others will not regenerate after cocultivation. At present the most prevalent crops which can be genetically engineered by this method are tomatoes, tobacco, cotton, and rapeseed.

The applications of genetic engineering to crop improvement can be split into two basic areas, agronomic or horticultural improvements and crop product quality improvement. In agronomic or horticultural improvement, there are several types of improvements including insect, disease and herbicide tolerances as well as yield improvements. Insect tolerance has been engineered into plants. Plants have been engineered to produce the insect specific toxin from the bacteria, **Bacillus thuringensis**. Tomato plants containing this trait have been field tested and do show insecticidal action against the targeted pest. Another approach to insect tolerance has been the engineering of the gene for trypsin inhibitor from cowpeas into plant leaves. This is a novel approach in that it takes an enzyme, which in nature is not an insecticide, and places it in plant leaves where, when eaten by a broad range of insects,it causes inhibition of digestion and a level of control of those insects.

Viral disease resistance has been developed by cross protection. Cross protection is found in nature where infection by a mild strain of a virus prevents the infection by more virulent strains. Using this observation, the genes for the coat protein of several viruses have been engineered into plants. Viral inoculation tests of plants transformed with viral coat proteins have shown a high level of tolerance in several cases. Tolerance to **TMV** in tomato has been field tested.

Table 1. Transgenic plants.

alfalfa	morning glory
arabidopsis	moth bean
birdsfoot trefoil	petunia
black walnut	poplar
carrot	potato
celery	rapeseed
corn	rye
cotton	sunflower
cucumber	tobacco
flax	tomato
horseradish	turnip rape
lettuce	

Herbicide tolerance in crops has been pursued extensively and several examples have been reported. At Calgene, we have two herbicide programs which we are currently field testing. Calgene has developed bromoxynil tolerance by a detoxification method. A gene encoding for an enzyme, nitrilase which converts bromoxynil to a non-toxic form, was isolated from a bacteria. When introduced into plants this gene causes a high level of tolerance to bromoxynil in otherwise susceptible species with no apparent effects on growth. Levels of tolerance in tobacco and tomato range from 3x high field rate (1.5 lbs./ac.) to at least the highest rate tested, 16x field rate. Tolerant tobacco was tested in the field recently and no symptom development was seen from plants sprayed at 2.0 lbs./ac. Sprayed transformed plants were indistinquishable from nonsprayed transformed plants or the parent variety, **Nicotiana tabacum cv. xanthi nc.** Tolerant tomato plants have been sprayed at 0.5 lbs./ac. in greenhouses and grown to seed. No affects on fruit production were seen. Tolerant tomatoes are being tested this season. Yield and quality characteristics will be examined. Cotton plants transformed for bromoxynil tolerance have been produced and testing should start in the coming months.

The other herbicide tolerance program we have in field testing is for the herbicide, glyphosate. This tolerance is based on a different mechanism than the bromoxynil tolerance. Glyphosate binds to an enzyme involved in amino acid production and prevents its functioning. The engineered gene in this case is an altered form of this enzyme, from a bacteria, which is not as severly affected by glphosate. Greenhouse grown plants in both tobacco and tomato sprayed at 1 lb./ac. have produced seed. Initial symptom development is found in the tolerant plants after spraying. Some plants recover from spray and grow from the original shoot tip and others grow from lateral shoots. This response is variable depending on environmental conditions.

Genetic engineering is being used to look at improvements that could affect yield. Enzymes involved in photosynthetic efficiency and carbon incorporation are being analyzed as possible sources for yield improvement.

There are many ways in which genetic engineering could be used to improve the quality of crop products. In order to give a general idea of some of the potentials, I will discuss areas of research Calgene is pursuing towards this goal. The first is in tomato. Processing quality of tomatoes and shelf life of fresh market tomatoes are being examined. Polygalacturonase (PG) is an enzyme involved in break down of the cell walls in tomato fruit during ripening. The gene for this enzyme was isolated from tomato. The objective of this project was to turn down the production of this enzyme in the fruit. By producing a gene with the opposite sequence from this gene (the antisense of the gene), the enzyme production in the plant will be

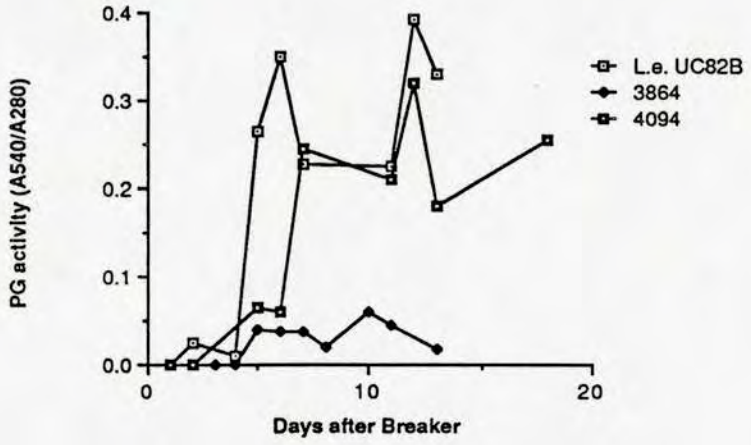
turned down. Figure 1 shows the level of PG enzyme activity in fruits from two different transformed plants and the parent variety. Levels of PG activity are reduced in the transformed plants. There also appears to be a delay in color development. Further study of these effects are underway. The lowered PG activity could affect shelf life, firmness and/or solids content of these tomatoes, which is also being analyzed. All three are important characteristics for commercial tomatoes.

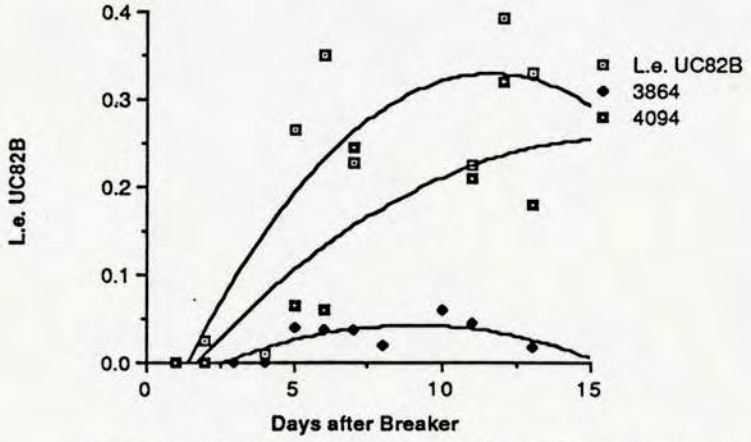
Oil quality in seed crops is a research area at Calgene. The goals of this research are to tailor rapeseed for the production of different types of oil, for example coconut oil. This is an integrated effort involving many disciplines. Biochemistry and physiology are being used to examine potential enzyme targets from other species for engineering into rapeseed and to isolate the enzymes. Molecular biology is isolating, cloning and engineering genes for transformation. Cell biology has been used to develop an optimized rapeseed transformation system and transform plants with the isolated genes as they are engineered. A plant breeding program is in place to develop varieties adapted to U.S. growing regions, to approach the goals with classical breeding and to incorporate the transformed plants into the breeding program. A product evaluation group bridges the gap between the lab and breeding and does evaluation of the effectiveness of the trait and determines genetic inheritance of the transformed plants. Rapeseed plants transformed with the gene incoding for the acyl carrier protein (ACP) have been produced and will be tested in the field to see what affects this gene has on oil content and to examine the effects of transformation on rapeseed.

Fiber quality is another area is pursing in coordination with a subsidiary, Stoneville Cottonseed Company. Natural fibers are made of two major components either cellulose (as in cotton. flax, and ramie) or proteins (as in wool, silk, cashmeer and mohair). Two approaches to fiber quality improvement are concievable. Through improvement of the cellulose or other components in the fiber affecting quality or through the development of novel fibers. We can engineer genes to be expressed in specific organs such as cotton fibers and we can transform cotton to produce specific proteins.

In conclusion, biotechnolgy is being used by breeders to increase their efficiency in both speed of variety production and in selection for quantitative traits. Biotechnology will have impacts in combatting pest problems through genetic engineering and through a greater level of sophistication in diagnosis (immunological techniques). It adds a greater versatility in the options open to breeders when dealing with specific problems in breeding. Biotechnology is a cooperative effort of many disciplines and rides on the strong foundation of high quality varieties developed by the more traditional techniques of breeding. Genetic engineering is probably the most flexible area in biotechnology, as there are no limits on the

Figure 1. Polygalacturonase (PG) activity in antisense PG transformed tomato





The second graph depicts the line of best fit for a second order polynomial
R = ~0.8

sources of genetic diversity open to molecular biologists and unusual solutions can be adopted for reaching goals. Commercial applications for genetic engineering are now being demonstrated in the field. Products from this work will be ready for commercialization in the early 1990's.