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COTTON BIOTECHNOLOGY

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Cotton growers have heard reports dating back to 1987 about the coming promise of this technology. Promise is about to change to reality as the first genetically engineered cotton varieties now await final regulatory approval prior to commercial release. In our first newsletter on biotechnology, we will review the science of genetic engineering and its potential role in cotton improvement in the future.

Biotechnology refers to any number of biological processes and products. Pharmaceutical companies use biotechnology to manufacture drugs. Crop protection companies manufacture new compounds or alter existing ones using biotechnology. Milk production can be increased dramatically by administering a biotechnology product, Bovine Somatropin (BST), to dairy cows. Shoppers can purchase genetically engineered tomatoes that can be vine ripened without losing shelf life. Microorganisms used in insect control, such as *Bacillus thuringiensis (Bt)*, are grown using a biotechnology process. Today the genes for *Bt* have been transferred to cotton plants which then produce the *Bt*, making the plant insect resistant. This has all been made possible through advances in molecular biology and tissue culture in collaboration with classic plant breeding. A brief review of each may help you appreciate their respective roles in the future of cotton varietal improvement.

Genetics

The appearance and performance of cotton and all other living organisms are determined by genes. Genes are composed of DNA, the basic unit of inheritance, and the arrangement of the DNA distinguishes each gene. Genes can come in more than one form, allowing variation in how organisms look and perform; for example, eye color. One form of the gene for eye color results in brown eyes, another in blue eyes. Sometimes, more than one gene is required to change the appearance or performance of an organism. The number of genes and gene combinations is virtually endless, allowing an infinite number of variations, although the combination of genes that act at any given time is much lower. These different combinations of genes must act in concert to maintain life.

Genes are arranged along DNA strands called chromosomes. Cotton has 52 chromosomes. During sexual reproduction, each parent randomly contributes 1/2 of these chromosomes, so cotton has 26 chromosome pairs. The complement of genes are thus set at fertilization and all future cells will contain that complement, no matter where the cells are in the plant. However, the tissue (root, leaf, flower) and environment (drought, cold, heat, wind, fungal attack) that the cell finds itself in, determine which genes will become active (have expression).

Sexual reproduction, therefore, enables organisms to reorder their complement of genes and confers the ability to adapt to changing environmental conditions. The complement of genes that makes one plant successful in a particular environment may not be suitable for another environment. Plants, such as cotton, that are competitive in a warm environment, are not competitive in a much cooler environment. Changing environmental conditions may render a plant species less competitive if the population does not include plants with a desirable gene complement to adapt to these conditions. Thus, variation in the gene complement brought about by the reordering of genes during sexual reproduction insures survival. It is this variation that classical plant breeders use to develop new varieties of cotton that perform better in one growing area than another or that produce lint which is different from one variety to another.

However, since variation comes about as a result of reshuffling of genes during sexual reproduction, it has certain limits, depending upon how sexual reproduction takes place. Sexual reproduction in cotton occurs within a single flower containing both male and female parts which contribute a random set of 26 chromosomes to the offspring. Because both sets come from the same plant (self pollination), the amount of possible gene shuffling (variation) that can occur is reduced compared to plants such as corn. In corn, one set of chromosomes is contributed by one plant and the second set by another plant (cross pollination).

To increase the variation in the gene complements available for developing new varieties of cotton, plant breeders have utilized variation inherent in different cottons from different parts of the world. They use the male portion of one plant (pollen) to fertilize a second plant. Sometimes these cotton plants are so different in their gene complement, that fertilization is

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impossible, or reduced, or the offspring are infertile. Additionally, the genes wanted for a particular trait, such as insect resistance, may not be present within either cotton's strains or its wild relatives. Thus, the plant breeder's ability to breed new gene complements into a cotton variety that might allow more improved productivity, quality or pest resistance is limited. In these cases, molecular biology offers a valuable tool to improve existing breeding populations.

Molecular Biology

The limitations inherent in reshuffling of gene complements during sexual reproduction can be overcome somewhat by the techniques of molecular biology. Some years ago, scientists discovered how to cut genes from one DNA strand and paste them into another DNA strand, thus molecular biology came into being. Not long after, other scientists discovered that different sections of a gene determined what product (protein) would be made while other parts of the gene determined when, where, and how much of the product would be made. The region of the gene that determines what product will be made is called a coding sequence, while the part that determines when, where and how much is called a promoter. Within the promoter, when, where and how much are also defined by specific regions. So molecular biologists now had a generic "road map" of genes.

Soon molecular biologists could transfer genes from the DNA of one organism into the DNA of a second organism and have the gene product made in the second organism. This process is called transformation and a plant containing such a new gene is called a transgenic plant.

If the entire gene is transferred, the gene is normally expressed in the transgenic plant in a manner similar to its original host. However, if the promoter is removed from the gene and a new promoter from a different gene is added, the gene will be directed in its new host by the new promoter. Now, molecular biologists could shuffle not only genes between sexually incompatible organisms, but could shuffle different parts of the genes themselves. Manipulation of promoters thus allows us to determine where and when in a plant we want to make our new product and even enhance how much product is made.

Much of the information about how a promoter behaves has been determined by attaching promoters to gene sequences, coding for a marker that is easily detected when it is made in the transgenic plant. For example, one marker gene makes a product called GUS that turns cells bright blue. The marker product is made only in the tissues of the plant, at times during development or under the specific environmental conditions which the promoter normally directs in its original host. This allows us to dissect promoters and determine their exact function in a plant and ultimately to regulate gene activity in a plant. This raises a question: Could we shut off a gene so that its product is not made at particular times or in particular places? By using the complementary coding sequence of the gene we want to shut off, and putting it under the control of a specific promoter, we can produce a messenger that cancels out that gene's activity (antisense technology) at specific times or in specific tissues. A promoter that works only in pollen drives a gene sequence that causes pollen cells to die.

So now we can shuffle different parts of a gene to make it behave the way we want it to in its new host. What genes do we want to shuffle? Sometimes we don't know what the gene is, but we know it confers some property to its host, such as the ability to kill insects. We know that certain bacteria will kill certain insects but not others. It would be impossible to transfer that gene from bacteria to plants by sexual reproduction. But we can isolate the DNA that codes for the gene product that allows the bacteria to kill the insect. The coding sequence can then be spliced to a promoter that will work in plants and placed into a plant by transformation. The plant then makes that product and kills the insect. In the case of transgenic *Bt* cotton, a bacterial gene was isolated and a promoter from a virus attached to the coding sequence. This promoter is very powerful and acts in all parts of the plant.

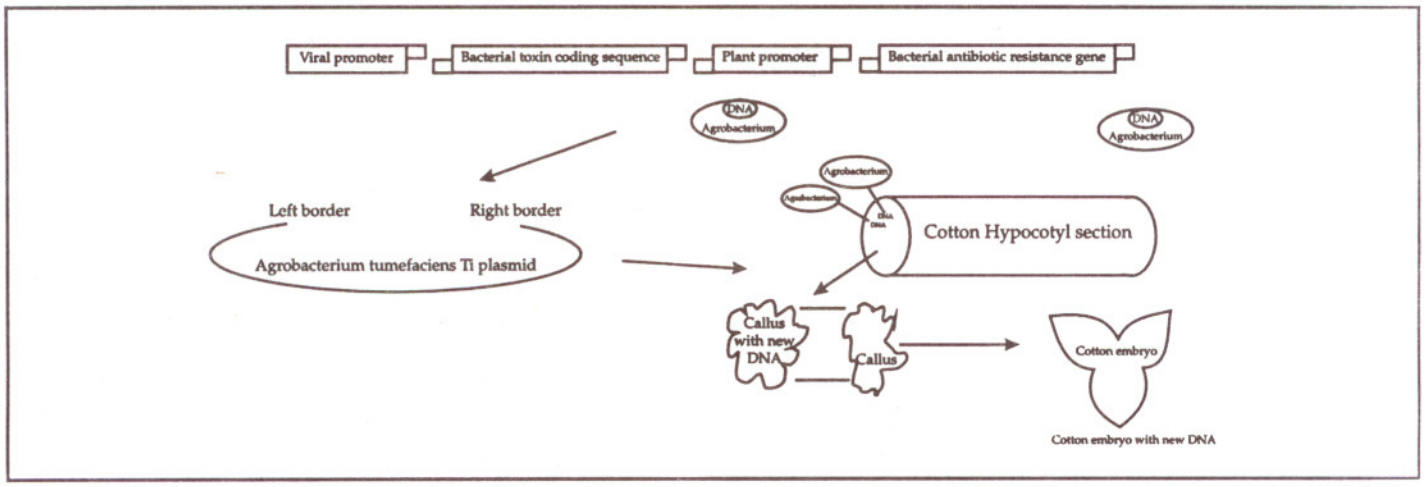
Such techniques open the door for fertile imaginations to speculate on the many possibilities of biotechnology. Some experts can envision that one day the cotton plant might produce its own nitrogen by transferring genes from a nitrogen fixing bacteria or produce fibers that contain a plastic core by transferring another bacterial gene to divert products into plastic. Transgenic plants that produce plastic are a reality. Transgenic plants that fix their own nitrogen likely would require the transfer of numerous genes. While we can transfer one to several genes, to date, we cannot transfer whole blocks of genes.

Tissue Culture

In order to transfer any gene, there must be a mechanism for getting the DNA into a cell that will eventually form an entire plant. Getting DNA into animal cells or other bacterial cells is no problem, because there are few barriers for the DNA to cross. However, plant cells are surrounded by a cell wall that prevents large DNA molecules from passing through. Fortunately, there is a bacteria, *Agrobacterium tumefaciens*, that is exceptionally good at transferring a piece of its own DNA into plant cells and having that DNA expressed in the plant cell to form a product. Normally, the product results in the formation of large galls on plant. Scientists used their knowledge of how to cut and paste DNA to cut out the part of the bacterial DNA that is responsible for the gall formation, but leave intact the part of the DNA that is responsible for the transfer of the bacte-

ria's DNA into plant cells. Using the same cut and paste techniques, new DNA coding for a desired product, i.e. *Bt* toxin, can be inserted where the gall forming genes were. The bacteria are then brought into contact with wounded cells and allowed to transfer their DNA (now also containing our new DNA) into plant cells. Thus, plant transformation became feasible. Once these individual cells are transformed, the whole plant containing the identical transformed genetic complement can be regenerated through a process called somatic embryogenesis.

Fortunately, too, most plant species have one or more genotypes that are capable of somatic embryogenesis. In cotton, the Coker cultivars are highly embryogenic but only a few other cultivars are. The embryogenic Coker cultivars are all sister lines derived from a cross made many years ago between Deltapine 15 and Coker 100W. Most transformation today is done with Coker 312 and the new genes backcrossed into the desired varieties.



To transform cotton, the stem section (hypocotyl) of a very young seedling is cut into small sections in the presence of *Agrobacterium* containing a hybrid chain of DNAs from different sources called a chimaeric gene construct. This chimaeric gene construct behaves like a micro factory in the plant cell which directs the transformed plant cell to start, and stop making a new product. For example, on each end of the DNA chain is a border sequence from *Agrobacterium* called the T DNA (Transfer DNA). In between the T DNA borders, one may place a promoter sequence followed by a coding sequence and a termination sequence. We already have discussed the function of promoter sequences and coding sequences. A termination sequence simply tells the cell's copy machinery to stop making the message that directs other parts of the cell to make the product.

After the tissue is exposed to the *Agrobacterium* for a short period of time, it is blotted to remove excess bacteria and placed on a growth medium which will allow the tissue to grow. The tissue is then incubated for two to three days. During this time, the bacteria multiply and cover the cut surface of the plant tissue. The cut surface of the plant tissue exudes substances that induce the bacteria to transfer its DNA into the plant's DNA. The tissue must then be placed on new media containing an antibiotic that will kill the bacteria or the bacteria will overrun and kill the plant tissue. If the chimaeric gene construct contains an antibiotic resistance gene, then all cells that contain the new DNA will be resistant to the antibiotic (or other selectable marker gene). If the antibiotic is added to the growth medium, then only those cells containing the new DNA can grow and develop into somatic embryos and eventually entire plants. The transformation process is summarized in the figure below.

Today, most transformation of cotton is done via *Agrobacterium* and somatic embryogenesis. However, there are other methods by which DNA can be introduced into plant cells. Particle gun bombardment is the second most used method. This does not require *Agrobacterium*. The chimaeric gene construct is shot through the cell wall into the cell by a number of different methods: compressed air (usually helium), electric shock wave, gun powder, to name a few. This method leads to multiple insertions of the new DNA and fragments of the DNA so sorting out the results is a bit more confusing. Some, but not all, particle guns are capable of delivering DNA into the cells of the plant meristem that eventually will give rise to offspring (germline cells). Unfortunately, the frequency with which this happens is very low and requires extensive labor to achieve acceptable results. No other method as yet has been successful in delivering DNA into the intact germline cells of cotton plants.

One of the most perplexing challenges associated with transformation has been the inability to regenerate all varieties necessitating repeated backcrosses to produce adapted varieties that carry the new trait. But, once the new trait is incorporated, they behave similarly to traits obtained through conventional plant breeding. With genetic engineering, plant breeders have a powerful tool to enlarge the pool of available traits and provide new and improved cotton varieties.

The transfer of a transgene into a conventional variety doesn't automatically mean the transformed strain will be acceptable to growers. This is because insertion of a major gene into an established high performing variety usually results in a lower yielding, later maturing, and poorer fiber quality strain than the established variety. However, the genetic engineering breeders continue to produce new transgenics until they find a strain that doesn't possess these undesirable characteristics. It only takes one successful transformation to result in a new high performing variety with the extra value trait added. As with all new varieties, growers need to know how transgenic varieties perform over a range of management regimes and test environments.

The value and cost of transgenic cottonseed will be higher. The least costly input for growers is cottonseed. The price of high quality, high performing varieties has not kept pace with the increasing cost of other inputs, such as herbicides and insecticides. However, since the genetic engineering breeder partnerships have gone to considerable expense to add the new traits, such as insect and herbicide resistance, into high performance varieties, growers can expect their seed cost to increase.

It also should be stressed that genetic engineering is a tool to use with conventional breeding. Conventional breeding's objectives have concentrated on yield, earliness and fiber quality. These are quantitative traits and are controlled by the action and interaction of many genes. In contrast, genetic engineering concentrates on traits that are controlled by a single gene, such as insect resistance or herbicide resistance. The first efforts to use transgenics will be to insert the transgene by backcrossing into a high performance conventional variety. The backcross method essentially reproduces the conventional variety with the addition of the transgene. Later, transgenic varieties and strains will be crossed with one another to produce genetically variable populations. The varieties selected from these populations will contain not only the transgenes but also will be improvements over the older varieties.

Wrap Up

The combining of conventional and genetic engineering promises to be an exciting time for cotton breeding and for cotton growers. At this time, no one knows how these new combinations of genes will perform, but within the next several years, the cotton industry should begin to get an idea of how genetic engineering will impact the entire cotton industry. The impact will be on every aspect of the cotton industry involving the grower, breeding organizations, biotechnology organizations, pesticide and chemical industries and the textile industry.

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