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Varieties: Development and Selection

That not all cotton varieties are alike is a given. Annually growers face the challenge of determining which varieties will perform best on their farms. Years in advance, seed companies weigh the advantages and disadvantages of increasing seed of one variety or another to meet and spur sales demands in future years. Breeders continually cull through diverse traits and decide which ones to introduce into new varieties. Could all of these individuals benefit from a crystal ball? At times, it certainly seems so. Their combined decision-making determines the future of the U.S. cotton industry.

Here we discuss where new traits originate, how new varieties (including transgenic cottons) are brought to market, and how cotton's future is affected by the decision-making of growers, breeders, and representatives of the seed industry. Last, we give some insights as to how to gain helpful information from statewide variety trials when deciding what is in that crystal ball for your farm.

JM Stewart

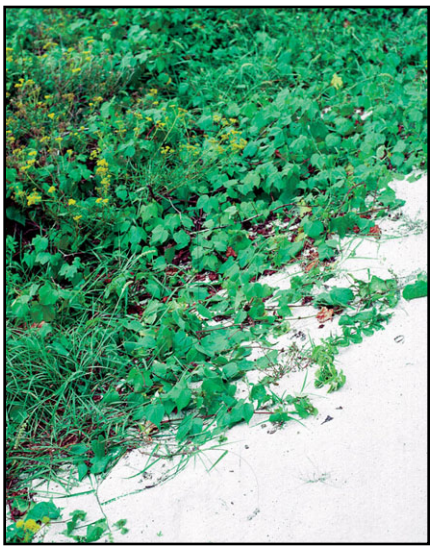

Figure 1. Ancestor of cotton, *Gossypium hirsutum* race *yucatanense* in its natural habitat along the coast of the Yucatan peninsula in Mexico (a) and in close-up view (b).

Bringing Cotton's Ancestors in from the Wild

Where did cotton originate? Sand dunes on the north coast of the Yucatan peninsula in Mexico are home to the most likely wild ancestor of Upland cotton, *Gossypium hirsutum* race *yucatanense* (Figure 1). This wild cotton remotely resembles today's commercial varieties. It

INSIDE:

The Cotton Physiology Education Program (CPEP) was created 9 years ago by a grant to the Cotton Foundation from BASF, makers of Pix®, the original plant regulator. CPEP's mission is to discover and communicate more profitable methods of producing cotton.

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has small bolls with only three locs. Its lint is short, sparse and brown. Its seeds are hard.

Before the days of the Mayan civilization, natives domesticated Yucatan cottons. They selected for mutants with white fiber, a trait which stood out among otherwise drab plants (Figure 2). Increased fiber length and easily germinated seed were other attributes selected. These early forms were distributed rather widely in southern Mexico and Central America by native people.

Continued domestication of these Yucatan cottons resulted in geographic landraces[1] sometimes commonly referred to as dooryard cottons (Figure 3). The varied environments represented by many locations imposed further selection pressure for resistance to pests prevalent in the different regions. As a result, these landraces contain greater genetic diversity than that found in the original wild ancestor.

JM Stewart

Figure 2. Pre-Mayans selected mutants with white fiber from cinnamon-colored wild cottons.

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Figure 3. Dooryard cotton, *Gossypium hirsutum*, from Tabasco, Mexico.

Length of Day Matters

Ancestral cottons were genetically programmed to bloom during short days which are typical of tropical regions. However, as cotton's domestication extended to temperate regions of the world, long summer days presented a significant problem. Plants would not bloom.

Fortunately, landrace cotton was discovered with a day-neutral trait. By moving this trait into existing varieties, breeders produced cotton that would bloom under both short and long day exposures. These day-neutral cotton varieties could be grown as annuals. As a result, cotton cultivation rapidly expanded in the southern U.S. Breeding in this century has placed strong emphasis on selecting for earliness, in reality, a process of making perennial plants produce like annuals.

Why is Genetic Diversity Important?

Although hundreds of cotton varieties are available to growers, approximately 30 varieties account for nearly 95% of the acreage grown in the U.S. When large acreages are planted to a single, or only a few varieties, diversity is greatly restricted in that particular area.

Consider the potential consequences in this example. In 1992, the cotton leaf curl virus (CLCV) epidemic in Pakistan caused a 30% decline in cotton production there. All their regionally adapted varieties were susceptible to CLCV. The first believed-to-be tolerant lines that were planted proved susceptible. In 1996, lines were identified that appeared to have some resistance. These lines are not yet a solution, but they are the first signs of any progress in the 5 years since the CLCV epidemic began. It is no wonder that production has yet to recover.

Our industry is not immune to similar problems. Although viruses are plentiful in the Americas, they have not been a serious problem. However, a mutation resulting in increased virulence in one of these viruses could occur. The mutated virus could wreak havoc on existing cotton varieties, much like the Pakistani scenario.

The pest of concern does not have to be a virus. New versions of insects (e.g. the whitefly in the West and Southwest), bacteria, nematodes (e.g. reniform in the Mid-South and Southeast), and combinations of them could have similar adverse effects on varieties susceptible to them.

[1] Landraces are passed down from generation to generation. They are primitive cottons that have been maintained in isolated regions through cultivation in gardens or small plots.

U.S. Cotton Germplasm Collection

As new problems arise, breeders can look for solutions in the genetic diversity represented in the U.S. Cotton Germplasm Collection. The United States Department of Agriculture maintains this collection of over 6,000 germplasm entries at Fort Collins, Colorado and College Station, Texas. Although a vast wealth of genetic diversity exists within the collection, useful traits must be identified and isolated from many less desirable traits before they can be introduced into existing varieties or used to create new ones.

All five categories in the collection (obsolete, landrace, *Gossypium barbadense*, Asiatic, and wild species) contain valuable material (Figure 4). Resistance to root-knot nematode was found in the landrace collection. Many of the Asiatics exhibit resistance to CLCV and some have resistance to the reniform nematode. *Gossypium longicalyx* in the wild collection is immune to the reniform nematode. Varying degrees of tolerance to drought and salinity are found in all the categories of the collection.

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Figure 4. *Gossypium mustelinum*, a valuable source of wild germplasm.

Not all available germplasm is in this collection. The seeds in the collection represent a mix of genetic material. Some were acquired by happenstance. Others, like those in the examples that follow, were obtained through explorations planned to rescue threatened genetic resources. Many original sources have never been collected.

Landrace collections made in the early 1900's around the village of Acala (Figure 5) in the state of Chiapas, Mexico were the source of today's superior Acala varieties. A trip back to that area in 1984 revealed that all the native cottons had been destroyed because they were believed to harbor insects that were major pests in the commercial fields. The insects won. Cotton is no longer grown commercially in the area and all the Acala landrace germplasm is gone.

JM Stewart

Figure 5. Village of Acala in Chiapas, Mexico. Landraces collected here provided the germplasm used to develop the fine Acala cottons of California and New Mexico.

The Moco cottons which are grown in northeastern Brazil have great genetic diversity. In 1988 when an invasion of the boll weevil threatened them, a rescue expedition was sent to collect them. Landrace 'Marie Galante' typically is grown in small family plots as perennials with a minimum of management. A rapidly disappearing sight is to see Moco cotton intercropped with forage cactus (Figure 6) or growing on hillsides among boulders (Figure 7). The boll weevil infestation has been forcing natives to abandon this perennial production of the Moco cottons.

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Figure 6. Moco cotton, landrace 'Marie Galante,' intercropped with forage cactus in Brazil.

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Figure 7. Moco cotton grown as perennial on rocky hillsides in Brazil.

Another wild relative of cultivated cottons, *Gossypium mustelinum* (Figure 8) may face extinction. The same rescue mission that collected Moco cotton germplasm also collected seed from this species. Subsequent evaluation showed that this cotton has very high levels of compounds (terpenoid aldehydes) in the gossypol glands of its leaves. These chemicals impart resistance to foraging insects and herbivores.

The value of saving diverse genetic material is obvious. Untapped traits housed in this collection, as well as in plants not yet collected, represent a reservoir of potential solutions to problems facing growers now and in the future. The process of moving desired traits into existing varieties, or of creating new varieties, is long and tedious. Here we give you a glimpse of just what is involved and who performs this invaluable effort.

JM Stewart

Figure 8. Wild cotton, *Gossypium mustelinum*, holds genes for high production of defense chemicals that deter chewing insects and animals.

Providing Useful Germplasm

As the U.S. cotton industry strives to maintain its competitive edge, active public breeding programs are more important than ever. Public breeders supply germplasm for use in public and private varietal development programs (Table 1). Some of these germplasm sources are almost ready to be used as varieties. Others are very basic materials that can best be used as a source of one or more desired traits to be incorporated in future varieties.

Because breeders have used more publicly-developed germplasm in recent years than any other gene source, they have maintained a fairly broad genetic base in cotton compared to that in other crops. In the development of their germplasm, public breeders make use of diverse genetic material that private breeders (such as those at seed companies) cannot afford the time to use in their quest for immediately-marketable varieties.

Table 1. Breeding programs with public support through universities (state) or USDA/ARS (federal)[2]. (Source: J Gannaway, B Meredith)

Breeder/Geneticist	Funds	Germplasm	Varieties
Arizona			
Hal Moser	State	✔	✔
Richard Percy	Federal	✔	
Arkansas			
Fred Bourland	State	✔	✔
J. McD. Stewart	State	✔	
Georgia			
Shelby Baker	State	✔	✔
Louisiana			
Gerald Myers	State	✔	✔
Mississippi			
Steve Calhoun	State	✔	✔
Johnie Jenkins	Federal	✔	
Reiner Kloth	Federal	✔	
Jack McCarty	Federal	✔	
Bill Meredith	Federal	✔	
Ted Wallace	State	✔	✔
New Mexico			
Roy Cantrell	State	✔	✔
North Carolina			
Daryl Bowman	State	✔	
South Carolina			
Lloyd May	Federal	✔	
Texas			
Charles Cook	Federal	✔	
Kamal El-Zik	State	✔	✔
John Gannaway	State	✔	
Russ Kohel	Federal	✔	
Ed Percival	Federal	✔	
Jerry Quisenberry	Federal	✔	
Wayne Smith	State	✔	✔
Peggy Thaxton	State	✔	✔

[2] No public breeding programs in Alabama, California, Florida, Missouri, Oklahoma, Tennessee, and Virginia.

Developing Varieties

Improved varieties are developed by crossing one variety or line with another to combine their best traits which are coded for by genes[3]. In most breeding programs, dozens of crosses are made each year. From any one cross, thousands of gene combinations exist in the offspring. The challenge to breeders is to select the very few new combinations which are better than the best varieties currently available.

The numbers game is important in variety development. Odds are stacked against success. Less than one cross out of 100 produces an improved variety. Within the offspring of a given cross, less than one plant in 10,000 results in an improved variety. Consequently, breeders screen very large populations to increase their chances of success.

Time constraints also limit the rate at which new varieties can be developed. After a cross is made, but before selections will breed true, four to six generations need to be grown. Unless off-season generations are produced in winter nurseries in the tropics, it takes 4 to 6 years to obtain true-breeding selections. These selections must be tested for yield, yield stability, and fiber quality.

Usually another 3 or more years are needed to adequately evaluate whether a new strain has sufficient worth to release it as a commercial variety, at which point another 3 to 4 years of seed increases and further evaluation in many environments are required. Assuming all goes well, a minimum of 10 to 15 years is required to go from an initial cross to a new commercial variety (Table 2).

Table 2. Steps used to produce new varieties by pedigree-selection including early generation testing. New varieties are released anywhere from the F9 through F15 generations, which can take from 10 to 16 years to produce. (Source: CW Smith)

Year		
1st	Parent 1 x Parent 2	Breeder makes crosses — up to 300 combinations
	F1 seed	Formed as a result of crosses
2nd	F1 generation	F1 seed planted to produce F1 generation (plants)
	F1⊗	F1 plants self-pollinate, form F2 seed
3rd	F2 generation	F2 seed planted in replicated yield/quality performance trials at one or more locations; resulting F2 plants evaluated; individual plants with desired traits selected (seed saved); some F2 families discarded
	F2⊗	F2 plants self-pollinate, form F3 seed
4th	F3 generation	F3 seed planted in single rows (from selected F2 plants so that seeds from one F2 plant go into one row); F3 plants with desired traits selected
	F3⊗	F3 plants self-pollinate, form F4 seed
5th	F4 generation	F4 seed planted in single rows (from selected F3 plants); rows of F4 plants (instead of individuals) with desired traits selected; strain numbers may be assigned to F4's
	F4⊗	F4 plants self-pollinate, form F5 seed
6th	F5 generation	F5 seed, saved from best rows of F4 plants, planted in replicated tests in at least one location; yield, fiber properties, and any other characteristics of interest (e.g. insect or disease resistance) evaluated in F5 plants
	F5⊗	F5 plants self-pollinate, form F6 seed
7th	F6 generation	F6 seed, saved from F5 plots, planted; evaluated at multiple locations
8th to 10th	F6 to F9	Generations evaluated as for F6 generation

[3] A gene is a functional unit of inheritance. It is a specific segment of DNA (deoxyribonucleic acid) that controls the synthesis of a polypetide (protein). Examples of traits controlled by one or more genes include plant hairiness, leaf shape (e.g. okra vs. normal), gossypol content (e.g. glanded vs. glandless), fiber quality, disease resistance, and lint color.

Advent of Transgenics

Cotton variety development and testing have entered a new phase with the advent of transgenic varieties. In current commercial practice, developing transgenics involves moving only one or two genes from a transgenic donor parent to an existing variety by means of the backcross method (Table 3). Whereas conventional breeding methods can be used to move traits only from close relatives, genetic engineering techniques allow the introduction of genes from unrelated species.

The backcross method takes less time than conventionally breeding an entirely new variety. It requires smaller populations which can be handled in greenhouses, instead of fields. Two to three generations can be produced in one year. The backcross method reduces both the time and numbers crunch faced by breeders using conventional methods.

The biggest disadvantage of the backcross method is that it does not result in totally new varieties, but only new versions of existing varieties with "value-added" traits. To achieve long-term genetic gain and global competitiveness, the U.S. cotton industry must supplement this approach by continuing to utilize new sources of genetic variability through the more difficult and time-consuming efforts of conventional breeding. The efforts of public breeders and geneticists are essential in this regard.

Table 3. Outline of simplified backcross method used to transfer a desired trait into an agronomically useful variety. Donor parent (containing the transgene for the "value-added" trait) is crossed with recurrent parent (containing the desired agronomic traits). Offspring are repeatedly backcrossed to recurrent parent until only genes for the new trait are exhibited in the "value-added" line which performs agronomically like the recurrent parent. Note that in each additional backcross generation another 50% of the remaining donor parent's genes are eliminated.

Year	Generation		
1st	**A x B** Donor x Recurrent Parent Parent	**➡ F1**	Breeder crosses donor (A) containing transgene with recurrent parent (B) having desired agronomic traits. F1 seed contains 50% genes from A and 50% from B.
2nd	**F1 x B**	**➡ BC1F1**	Breeder plants F1 seed to produce F1 plants; crosses F1 plants with B (recurrent parent). BC1F1 seed contains 25% genes from A and 75% from B.
3rd	**BC1F1 x B**	**➡ BC2F1**	Breeder plants BC1F1 seed; crosses BC1F1 plants with B. BC2F1 seed has 12.5% genes from A and 87.5% from B.
4th	**BC2F1 x B**	**➡ BC3F1**	Breeder plants BC2F1 seed; crosses BC2F1 plants with B. BC3F1 seed contains 6.25% genes from A and 93.75% from B.
5th	**BC3F1 x B**	**➡ BC4F1**	Breeder plants BC3F1 seed; crosses BC3F1 plants with B. BC4F1 seed contains 3.13% genes from A and 96.87% from B.
6th	**BC4F1**$^{\otimes}$		BC4F1 plants self-pollinate.

Transformation Timeline

Because the backcross method is relatively quick and simple, one might ask why seed for currently available transgenic cottons costs more than that for conventional varieties. Here we briefly outline some of the steps involved for molecular geneticists to use gene cloning to move desirable traits from a totally unrelated species into cotton as transgenes. Creating transgenes of value by this process takes years of coordinated molecular biology and tissue culture work.

Once a gene of value (such as that coding for Bt toxin) is identified and its structure is mapped, molecular biologists use enzymes to cut out the segment of interest and insert it into DNA of individual cotton cells. This process is called transformation.

Transformed cotton cells are grown on nutrient medium in petri plates (Figure 9). The cells divide and form a mass of undifferentiated tissue (callus) in which all the cells contain the inserted DNA. By changing relative amounts of specific nutrients and growth regulators, researchers can induce callus to differentiate into entire cotton plants containing the newly introduced genetic material. This step is called regeneration.

T Kerby

Figure 9. Introduction of genes from other species begins in cotton cells cultured in media that can be reformulated to regenerate transformed plants.

The insertion of foreign DNA into cotton's DNA in the transformation process is a random event. If the DNA winds up in the middle of another gene, for instance, that gene will no longer function and will cause problems in the transformed plant. Often these problems are evident early and the plants can be thrown out. However, sometimes no outward bad effects are seen. In these cases, the plants are studied to verify that the gene has no ill effect on the transformed plant.

Most cotton varieties can be transformed easily, but only a few can be regenerated. Coker 312 is more easily regenerated than other cotton varieties. However, Coker 312, an old variety, cannot compete successfully with current commercial varieties. Consequently, transformed Coker 312 is repeatedly backcrossed to the agronomic variety (recurrent parent) for four generations (Table 3). The resulting fourth generation (BC4F1) is allowed to pollinate itself (the process of selfing) for an additional two generations to produce a segregating population, a total of six generations after transformation.

Plants in the segregating population will show different traits. Seeds of plants that are pure for the transferred gene are planted out the next generation as progeny (offspring) rows. The next year, seed from these plants is replicated in plots. The best lines are increased. After 2 years of seed increase, commercial introduction can be done on a small scale, a total of nine generations after transformation.

Off-season seed increases typically are done in the tropics. Limited seed is available for these winter increases. The increased seed, which is often below commercial standards for quality, is further multiplied and tested by the seed company.

Company testing in small plots is used to cull lines identified as being lower in yield, quality, or any growth factor. Large scale replicated trials are performed in the targeted market. Only good performers are increased again that winter. Seed from this second winter increase are made available for company and university testing.

Further Steps in Bringing Varieties to Market

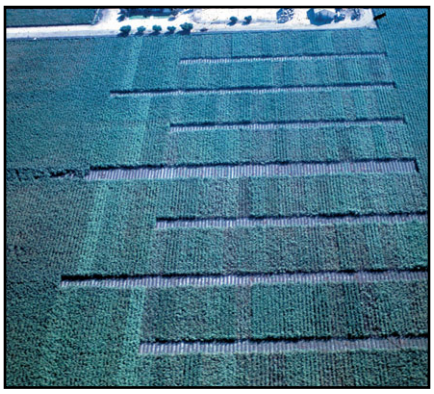
Historically, varieties have reached the market in a unique way in California's San Joaquin Valley, which accounts for 97% of the state's production. From the inception of a one-variety law in 1925 until the 1970's, only Acala varieties developed by the USDA could be grown. When a new variety was released, the variety grown previously was no longer maintained. Growers had only one variety to plant.

In 1978, the law was amended to permit breeding in the private sector and the growing of more than one variety. Any varieties grown had to be tested and found to meet a standard. This revision in the law made the San Joaquin into a high-quality, rather than a one-variety district.

Today there are about a half-dozen private breeders with active programs. Newly developed varieties are tested for 3 years in university trials that compare production and fiber quality characteristics to those of a variety that has been designated the standard. Currently Acala Maxxa is the standard.

Each year a total of 36 entries submitted by the breeders begins the first year of the 3-year testing cycle. Varieties are planted at three or four locations in small, replicated plots four rows wide and 50 feet long (Figure 10). Those entries that perform well are advanced to the second stage, an expanded scale of testing.

Replicated trials in grower fields are done at eight locations for second stage testing. Individual plots run the length of the field which is usually 1/4 mile. The cooperating grower carries out his normal management with respect to row spacing, planting date, irrigation, fertilization, pest control, etc. Detailed measurements are made of yield, earliness, disease (e.g. *Verticillium* wilt) tolerance, growth and fruiting characteristics (Figure 11). In addition to fiber quality measurements, spinning tests are performed to measure processing efficiency and yarn quality.



D. Bassett

Figure 10. Small, replicated plots are used to test first year entries in San Joaquin Cotton Board's[4] 3-year testing cycle of experimental varieties.

D. Bassett

Figure 11. Dramatic changes have occurred in cotton varieties as a result of breeding efforts. Examples include *Verticillium* tolerance (left), a marked improvement over susceptible variety (right).

[4] Growers and representatives of various segments of the cotton industry are elected to serve on this board.

After an experimental cotton completes 2 years in the second stage (Figure 12) and a total of 3 years' testing, it can be considered for approval by the San Joaquin Valley Cotton Board. A summary of a variety's performance over years is prepared. If the candidate variety is found to meet or exceed the existing standard, approval is granted. It then becomes Acala - a cotton that meets the prescribed Acala standard. A separate, but parallel, program has been in effect for Pima varieties since 1991.

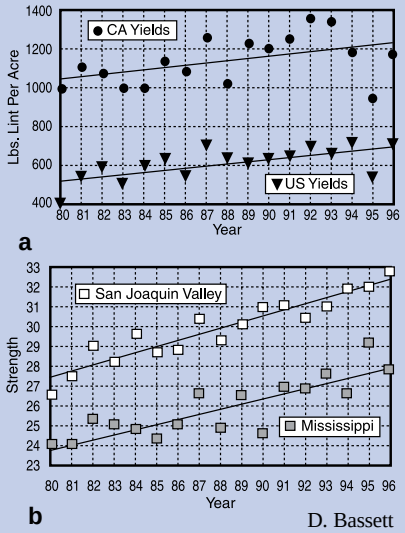
Breeders can market their approved varieties. Growers can choose to plant any approved variety. Since 1978, nearly 2 dozen Acalas have received approval. Only a few of them command nearly all of the current cotton acreage.

D. Bassett

Figure 12. Expanded scale tests are performed in grower fields for the second and third years of the 3-year testing cycle.

After a variety is approved, it is dropped from the testing program to make room for new entries. Farm advisors with the University of California Cooperative Extension continue field testing the newly approved varieties alongside previously approved varieties in each of the cotton-producing counties. These approved variety tests are carried out in replicated field-length plots similar to those used in the second stage of testing previously described. Ten varieties are tested in these trials.

This program's success is clearly illustrated when yields and fiber strength are plotted against time (Figure 13). Significant improvement in both yield and strength for both San Joaquin Valley and U.S. cottons is seen. These graphs clearly illustrate the value of our breeders' efforts.



D. Bassett

Figure 13. Steady improvement of varieties over time reflected by a) yield increases and b) improved fiber strength.

Variety Trials Are Useful Tools

Growers look at the bottom line. When selecting a variety, they consider two main characteristics: yield and demand. The number of pounds produced provides money in their pockets and demand determines the fiber quality required. Variety trials are a source of yield and quality figures.

Geographic and climatic differences influence both production practices and the varieties available in a particular region. Consequently, most statewide variety testing programs include diverse geographic locations and soil types typical of cotton producing areas. Trials are conducted in grower fields and at university experiment stations (Figure 14).

S Calhoun

Figure 14. Cotton variety trials in Stoneville, Mississippi.

Trial sites include those that are rainfed, irrigated, infested with nematodes, conventionally cultivated and planted no-till (Figure 15). Some reflect the different row spacings used by growers, including ultra narrow row spacing of 7 or 10 inches. In most areas, time of planting trials is determined by availability of equipment and suitable weather, much as it is for growers.

If one cultivar performs well in all locations, the seed company is pleased and so is the farmer. However, if one variety performs well in the Lower Rio Grande of Texas, for example, and another does not, growers in the Lower Rio Grande will want to plant the good performer. That same variety may not do well in the Blacklands. Growers there would need to plant a variety responsive to their conditions.

B Phipps

Figure 15. Cotton planted into killed wheat cover crop.

Advice from across the Cotton Belt is the same. It is not necessarily the top performer in a variety trial that is best-suited to your farm. Look at variety trial data from multiple years and locations (including some near your farm) as guides to how a variety will perform. First try new varieties on limited acreage. Use variety trials only as guides in deciding which new varieties to try.

Conclusions

Cotton has a long and colorful history. Much work has gone into developing the modern high-yielding varieties that we often take for granted. Traits from wild cottons collected around the world have been pulled out and used in varietal development to enhance our commercial varieties. Main selection pressure has been for yield and fiber quality traits.

Relatively recently, the tools of biotechnology have been used to place value-added traits into existing commercial varieties. The resulting transformed varieties target pests that require the greatest dollar input to control. This exciting approach is but one more tool to supplement, but in no way replace, traditional breeding methods.

Undoubtedly, growers, breeders, and seed company representatives all could benefit from a crystal ball when making decisions as to what to plant, which traits to select, and what varieties to market. Their decisions to date have created a healthy U.S. cotton industry.

NEW LOOK for *Cotton Physiology Today*

Cotton Physiology Today sports a new look which we hope you will find easy-to-read and eye-catching. We would enjoy hearing from you about anything you see or read in the bi-monthly issues. You can write to us C/O *Cotton Physiology Today*, National Cotton Council, 1918 North Parkway, P.O. Box 12285, Memphis, TN 38182-0285.