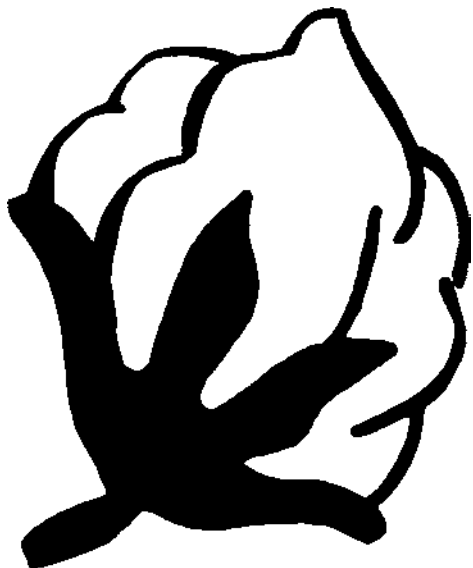




# **THE ICAC RECORDER**

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International  
Cotton  
Advisory  
Committee

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Technical  
Information Section

VOL. XX NO. 1  
MARCH 2002

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Update on Cotton  
Production Research

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## Introduction

The *ICAC RECORDER* has been published in three languages since 1989. Since then, there has been only one issue (Volume XI, No. 3, September 1993) that did not include the French and Spanish versions. The Technical Information Section (TIS) of the ICAC started the publication in 1983, soon after the Section was created. Articles written by the Section staff and researchers from many countries have been published on all aspects of production research. Emphasis has changed as researchers' focus in the world has changed, but efforts have been made to keep a consistency in the publication.

In the past, the English, French and Spanish versions were included in one publication. Starting from this issue, the three languages will be published in three separate publications. If you are used to consulting more than one language, or if you have a specific language preference, you are advised to contact the ICAC at <publications@icac.org> There is no special reason for separating the languages other than the ICAC will spend less on the publication and mailing of the French and Spanish versions, as the demand for them is much lower compared to English.

In the past, *THE ICAC RECORDER* included contributions by researchers from various countries, but the number of articles published every year was not many. We are going to increase this frequency and encourage researchers to send articles to the editor at <rafiq@icac.org>. However, it may be noted that any article/paper submitted for publication must have a wide applicability of results.

The mandate of the TIS is to facilitate communication among researchers and bring them together for exchange of information. The objective is being achieved through regional networking and the organization of world cotton research conferences. The Latin American Association for Cotton Research and Development (ALIDA) is the oldest and one of the most active networks established on cotton production research. The network was started in 1986 and has met regularly almost every two years since. Meetings are held in different countries and

are jointly organized by the TIS in collaboration with local organizers. The 8<sup>th</sup> Meeting of ALIDA was held in Asunción, Paraguay, from November 25-29, 2001. The National Project of Cotton Reactivation (NPCR), a technical branch of the Ministry of Agriculture and Livestock (MAG), Government of Paraguay, hosted the meeting. Mr. Cirilo Centurión of NPCR served as the coordinator, with significant help from Dr. Graciela Gómez, a consultant of the Ministry. The FAO Regional Office for Latin America and the Caribbean supported the meeting. Mr. Pedro Lino Morel, Senior Minister of Agriculture and Livestock, inaugurated the meeting. Mr. Ubaldo Tadeo Britos, Technical Coordinator of the NPCR – MAG, was elected as the new president of ALIDA. The Ministry of Agriculture, Livestock and Supplies of the Government of Brazil offered to host the 9<sup>th</sup> meeting of ALIDA in Brazil in 2003 and the meeting accepted the invitation. A full report is published in this issue.

The current hybridization approaches for increasing variability and ultimately selecting better and desired genotypes are based on crossing two parents and then relying on phenotypic performance. Breeding, although said to represent the application of genetic principles, has utilized genetic principles the least. Since the adoption of transgenic cotton on a commercial scale, it is increasingly realized that genetic engineering can contribute to faster and significant improvements. DNA marker technology allows breeders to select desirable plants on the basis of genotypes instead of phenotypes. Now, various new marker techniques and breeding strategies, tailored for the inclusion of DNA markers, have been designed and as a result a number of molecular markers for agronomically important traits have been identified. Mr. M. Arshad, Mr. Sajjad Haider and Dr. Iftekhar Ahmad Khan, of Pakistan, have discussed a number of techniques used in molecular markers and their potential application to cotton improvement in the third article on "Molecular Marker Technology for Cotton Plant Improvement."

Fiber strength being the most positively correlated fiber characteristic with yarn strength is one of the most important pa-

rameters for high speed spinning. Growing conditions affect fiber quality but fiber strength is more dependent on variety than on the growing environment. Fiber strength is said to have a negative correlation with yield and its inheritance is more complicated compared to other parameters. Three types of instruments are used to measure fiber strength, and the data from each are different. Stelometer readings and HVI data can have some relationship but Pressley data cannot be related to the other instruments. Many countries have already moved to 3.2 mm gauge measurement as the data have a higher correlation to yarn performance. Conventional breeding has a limitation to improve fiber strength due to the non-availability of high strength germplasm within *hirsutum*. A number of options are being tried to improve fiber strength through genetic engineer-

ing approaches, and some progress has already been made. All these issues are discussed in the third article.

Preparations for the World Cotton Research Conference-3 to be held in Cape Town, South Africa, from March 9-13, 2003 are in full swing. By the end of March 2002, 189 researchers from 35 countries had pre-registered. The Organizing Committee in South Africa has finalized the full registration package which will be mailed in April to all who pre-registered. Pre-registration will be now closed and full registration will be opened. The full registration package includes information on hotel reservation, submission of abstracts and papers, and social events. You can receive the registration package by sending a request to Dr. G. D. Joubert, Chairman of the Organizing Committee, at <director@nitk1.agric.za>.

## VIII Meeting of the Latin American Association for Cotton Research and Development

Asunción, Paraguay, November 26-29, 2001

### Introduction

Cotton researchers from the South American region decided to form an association in 1986. The Latin American Association for Cotton Research and Development (ALIDA) was created in Sáenz Peña, Argentina in 1986 with the objective of exchanging information through regular meetings. Since then, ALIDA meetings have been held almost every other year. Argentina has hosted two meetings, in 1986 and 1997, while Perú, Brazil, Colombia, Nicaragua and Bolivia have hosted one meeting each in 1988, 1991, 1993, 1995 and 1999, respectively. At the invitation of the Ministry of Agriculture and Livestock, Government of Paraguay, the 8<sup>th</sup> Meeting of ALIDA was held in Asunción, Paraguay from November 26-29, 2001. The National Project for the Reactivation of Cotton (NPCR), a technical branch of the Ministry of Agriculture and Livestock (MAG), served as the primary host of the meeting.

In addition to the Ministry of Agriculture and Livestock of Paraguay, the Food and Agriculture Organization of the United Nations (FAO), the International Cotton Advisory Committee and many private companies also sponsored the meeting. All cotton producing countries in the region were invited to attend. Eight countries sent their representatives to participate in the meeting. Representatives of CIRAD-CA of France, FAO and the ICAC attended the meeting. A large number of people from the private sector actively participated in the deliberations. In total, one hundred and eleven people registered for the meeting. The list of participants is attached.

Over twenty papers were presented in two days on various aspects of cotton production research and marketing.

The Minister of Agriculture and Livestock, Mr. Pedro Lino Morel, remarked in the opening ceremony that the economy and social structure of Paraguay mainly depend on the work and revenues of more than 150,000 rural families who cultivate cotton. Cotton growers comprise close to 50% of the total peasants in the country. Obviously, the current low prices that prevail in the world textile market outline big challenges for all links of the cotton chain, from the producer to the exporter and the industry, without excluding the technical and financial agencies. However, and despite the setbacks, it has been categorically demonstrated that cotton production continues to be an irreplaceable item in the Paraguayan model of family agriculture, because instead of abandoning cotton growers, the national government has resolved to support and strengthen cotton production as far as conditions in the world market allow. The Minister said that the Paraguayan government continues to grant high priority to cotton and, thanks to the spirit of encouraging authentic solidarity, the ALIDA forum will serve as a means to generate strategies and types of appropriate cooperation to successfully face the critical moment that burdens cotton in every country and region.

Mr. Morel said that cotton growers in Paraguay are currently faced with three main problems: poor soil fertility, lack of suitable varieties and pest problems. Deltapine varieties, introduced in Paraguay in the early 1990s, initially proved very successful. Increases in yields were attributed to the high quality and high yielding ability of Deltapine seed. But problems soon developed and growers in Paraguay do not use Deltapine varieties now. Regarding pests, fusarium wilt, Alabama cotton leaf worm *Alabama argillacea*, and boll weevil *Anthonomus grandis*

are the most prevalent. Boll weevil is responsible for heavy yield losses. Early planting may reduce such losses, but late planting poses a high risk. On average, each farmer owns 2.2 hectares, and all cotton growers are provided a free "boll weevil attract and kill tube" by the government. The problem with Alabama is that it has developed resistance to insecticides, particularly pyrethroids. However, cotton growers in Paraguay still believe in cotton production, and the future of cotton in Paraguay depends on international prices.

On behalf of ICAC, Mr. Carlos Valderrama welcomed participants to the 8<sup>th</sup> ALIDA Meeting. Mr. Valderrama mentioned that Paraguay has been an active and important member of the ICAC and that the country successfully participated in a 5-year project on boll weevil control financed by the Common Fund for Commodities (CFC). In reference to the world cotton economy, Mr. Valderrama noted that international cotton prices had reached a 27-year low in October 2001 and that subsidies offered by some countries, new area dedicated to cotton, new technologies, and currency devaluation in some countries have resulted in a rapid increase in world supplies, pushing prices downward. He said that prices would not be low forever and that it was necessary to prepare for higher prices in the future.

The two-day paper presentation program was divided into four sessions: Production, Marketing and Research; Varieties and Seed Market; Farmers and Research; and Integrated Pest Management. Summaries of presentations and the main observations are given below.

## **First Session: Production, Marketing and Research**

Presentations from the participating countries visualized that the cotton sector in most countries is facing difficulties due to low international prices rather than to domestic problems. International cotton prices are the lowest in many years and this seriously affects the economics of growing cotton without relief from high costs of production.

Lower production and continued increases in local consumption have converted some traditional producing and exporting countries in the region, such as Perú, into importing countries. Growing imports of fiber are necessary to satisfy industrial raw material needs.

From an analysis of world statistics on production, consumption and final stocks, it is observed that the current crisis due to low international prices cannot be attributed to fluctuations in global quantities but to distortions of the market caused by direct and indirect subsidies applied to production in the industrialized countries.

Direct subsidies to cotton production and trade cause great harm to growers worldwide. The International Cotton Advisory Committee has discussed the issue of direct financial support in producing countries at various meetings. At the last plenary meeting held in Zimbabwe from September 16-21, 2001, member

governments decided to create a working group to discuss government measures and identify strategies to reduce and eventually eliminate the negative effects caused by direct subsidies to cotton production and trade. The group will outline solutions to this problem so that practices harmful to cotton's international trade can be minimized, if not eliminated.

As an answer to low international cotton prices, more and more countries are modernizing their production practices based on the latest technical developments, and they are trying to find ways to reduce direct costs of production. More efficient production practices are a key to lowering production costs and improving cotton economics.

Brazil is revitalizing its agro-industrial sector pointing at the whole chain of the textile production industry. With regard to quality, poor ginning was identified as one of the weakest links. Recently, Brazil started a school for training ginning technicians. Such a facility was not previously available in the region. The facility is open to other countries in the region at a nominal tuition fee. The school is expected to improve the current work force, anticipate future training needs of the industry, and provide trained manpower for the ginning industry in Brazil and in the surrounding area.

A systemic vision of the production sector was discussed in the meeting. It was emphasized, for example, that integrated pest management (IPM), together with other analysis or means to approach the decisions to be taken, such as the Geographical Information System (GIS), macroeconomic policies, extension, etc., should be considered as a strategic point to find solutions to main problems in cotton production.

## **Second Session: Varieties and Seed Market**

Cotton production took off in Paraguay in the early 1970s. A bacterial blight resistant variety, Reba B-50, dominated the industry until 1990. A number of varieties have been tried from many countries, particularly in the last ten years. Paraguay has not been able to develop its own variety so far. If adopted on large areas, IAN 338 will be the first locally developed variety grown on a commercial scale in Paraguay.

Colombia continues to depend on varieties developed elsewhere, particularly in the USA. Deltapine varieties were grown on almost 100% of the area during 2001/02, mainly DP 5415, planted on almost 70% of the total area. Other popular varieties grown in Colombia on 5-15% of the total area are DP 90, DP 50, DP 5414 and DHS.

Argentina and Brazil have their own strong breeding programs. Argentinean varieties have been tried on a large scale in other countries, including Paraguay. The variety development process has been slow in Perú due to differences in the types of varieties required to meet growing needs in the five production regions. The three main types of varieties required are Del Cerro, Pima and Tanguis.

The private sector has grabbed a significant portion of the cottonseed industry in Brazil. Varieties developed by the Empresa Brasileira de Pesquisa Agropecuária-EMBRAPA (Brazilian Enterprise of Agricultural Research) were grown on only 10% of the total area in 2001/02. The private sector is very active in Brazil and its contribution to the variety development process is significant.

Every country in the region can choose from a list of varieties with excellent technological qualities and great diversity of agronomic adaptations for planting every year. The work on variety development, and the genetic material acquired from other countries, which is available for production, shows the importance and achievements made in genetic improvement in various countries. However, it was pointed out that recent changes in the seed industry in the form of introduction of genetically engineered varieties demand higher participation of the private sector in seed production and distribution to cotton growers.

There was a cautious warning with regard to biotechnological approaches to the improvement of cotton, particularly the use of transgenic varieties. Papers presented showed great benefits in the use of genetic engineering, but underlined the fact that researchers must elucidate the useful aspects of this technology to the public in order to avoid any rejection of biotech products by consumers. It was recognized that the technology could contribute a number of potential benefits to the environment and animal and human health.

With greater involvement of the private sector in variety development, seed production and development of genetically engineered varieties, rigorous implementation of intellectual property rights in agricultural production has become more important. Countries that intend to follow the path of private sector participation in the development of technologies and products for direct use by farmers need to have an in-house framework of assurance that developments from the private sector will belong to the private sector and that there will be a legal framework for their commercial use in each country. The private sector may not be able to invest in today's agro-industrial competitive world with much confidence without such an assurance.

### **Third Session: Farmers and Research**

Countries in the region share a common concern regarding more efficient application of the process of dissemination of the available technologies for increasing productivity in cotton. Countries represented in the meeting noticed the limited use of recommendations by producers, especially in those countries where small growers form the bulk of the farming community.

In Paraguay, the official Agricultural Extension Service is expanding its scope with the participation of the Unidades Técnicas Tercerizadas (UTT). In a management and soil conservation project, the gender focus and the participation of the

private sector have been shown to increase the adoption of the technology, particularly with respect to minimum tillage or no till, crop rotation and the use of green manure fertilization.

Credits granted to producers by Cooperativa Coronel Oviedo in one of the main regions of Paraguay are conditioned on protection of natural soil resources, production of items of self-consumption, and breeding of small animals, with the objective of assuring the adoption of technologies.

An experience of participative research was presented where producers collaborated actively with specialists in the definition of the most advantageous varieties for their agro-ecological conditions (Department of Ñeembucú). This seems to be an alternative that can contribute to increase technology adoption in cotton cultivation. This methodology has been successful in other places applied to food items and of self-consumption with small farmers.

### **Fourth Session: Integrated Pest Management (IPM)**

More and more consensus exists on the need to stimulate the application of all the methodologies that comprise IPM: genetic improvement; cultural and agronomic practices; pest monitoring and natural enemies; and application of selective pesticides; in order to achieve "an integrated cultivation management" for avoiding or reducing the use of pesticides that unnecessarily contaminate the environment.

The tri-national project "Integrated Pest Management of the Cotton Boll Weevil in Argentina, Brazil and Paraguay" resulted in very useful information on the biology, ecology and management of the cotton boll weevil in the region. The project was sponsored by the International Cotton Advisory Committee and funded by the Common Fund for Commodities, an international intergovernmental organization based in Amsterdam, Netherlands.

### **Recommendations and Final Comments**

In the final session, the outgoing President of ALIDA, Mr. Juan Campero Rojas from Bolivia, once again raised the issue of direct subsidies. He stated that direct subsidies to production and to cotton trade cause great damage to producers in many countries worldwide. The representative of the ICAC repeated that member governments of the ICAC are actively discussing the issue.

Speakers reiterated the fact that the boll weevil is a major common enemy of cotton in the region. It was suggested that the three countries involved in the tri-national project must make extra efforts for maximum diffusion of the results obtained, in order to achieve more significant effects in the fight against this harmful insect.

It was also proposed that such multinational projects should continue with an enlarged focus and inclusion of more coun-

tries like Bolivia and others affected by the boll weevil in the region.

Bt cotton is currently grown on a commercial scale only in Argentina. A number of countries in the region have yet to complete formalities for proper introduction and adoption of genetically engineered cotton varieties. Some participants suggested that governments should solve the problem of restrictions that limit farmers' access to the use of new technologies, like Bt cotton.

The ICAC's viewpoint on genetic engineering of cotton was made clear at the meeting. The technology has tremendous applications in agriculture but should be used carefully. Since the appearance of transgenic cotton, over ten years ago, the ICAC Secretariat has published many reports on various aspects of biotechnology, commissioned two extensive review articles (one published and the second in process), and constituted an expert panel that also prepared a report for the ICAC. Biotechnology with reference to currently available genetically engineered varieties also has been discussed at plenary meetings of the ICAC. ICAC has put together all these papers/reports on the ICAC web page at <http://www.icac.org>. All publications on this subject, except review articles, can be accessed free of charge.

The meeting also observed that the private sector is an integral part of the cotton production and processing chain. It is important that the issues confronted by various sectors of the industry are tackled jointly. The meeting decided that new developments and improvements in the existing technologies would be discussed at the next meeting of ALIDA.

A representative of the Ministry of Agriculture, Livestock and Supplies of the Government of Brazil offered to host the 9<sup>th</sup> meeting of ALIDA in Brazil in 2003, and the meeting accepted the invitation.

In order to keep communications alive in the interim, the ICAC representative reminded participants to use the ICAC electronic mailing list. It is a free service, and messages can be posted at "ALIDA List" [alida@liststar.icac.org](mailto:alida@liststar.icac.org).

The meeting urged governments present to strengthen the primary sector, especially cotton cultivation, with genuine and non-distorting economic measures in their countries.

The government of Paraguay proposed Agr. Eng. Ubaldo Tadeo Britos, Technical Coordinator of the National Project for the Reactivation of Cotton (NPCR) as the new president of ALIDA. The meeting unanimously elected Mr. Britos as the 8<sup>th</sup> President of ALIDA for the period until the meeting in Brazil in 2003. Mr. Britos can be contacted at the following address:

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## Molecular Marker Technology for Cotton Plant Improvement

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Cotton belongs to the genus *Gossypium* which contains about 50 species of which 44 are diploid species ( $2n=2x=26$ ) and six are allotetraploid ( $2n=2x=52$ ). The diploid species comprise genomic groups A, B, C, D, E, F, G and K and allotetraploid species are made up of two sub-genomic groups having an affinity with A and D genomes (Stewart, 1995). The cultivated cottons include *G. arboreum* L and *G. herbaceum* L, both diploid species with an A genome native to Southern Asia and Africa, and two allotetraploid species, *G. barbadense* L and *G. hirsutum* L, with an AD genome from Central, North and South America (Endrizzi et al., 1985).

During the past decade, the prospect for genetic engineering of cotton has soared with recent advances in the field of biotechnology. In 1996, the first two transgenic cotton cultivars, engineered for insect and herbicide resistance, were released for commercial production. The production of transgenic cotton exhibiting increased yields, enhanced fiber quality, and/or novel fiber properties holds significant potential for widespread impact on the global economy. Although small gains in yield and fiber quality continue to be made by conventional breeding programs, genetic improvement of agronomic traits is beginning to plateau as a result of an increasingly narrow germplasm



base for selection. To counteract this trend, the use of genetic engineering of cotton will become increasingly common as a means of bolstering breeding efforts.

Presently, plant breeders select desirable plants by looking at the phenotype. Most of the economically important plant traits are polygenic with complex non-allelic and environmental interactions. The biometrical genetic analysis has also been used for about half a century to gain an understanding of the genetic architecture of quantitative traits, which has helped to guide genetic improvement programs. Genetic variation between plants for a trait controlled by a single or a few genes inherited in a single, Mendelian fashion, is easy to manipulate in a breeding program. The biometrical genetic analysis determines the cumulative effects of all the genetic loci involved in a quantitative trait, but it is unable to identify the specific locus involved. If quantitative traits could be resolved into individual genetic components by finding DNA markers closely linked to each trait, it might be possible to manipulate them with efficiency for single gene traits. This would help the advancement of breeding material through consistent progress. DNA markers technology has provided plant breeders with a tool to select desirable plants directly on the basis of genotype instead of phenotype.

Conventional breeding of the cotton plant generally aims to improve agronomically relevant, or otherwise interesting traits, by combining characters present in different parental lines of cultivated species or their wild relatives. This has been achieved by generating  $F_2$  populations and screening phenotypes of pooled or individual plants for the presence of desirable traits. Then, a time consuming and costly process of repeated back crosses, selfing and testing begins. All this depends upon accurate screening methods and the availability of lines with clear-cut phenotypic characters. Therefore, the combination of complex characters encoded by multiple genes with additive effects (quantitative trait loci), recessive genes, or accumulation (pyramiding of genes encoding the same trait) is difficult to achieve with classical methods. Molecular markers, however, facilitate all these processes, and can accelerate the generation of new varieties and allow connection of phenotypic characters with the genomic loci responsible for them. Both of these properties make molecular markers indispensable for cotton plant improvement.

Morphological and physiological features of plants have been used by plant scientists to understand genetic diversity. Some genes named by their physical appearance irrespective of their DNA nature and location on chromosomes were identified. Scorable morphological characters are very few in the cotton plant compared to biologically active genes. In closely related cotton plant varieties and species, there are very few morphological differences, which as a matter of fact do not represent the true genetic differences at the DNA level. Moreover, in most cases a plant genome has large amounts of repetitive DNA, which is not expressed and does not contribute to the physiological and morphological appearance of the plant. Therefore,

there is a need to study polymorphism at the DNA level, which can be indicative of genetic diversity in cotton.

The number of polymorphic morphological markers is limited in the cotton plant, especially in intra-specific crosses, and their expression is influenced by the environment. Therefore, more reliable markers such as protein or, more specifically, allelic variants of several enzymes so called isozymes, and other biochemical characters such as lipid or sugars must be considered. Isozyme numbers are limited and their expression is often restricted to a specific developmental stage of tissues; and their presence can be determined by electrophoresis and specific staining. Unlike isozymes, the number of DNA markers is unlimited, their expression is not necessary for their detection, and all markers can be detected with a single technique. Polymorphism has been detected in restricted genomic DNA of plants, which paved the way for the development of molecular markers for cotton plant breeding. Today, various new marker techniques and breeding strategies, tailored for the inclusion of DNA markers, have been designed. The result of these efforts is an ever-increasing number of molecular markers of agronomically important traits, available for all crops. In the most advanced projects, this has already led to map-based cloning of the responsible genes.

## Techniques

The techniques for molecular markers include restriction fragment length polymorphism (RFLP) and polymerase chain reaction- (PCR) based random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP); and mini and micro-satellites. Each is described below.

### Restriction Fragment Length Polymorphism (RFLP)

Restriction endonucleases cut genomic DNA at specific palindrome recognition sequences, generating thousands of fragments of defined length, the number of which depends on the number of recognition sequences in a given genome. If a recognition sequence is present at a distinct genome location in one individual but not in the other, the enzyme generates different sized restriction fragments of this locus. This length polymorphism is detected by a radioactively labeled complementary DNA probe derived from the same locus. Such RFLPs were the first DNA markers applied to genome mapping. In principle, RFLP probes are single copy markers that detect only one defined genomic fragment each, although multilocus probes such as repetitive DNA or cDNA, that detect several fragments at a time, are also used. RFLPs are co-dominant markers, and their coupling phase can be determined experimentally, because DNA fragments from all homologous chromosomes are detected. RFLPs are therefore very reliable markers in linkage analysis and breeding, particularly if a linked trait is present in a hetero- or homo-zygous state in an individual. This information is highly desirable for recessive traits. The suitability of RFLP for the elucidation of QTL has been documented by sev-

eral researchers. Despite their usefulness, the generation of RFLP markers and their application is time-consuming and expensive. First, only one out of several markers provides polymorphism. This problem is serious, especially in a cross between closely related cultivated breeding lines. Second, for every polymorphism locus tested in a cross, a single experiment has to be performed and this is a formidable task with saturated maps such as those of tomato or maize, with hundreds of markers. As the method also requires a large amount of DNA, the material from a  $F_2$  generation is soon exhausted and this limits the number of markers that can be tested. To overcome these problems, the application of stable mapping populations has been suggested. RFLP markers can be used for the mapping of QTL, genes involved for resistance to diseases and the relationship between the components of tetraploid genome of *Gossypium hirsutum* and its ancestors.

### Random Amplified Polymorphic DNA (RAPD)

PCR-based RAPD is much faster and cheaper than RFLP analysis and is used only in minute amounts of DNA. Instead of primers complementary to known sequences, as in normal PCR, randomly generated synthetic oligonucleotides of 9-12 bases are used as starting points for thermostable DNA polymerases (Welsh and McClelland, 1990; Williams et. al. 1990). This approach produces 1-10 fragments from a single primer PCR reaction, which are highly polymorphic and can be easily detected on ethidium bromide stained agarose gels. This approach has several modifications. For example, the use of shorter oligonucleotides, typically 5-15 nucleotides in combination with PAGE and highly sensitive silver staining, results in many more visible bands in DNA amplification fingerprinting (DAF). The high sensitivity of the method applied to pre-digested DNA permits the tagging of specific locus for some characters in nearly isogenic lines of cotton. RAPD markers can be used for map-based cloning of disease resistance genes in cotton. The random amplification process introduces some difficulties in reproducing RAPD patterns in different laboratories and in different thermocyclers. Recent reports claim that these problems arise from impurities and thus may be solved.

### Minisatellites, Microsatellites and Dispersed Repetitive DNA

Although some RFLP markers, as well as many RAPD markers, recognize more than one locus in a given genome, the number of loci recognized is limited, as is their polymorphic information content. Markers derived from small, tandemly arranged repetitive elements offer a way around this limitation. Such markers are called micro- or mini-satellites or simple tandem repeats (STR), because their sequence organization resembles the tandem arrangement of classical satellite DNA. These sequences are arranged in the eukaryotic genome in many copies of varying repeat-unit numbers. Due to their varying repeat numbers at a given locus, the elements frequently change their length by slipped-strand mispairing and other, less understood, processes. The surrounding single copy sequences are normally

not affected, and therefore provide a valuable source of polymorphism for many purposes, including linkage analysis (Nakamura et al., 1987), identification of species and cultivars and marker assisted breeding (Beckmann and Soller, 1990)

Minisatellites (repeat units of 9-20 nucleotides) can be hybridized to restricted and electrophoretically separated DNA blotted on to nylon membranes (Jeffreys et al., 1985). Microsatellites (repeat units of 1-5 nucleotides) can be hybridized to DNA in dried gels (Ali et al., 1986) or microsatellites can be cloned, sequenced, and amplification fragment length polymorphism detected by PCR, using oligonucleotides from the surrounding monomorphic DNA as primers. These sequenced tagged microsatellite sites (STMS; Beckman and Soller, 1990), like RFLP are co-dominant markers and therefore highly informative, which justifies the large amount of work needed for their generation. Microsatellites are present in different forms in plant genomes.

### Amplified Fragment Length Polymorphism (AFLP)

This technique is based on the detection of genomic restriction fragments by PCR amplification. DNA is cut with restriction enzymes and double stranded adapters are ligated to the ends of the DNA fragments to generate template DNA for amplification. The sequence of the adapters and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments. Selective nucleotides are included at the 3' ends of the PCR primers, which therefore can only prime DNA synthesis from a subset of the restriction sites. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified.

AFLP produces a higher number of polymorphisms than RAPD or RFLP markers. It is a robust and highly reproductive technique.

## Applications

### Genetic Diversity

DNA polymorphisms are exploited in cotton plants by an ever-increasing number of molecular marker techniques for differentiation between individuals, accessions and species of plants, pathogens and pests. Their high resolution compared with all other markers makes them a valuable tool for varietal and parental identification for the protection of cotton breeders' rights. DNA markers further add to the repertoire of tools for the determination of the evolutionary relationship between *Gossypium* species and families. Molecular markers also allow an understanding of the relationship between chromosomes of the related *Gossypium* species. Cotton plant pathogens and pests also display genetic diversity, therefore, their distribution, variation and gene flow can be measured in time and space. Together with aggressivity tests, the study of genetic diversity provided the basis for the detection of annual changes in pathogen distri-

bution that can be used in forecasting and preventing future epidemics.

### Marker-assisted Breeding

The use of molecular markers enables cotton breeders to connect the gene action underlying a specific phenotype with the distinct regions of the genome in which the gene resides, e.g., the phenotypic expression of fiber quality is confined to domesticated species. The genetic advances in fiber quality can be made indicative of the existence of genes that contribute to fiber quality in germplasm that does not express the phenotype. Molecular markers could provide the opportunity to use precision in identifying the phenotype of these traits. Molecular markers will allow direct selection for genotypes, thereby providing a more efficient means of selection for fiber properties. The genetic manipulation of cotton fiber properties using molecular strategies relies on the identification and isolation of genes that control fiber development and/or directly affect a particular structural property of fibers. Molecular markers provide an opportunity to identify and isolate the genes relating to fiber characters by map-based cloning. Once markers for an interesting trait are established, these should allow prediction of fiber characters, yield or resistance of individual offspring derived from a cross, solely by the distribution pattern of markers in the offspring genome. Besides the exploitation of genomic polymorphisms for germplasm utilization and protection of varieties, cotton breeders' interest in molecular markers currently focuses on three major issues.

- The acceleration of the introgression of single resistant genes for plant pathogens from wild species or cultivated donor lines into otherwise superior cultivars.
- The accumulation (pyramiding) of major and/or minor resistance genes into cultivars for generating multiple and more durable (horizontal) resistances against several pathotypes of the same pathogen.
- The improvement of the agronomic value of cotton by breeding for quantitatively inherited traits, such as yield, drought and cold tolerance.

### Breeding for Resistance

In cotton, the main advantage of using molecular markers is introgression of resistance genes into the cultivars to save time. The use of DNA markers could speed this process by three plant generations, by allowing selection of resistant offspring that contain the lowest amount of the donor genome in every generation (Tanksley et al. 1989). Single gene resistances (vertical resistance) are often rapidly broken by pathogens, thus converting the previously resistant cultivar into a susceptible one. Breeders therefore aim to accumulate several minor and major resistance genes into one cultivar to achieve more durable resistances. With conventional breeding, it is nearly impossible to combine so many different genes. The action of different resistance genes cannot be distinguished even with a set of different pathotypes. However, many resistance loci from

different sources can be determined, and their presence detected, by linked markers in parallel crosses.

### Quantitative Trait Loci Analysis

Many agronomically interesting traits, such as yield, are controlled by polygenes, with every gene contributing only a small percent to the expression of the trait. Tagging of polygenes with molecular markers requires a saturated linkage map with a marker spacing of no more than 20 cM and at least 250  $F_2$  individuals from a cross between parental lines that differ markedly with respect to the trait in question (Tanksley, 1993). First the offspring is tested for the trait and its genotype determined for every marker locus. Then the likelihood that the observed data rely on the presence of QTL is calculated, against the likelihood that no QTL is present, using specially designed computer software such as MAPMAKER.

### Population Development for DNA Markers

Population development is an important step in DNA marker studies. The appropriate design of a test population is a crucial step in the development of markers for agronomic traits. Several strategies for such a design, including different segregating populations and sampling regimes, have recently been developed. The following types of populations can be used for DNA marker studies.

#### Interspecific Crosses

In cotton, crosses of wild species with cultivated lines have generally been found useful for the generation of genetic maps, because of the relatively high degree of morphological isozyme and DNA polymorphisms in wild species. Such crosses helped in the generation of linkage maps and the identification of resistance loci. Once a map has been established and linkage markers found, commercially important accessions may also be included for linkage analysis using these markers.

#### $F_2/F_3$ Segregating Population

$F_2$  or  $F_3$  populations can be used for DNA markers. While making crosses, the parents should be screened carefully. These parents should be widely different for the desirable trait. The segregating population should be phenotypically screened and the population to be screened should be large, normally about a population of 500 plants, depending upon the expected number of loci involved for the trait. It is more useful if after the screening of  $F_2$  plants, the families of selected  $F_2$  plants are raised and final selection of plants for DNA marker studies is based on the  $F_3$  population. The progeny of the  $F_2$  plants showing segregation should not be included. About 25 plants are finally selected for each contrasting trait. The presence of polymorphic DNA molecules present uniformly in one group of plants and absent in the other contrasting group of plants show its linkage with the trait of interest. A large  $F_2$  population is most informative for genome mapping, especially if the map is at an early stage and only a few markers are mapped. However,

the  $F_2$  selection has three major drawbacks for the development of markers for the agronomically interesting traits concerned.

- The same individual tested for the trait also has to be used for linkage analysis, e. g., some of the plants are too affected with pathogens and pests to provide enough DNA for linkage analysis.
- After completion of their life cycle, plants die and will no longer be available for backcrosses or further genetic analysis (especially the pheno- and geno-typically characterized individuals).
- Most multilocus markers, including RAPD and mini and micro satellites are dominant markers, whose homo- or heterozygous state cannot be determined. The  $F_2$  generation does not allow these two possibilities to be distinguished and much information is therefore lost.

## Recombinant Lines

Advanced lines developed from the cross of two plants can also be used for DNA markers. A population of at least 500 recombinant lines is screened, and about 20 lines from each of the contrasting pairs are selected for DNA analysis. When using  $F_2/F_3$  or recombinant lines, after DNA extraction of individual plants, bulked segregant analysis (Michellmore et al., 1991) can also be used instead of using DNA of individual plants in RAPD, RFLP, or AFLP, etc., analysis.

## Isogenic Lines

Isogenic lines developed for a particular trait, using the recurrent back cross procedure, can also be used for DNA markers studies. The use of isogenic lines reduces the number of RAPD/RFLP/AFLP, etc., reaction as only two lines contrasting for the trait are used. However, it is a time-consuming and tedious procedure to develop isogenic lines. For more advanced studies with very tight linkage between markers and traits, which is a prerequisite for pyramiding of resistance genes or map-based cloning of genes, nearly isogenic lines (NILs), derived from several rounds of backcrossing of the recipient of an agronomically interesting gene with the recipient genotype, are used with high efficiency in several species. NIL contains normally only small amounts of donor DNA surrounding the gene of interest. To avoid the time consuming generation of NIL, specific genes can be tagged by bulking DNA from individual  $F_2$  plants with identical performance in phenotypic (resistance) tests (bulk segregant analysis; Michellmore et. al. 1991). Individual DNA from existing mapping populations can also be grouped according to the distribution of RFLP markers and then RAPD analysis can be used to tag the region surrounding the gene(s).

DNA markers thus offer two advantages:

- Faster recovery of the recurrent genome.
- More efficient selection of genomes that have recombination events close to the target gene.

Marker-assisted selection is still limited by three main factors:

- The number of samples that can be analyzed.
- The number of lines that can be improved within a given time.
- The belief that QTL identification is required whenever additional germplasm is used.

PCR based markers have also opened new doors for genome manipulation, since their use allow:

- Earlier sampling, because of the small amount of tissue required.
- Faster DNA preparation, because of the small amount of template DNA required.
- More efficient handling of large sample sizes, because of the efficiency of PCR technology.

Now is the time to consider the development of new breeding strategies that take into account genetic characteristics, such as the complexity of the genome; the nature and the number of the molecular markers available; the complexity of the traits to be improved; the number of plants that can be screened at each selection step; and the number of populations that can be concurrently manipulated. It is also crucial to explore the complementarity between marker-assisted selection and conventional breeding, and to develop overall strategies that tightly and interactively integrate the two approaches.

Marker-assisted selection for polygenic trait improvement is an important transition phase, and the field is on the verge of producing convincing results. Recent efforts in comparative genetic analysis allow identification across different plant species of gene sequences involved in the expression of target traits. The superior alleles identified among genomes in those target genes can be used as DNA markers to develop efficient screening techniques. Finally, technological developments, including automation, allele-specific diagnostics and DNA chips, will make marker-assisted selection approaches based on large scale screening much more powerful and effective.

## Map-based Cloning

The detection and cloning of distinct genes in cotton of unknown sequence and function, when only their involvement in specific traits and their chromosomal location is known, has been termed reverse genetics. In contrast, the conventional approaches, where a gene is cloned on the basis of a known product or sequence and then localized to a chromosomal region starts with the localization of a gene on a specific chromosomal region by determining the linkage of the phenotype it specifies to a set of flanking molecular markers. These linked markers are then used as starting points for physically mapping the gene-flanking region with pulsed field gel electrophoresis and rare-cutting restriction enzymes. Large-scale restriction-site mapping is necessary because physical and genetic distances between markers may vary over several orders of magnitude.

Physical maps are especially useful in polyploid crops such as cotton where duplicated sequences could prevent the assignment of markers to a single distinct location.

The construction and screening of yeast artificial chromosome (YAC) libraries is a next step towards isolation of desired genes. Clones containing at least one of the markers are selected. End fragments of these YAC are then used to construct contigs of the genomic region defined by the markers, to identify cDNA derived from that location. The cDNA are sequenced and candidate sequences retransformed into suitable heterologous and homologous hosts for proving the gene identity by complementation in transgenic plants, using different transformation techniques.

An absolute prerequisite for map-based cloning of genes in cotton is the availability of tightly linked markers flanking the locus of interest. These may be found by chance but are normally the product of a systematic effort to saturate a genome with polymorphic DNA markers.

Due to the economic dependence of many countries on cotton production, it is necessary to focus on reducing the cost of production, increasing yield and quality, and diversifying the product spectrum of fiber. Another objective for cotton research is to enable farming practices, as well as processing of fiber, to become more environmentally friendly. Molecular markers technology has made it possible to identify genotypes carrying desired characters efficiently and correctly. This may help in the selection of genotypes and boost conventional breeding programs.

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# Fiber Strength

Cotton is grown for lint and unlike other commodities it is traded based on quality parameters rather than just weight. Fiber quality affects the end products made of cotton. If fiber quality is low, spinning performance and efficiency will be affected negatively. Weak fibers give rise to a number of problems in today's high speed spinning industry, and to yarn breakage during processing. Weak fibers contribute to weak yarn and consequently to

low quality fabric. Strong fibers can be spun at higher speeds thus improving the economics of yarn formation. Therefore, stronger fibers are needed to offset the resistance losses of yarn obtained by the new high speed spinning processes.

Fiber strength started to be routinely measured only when the Pressley strength tester was developed about 60 years ago. When the Pressley tester became popular, most varieties did not measure stronger than 75,000 pounds per square inch (PSI). At present, most varieties in the world measure over 90,000 PSI.

Strength is not the only criteria that determine the performance of a given cotton. Other important characteristics include fiber length, micronaire and length uniformity. Based on changes in the spinning industry toward high speed machinery, requirements for raw mate-

**Fiber Quality Requirements for Different Spinning Systems**

| Ring Spinning | Rotor Spinning    | Friction Spinning | Airjet Spinning   |
|---------------|-------------------|-------------------|-------------------|
| Length        | Strength          | Strength          | Micronaire        |
| Uniformity    | Micronaire        | Micronaire        | Length            |
| Strength      | Length            | Length            | Length uniformity |
| Micronaire    | Length uniformity | Length uniformity | Strength          |
| Elongation    | Cleanliness       | Fiber friction    |                   |
|               |                   | Cleanliness       |                   |

rial have been changing, and the latest requirements for different types of spinning systems by order of priority for a characteristic are shown in the table.

## Improving Fiber Strength

Data from the USA show that on average fiber strength has increased at the rate of 0.28 gm/year, from 24.0 gm/tex in 1980/81 to 28.5 gm/tex in 1993/94. In 1994, Sasser stated at the 53rd Plenary Meeting of the ICAC that if this rate of increase continued, the average fiber strength in 2000 would be 30.0 gm/tex. He also indicated that this increase has come from all production regions though the contributions have been different among regions. The USDA data show that the average fiber strength value for the 1999/00 crop was 28.3 gm/tex, but it was lower in 2000/01. The main reason for not reaching the target seems to be the slower rate of introduction of new varieties, but at the same time it shows that there is a limit to the improvement of fiber strength. Since the introduction of transgenic varieties in the USA in 1996, efforts have been made to induce Bt and herbicide tolerant genes in established varieties. The companies responsible did not want to take the chance of introducing the technology into new varieties and, consequently, the quality improvement process was stabilized. However, as transgenes are inserted into new varieties and the rate of introduction of new varieties is revived, quality improvement may restart again.

## Measurement of Strength on Different Gauges

It is now over forty years since it was recognized that “zero” gauge strength testing does not give reliable results. The zero gauge was only slightly correlated with yarn strength but the cotton industry continued to use this gauge for many years. In February 1986, the ITMF International Committee on Cotton Testing Methods recommended the adoption of 3.2 gauge gm/tex as the recognized interpretation of fiber bundle strength. At the time of the ITMF Committee recommendation, the industry was using different types of instruments and, in several countries, also different strength values like Pressley zero gauge, Pressley 3.2 mm gauge, Stelometer 3.2 gauge, HVT and HVI.

**Fiber Strength Data for Two Gauges**

| Variety   | "0" Gauge | 3.2 mm Gauge |
|-----------|-----------|--------------|
| LRA 5166  | 44.0      | 21.7         |
| MCU 5     | 46.6      | 22.3         |
| G. Cot 13 | 43.4      | 19.1         |
| JK.HY 1   | 47.7      | 23.0         |
| H 4       | 41.8      | 21.4         |
| SUVIN     | 58.1      | 31.1         |
| DCH 32    | 45.6      | 23.3         |
| H 6       | 46.1      | 21.1         |

Source: Central Institute for Research on Cotton Technology, Mumbai, India

Many countries have already changed or are planning to change to 3.2 mm (1/8 inch) gauge. In India, for example, the Central Institute for Research on Cotton Technology—one of the oldest research centers on fiber testing in the region—started reporting the data in 3.2 mm gauge in 1996. The reason for the shift is that the strength at zero gauge length reflects the quality of fibrillar structure, which is largely a genetic character. The strength at 3.2 gauge length, on the other hand, is decided by the presence of “weak links” along the length of the fiber. It is not clear whether weak links are genetically controlled or whether environment influences their occurrence. It is known that strength decreases with the increase in gauge length and this decrease is not uniform for all varieties. The zero gauge measurement of fiber strength accords extremely high values, thus giving a false impression of fiber quality. Similarly, experiments made around the world on various gauges have proved that the 3.2 mm gauge is better correlated with yarn strength. If breeders continue using zero gauge, it is quite possible that improvements in strength (at zero gauge) may have no effect on the 3.2 mm gauge reading and thus no effect on yarn performance, which is determined by the 3.2 gauge. HVI data are of course reported at 3.2 mm gauge. The use of spacers in the Stelometer clamp ensures a gauge length of 3.2 mm during testing.

## Reasons to Adopt Stelometer/HVI Strength Data

The International Cotton Advisory Committee (ICAC) undertakes a survey of cotton production practices every three years wherein data on varieties planted and fiber characteristics are collected. The survey and other sources of information indicate that bundle strength data are still reported in PSI in Ecuador, Iran, Mexico, Mozambique, Nigeria, Pakistan, Peru, Sudan, Syria, Tanzania, Turkey and Turkmenistan, although some of these countries also have the option of using Pressley or Stelometer.

The list of countries using Pressley for strength measurement is decreasing continuously. As the list becomes smaller, uniformity in the data interpretation is improving. There are two main reasons why Pressley use has decreased significantly.

- Spinners are demanding 3.2 mm gauge data because the Stelometer data are better correlated to yarn strength data compared to the Pressley zero gauge.
- Expanding use of HVI in the USA and elsewhere has forced the use of Stelometer 3.2 mm readings, as the data from Pressley could not be converted into values equivalent to 3.2 gauge by a general formula for all varieties.

The fundamental difference between the Stelometer data and the HVI data lies in the way the data are recorded. The Stelometer gives a basic strength value and provides bundle fiber breakage following the rules of constant load. After the fiber bundle breaks, the actual sample weight is used to determine the strength value. The equipment gives bundle strength

and elongation values. A tex unit is equal to the weight in grams of 1,000 meters of fiber. The strength reported in gm/tex is the force in grams required to break a bundle of fibers one tex unit in size.

The HVI system does not allow measurement of fiber bundle weight at the time of fiber breakage and therefore HVI needs cotton standards with known strength values and micronaire values for comparison purposes. This means that HVI strength measurement is not a direct measurement of strength, rather it is an indirect determination of a value by comparison with a standard sample.

It is strongly recommended that for measurement of bundle strength, only Universal HVI Calibration Cottons should be used to calibrate the equipment instead of International Calibration Cottons. In the USA, where HVI data is used, fibers having over 31 gm/tex are considered to be very strong, while 29-30, 26-28 and 24-25 gm/tex form strong, average and intermediate strength values. Fibers with a bundle strength value of 23 gm/tex and lower are considered to be weak and can create additional problems during spinning. Stelometer readings are always lower compared to the HVI data and thus values in the USA are generally higher. There is no exact conversion factor, but the Stelometer readings could be multiplied by 1.28 in order to have an estimated HVI equivalent value.

## Breeding for Stronger Fiber

Fiber strength can be improved either by developing varieties that have stronger fibers or by changing the growing conditions so that a stronger fiber is realized from the available genotypes. Realization of good fiber strength through agronomic management has a limit and cannot be regarded as improvement. For actual improvement in strength, breeding for stronger fiber producing genotypes is the only option and genetic analysis has shown that strength has a high heritability. If this is true, then why have breeders not been able to develop even stronger fiber varieties? The answer to this question lies in the association between yield and strength, which has been determined to be negative. Yield has been the priority so far, and breeders have sacrificed at least some fiber strength at the cost of higher yields.

Much of the genetic variability for fiber strength has come from wild *Gossypium* species, and many times strength genes are linked to vegetative growth at the expense of reproductive growth. However, crossing between stable genotypes and segregating populations has resulted in minimizing the negative correlation between yield and strength.

At the time of making selections in the field, with single plant selection, breeders always look for the best looking high yielding plant. In this process, there is a good chance of selecting plants with weak fiber but which are good looking. This applies to progeny row selection and also to single plant selection, and even when the bulk selection method is followed. Progress has been made gradually but comparatively at a slower rate.

To improve fiber strength through breeding, scientists need to improve steeper spiral and lower reversal frequency and weakness magnitude. But the complexity of genetic control of these characteristics is not properly understood. The empirical approach of crossing parents with stronger fiber continues to be the only approach to improve fiber strength. Improvement in fiber strength is more complicated and limited compared to many other quality characteristics, particularly micronaire.

May (2000) concluded that fiber strength is a genetic property and that genotype x environment interactions are small relative to genetic influences. Additive gene action determines improvement but inter-crossing even between desirable parents may result in a small, and not generally meaningful heterosis, if any. It is reported that from five to fourteen genes are involved in the genetic control of fiber strength. Some data also suggest that strength may not always segregate in a quantitative manner. Literature also shows that single dominant or double dominant genes might condition strength.

Brown colored lint is usually weak, and efforts made in various countries have not been able to develop brown colored cotton equivalent to white cotton for fiber strength, though some claims of success have been made. It seems that strength genes are in the close vicinity of brown pigment-forming genes. May (2000) has reviewed the genetic control of fiber strength in the above-referred book.

Of the three methods available to test bundle strength—Pressley, Stelometer and HVI—which instrument is more suitable for testing breeding lines is still a controversial issue. Though the textile industry supports using HVI, Pressley and Stelometer seem to be more useful for testing breeding lines and improving strength.

## Application of GE Technologies for Fiber Modifications

Fiber strength is one of the least likely characteristics being improved, particularly beyond limits already achieved in most countries, and significant improvement through empirical approaches is not expected. Whether genetic engineering technology can be employed to bring modifications not achievable through conventional breeding, has been discussed often in the biotechnology of cotton. With the introduction of agronomic characteristics, there were hopes that fiber modification would be the next stage. Now, it is six years since transgenic cotton was commercialized, and there is no indication yet of any significant development in fiber modification on the way to commercialization, although work started many years ago and some of it has been published. Greater success than what has been reported in the literature may have been achieved, but it has not come to the surface yet. Most research on the use of genetic engineering to improve fiber strength is in the private sector, and companies have not made enough progress to share the information yet.

Based on what is publicly available, there is not much to report

on the genetic engineering applications to fiber modification. It is almost certain that there will be more approaches researched and John (2000) has discussed at least three options: manipulation of the existing cotton genes, manipulation of hormone levels in developing fibers and synthesis of new biomaterials in fiber. However, on the lines of Bt and herbicide resistance, induction of cotton and new non-cotton genes into existing varieties remains another choice.

John (2000) reported that there are a number of fiber-specific mRNAs that have an exclusive and critical function in the development and architecture of fiber cells. If some of the genetic material has a dual or even complex role affecting more than one or many plant features, their exploitation could have a negative effect on some other plant characteristics. But the fiber specific mRNAs are a hope, though many proteins may also be involved in the formation of a complex character like strength.

Abscisic acid, cytokinins, gibberellins and indoleacetic acid are among the hormones tried most often on cotton. Cotton fibers are single cells and many references in the literature show that indoleacetic acid (IAA) is one of several hormones that collectively regulate growth and differentiation of plant cells. But, work done in India and many other countries also shows no effect on fiber quality. More recent work has been done in the USA on the commercially grown variety DPL 50, where IAA levels were increased from two to eight-fold with no effect on fiber length, strength and micronaire noted. According to John (2000), over-expression of auxins and cytokinins also failed to show any influence on fiber elongation, strength and micronaire.

One of the other pragmatic approaches under current investigation is the synthesis of new biomaterials in fiber. It is hoped that, depending upon the biopolymer selected and its level, existing quality traits in cotton fiber can be improved. Though not commercialized, some progress in this regard has been achieved and transgenic cotton with different thermal properties has been realized. Such transgenic cotton has the ability to retain 8% more heat compared to its non-transgenic parent.

Some wild species like *G. thurberi* and *G. raimondii* carry higher fiber strength genes than cultivated species. If these genes cannot be transferred to cultivated varieties through conventional breeding techniques, opportunities are now available to insert them into desired genotypes through genetic engineering techniques. It would be easy to adopt such a transgressive population rather than adopting a non-cotton genetic material in the cotton genome. Recently, focus has shifted to such approaches, but such genetically engineered genotypes are yet to be seen.

Many other avenues may be in the offing. Now the issue is not the insertion of the genetic material but the identification of a material that, if inserted into cotton, will produce the desired result. Whether it is a cotton or non-cotton gene(s) addition, utilization of hormone activities, synthesis of new biomaterials within the fiber, or any other approach, it is very important to

make sure that the changes brought do not have a negative impact on any other existing characteristic, including yield potential.

## Need for Single Fiber Testing

Testing equipment currently used, either the Stelometer, Pressley, Universal Tensile Tester, HVI system or any other equipment, measures bundle strength. The tests measure the forces transmitted by a bundle consisting of a defined mass of fibers and the corresponding elongations. The equipment provides bundle-specific parameters. While within a bundle, in the case of a Pressley, weak fibers may break at a very low breaking load and some others may break at a very high breaking load. Assuming that all other fibers in the bundle broke at a reading within the range, the bundle strength data do not depict the weaker fibers and the stronger fibers. The result is the average of a bundle performance, which does not constitute a true characteristic of strength and elongation because interactions occur among fibers in the bundle.

Reliable and true values can be obtained by measuring single fiber strength. It is not a question of availability of the equipment to measure single fiber strength, it is the accuracy of the data which is required. Equipment not available today may become available tomorrow. Single fiber strength measurement is more desirable because of the following reasons:

- Bundle strength and bundle elongation do not represent the mean fiber strength and break elongation ratios of individual fibers in the sample. And, it is not possible to convert the bundle strength data into a single fiber strength data and vice versa.
- Bundle strength and elongation are influenced by the length of the fibers in the sample. Bundle strength does not take into account fiber length as the current bundle strength methods choose only the upper half of the length distribution.
- Fibers within a sample may also have a different fineness value and accordingly the interaction among fibers in the bundle will also be different. Finer fibers have better cohesion and thus could give higher value for the bundle strength.

## Fiber Strength vs Yarn Strength

Cotton fiber cells grow from the epidermis of the seed coat, and growth is completed in 15-25 days. The fiber consists of a primary wall and a secondary wall, both mainly comprised of cellulose fibrils. The primary wall is covered with a cuticle consisting of mainly wax, pectin substances and reducing sugars. Entomological sugars, if any, are deposited on the cuticle and may cover the natural wax. The position of cellulose fibrils in the walls is what determines their effect on fiber strength. In the primary wall, fibrils are placed in a wide angle with the fiber-long axis, and thus the primary wall has no effect on fiber strength. In the secondary wall, the cellulose fibrils are laid side by side and follow a helical course making an angle known as the spiral angle. Thus, the secondary wall thickening has a



significant effect on fiber strength. The spiral angle in varieties differs greatly. As a general principle, a smaller spiral angle forms steeper spirals and thus stronger fibers.

The secondary wall is more than 90% of the fiber weight from where the fiber derives almost all of its strength. Fiber strength is directly related to yarn strength:

| <b>Correlation Coefficients<br/>for Fiber Properties<br/>and Yarn Strength</b> |                     |
|--------------------------------------------------------------------------------|---------------------|
| <b>Fiber Property</b>                                                          | <b>Coefficients</b> |
| Micronaire                                                                     | -0.40               |
| Fiber strength                                                                 | 0.88                |
| Fiber length                                                                   | 0.75                |
| Length uniformity                                                              | 0.28                |
| Short fiber content                                                            | -0.61               |
| Elongation                                                                     | 0.15                |
| Reflectance (Rd)                                                               | 0.59                |
| Yellowness (+b)                                                                | -0.10               |
| Maturity ratio                                                                 | -0.18               |
| Maturity percentage                                                            | -0.19               |
| Standard fiber fineness                                                        | -0.21               |

strong fibers make a strong yarn. However, this relationship is affected by fineness, length and length uniformity. The relationship may be linear up to a certain limit, particularly with regard to fineness.

Gülyasar and Gençer (2000) have studied the relationship of eleven fiber quality parameters and yarn strength. They produced three varieties in replicated trials under uniform conditions and subjected lint to testing for various characteristics after roller ginning. The results given above clearly show that fiber strength has the highest correlation with yarn strength compared to other measured characteristics.

The high correlation clearly indicates the need for breeding stronger fiber varieties. How strong a fiber is strong enough to be spun smoothly may vary according to the spinning process and other quality characteristics. But, on average, if the bundle strength value is 25 gm/tex, it is assumed to be strong enough to withstand the wear and tear effects of the spinning machine. Once strong cotton has been produced, the objective will be to make the maximum utilization of this property and keep the yarn-strength to fiber-strength ratio high, which is influenced by the orientation of fibers in the yarn, the art of spinning.

Even the gentlest ginning is not capable of improving the inherent fiber quality characteristics. However, poor ginning has the potential to deteriorate many quality characteristics. Staple is the most affected characteristic, while strength is among the least affected by ginning.

Literature shows that fiber strength is not related to the force with which fibers are attached to the seed coat. Fibers can be weak and still attach to the seed coat with a strong force and vice versa. The fiber-to-seed attachment force is not a commonly measured property and it is understandable that stronger fibers could snatch more seed coat pieces in the process of removing them and thus result in more cotton contamination. The force with which fibers are attached to the seed coat and not the fiber strength is what causes seed coat fragments in cotton.

During the mid 1980s, when high speed airjet and friction spinning were becoming popular in cotton, it was anticipated that the textile industry would demand finer cotton. But finer cotton does not always mean mature and strong cotton. Cotton may be immature and weak but still very fine. So, with the increase in demand for finer cotton, demand for stronger cotton also became apparent. Also, because of the variability in the number of fibers in the yarn cross-section, fiber strength became more important in friction and airjet spinning compared to ring spinning.

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## Short Notes

### • “Remote Control” of Genes

According to news published in the February 2002 issue of the Biotech Reporter, researchers at the Massachusetts Institute of Technology in the USA have demonstrated control of DNA with radio waves. This is an indication that gene actions can be turned off and on as desired. The target genes exist but their phenotypic effect can be active or dor-

mant for some time. If the gene has been turned off using a remote control, it can be turned back on to perform its normal function. Researchers attach a metal nanocluster of less than 100 atoms to DNA and when a radio-frequency magnetic field is transmitted into the little antennae, the molecule is zapped with energy and responds. Using this technique, researchers can dehybridize the double stranded DNA

in a matter of seconds. The switching is reversible and does not affect neighboring molecules. There is also the possibility of applying the same principle to more complex processes like enzyme activity.

### • Area Under Genetically Engineered Crops

According to the International Service for the Acquisition of Agri-biotech Applications, global area planted to genetically engineered (GE) crop varieties increased to 52.6 million hectares in 2001. The increase in area between 2000 and 2001 was 19%, equivalent to 8.4 million ha. The increase was twice the corresponding increase of almost 4.3 million hectares in 2000 over 1999. Commercial cultivation of transgenic cotton started in 1996, and at that time only 1.7 million hectares were planted to GE crops. During 2001/02, cotton was planted on 33.5 million hectares out of which GE cotton varieties accounted for 6.8 million hectares in seven countries, Argentina, Australia, China (Mainland), Indonesia, Mexico, South Africa and the USA. Indonesia started commercial production of GE cotton in 2001.

GE cotton varieties were only 13% of the total GE crops area in the world in 2001. 33.3 million hectares, or 63% of 52.6 million hectares, were planted to GE soybeans, followed by GE corn at 19%, or 9.8 million hectares. Only 5% of the total GE crops area was planted to crops other than soybeans, corn and cotton. Among others, canola is a major GE crop. Herbicide-resistant cotton has been approved for commercial cultivation in Argentina, Australia and the USA, but herbicide resistance is the most adopted trait in all crops since 1996. Herbicide resistant varieties were planted on 77% of the total GE cropped area in the world in 2001. 15% of the total area was under Bt varieties and 8% under stacked gene varieties. Stacked gene varieties are also available in corn, in addition to cotton. Developed countries continue to embrace GE crops at a faster rate than in developing countries. It is estimated that four countries, Argentina, Canada, China (Mainland) and the USA, grew about 99% of GE crop area in 2001.

In 2001/02, GE cotton was planted on 78% of the total area in the USA. More than 60 GE varieties were available for commercial production though less than ten occupied almost 50% of the total cotton area in the USA in 2001/02. Almost 76% of the total GE cotton area in the USA had the herbicide resistant trait.

The Yellow River Valley in China (Mainland) suffered greatly due to the resistance problem which has made Bt cotton extremely popular there. It is estimated that in 2001/02, 1.5 million hectares were planted to GE varieties in China (Mainland), which has locally developed varieties as well as varieties having the Bt gene from Monsanto. The GE area is expected to increase in 2002/03. It is anticipated that India will also approve commercial production of Bt

cotton in 2002/03. Indonesia is the only country where GE cotton is approved for commercial production on a yearly basis and also limited to certain areas. Indonesia will continue to produce GE cotton in 2002/03.

### • Physiological Effect of Aphid Attack

Aphid *Aphis gossypii* may not be a major pest in many countries but it certainly affects cotton at various stages of development. In some countries it appears at a very early stage, soon after germination, and may not require any pesticide use. Insecticide applied in the seedbed at the time of planting cotton is one of the most successful ways to avoid spraying at an early stage. Researchers at the University of Arkansas, USA, have studied what happens to the cotton plant beyond physical damage if the aphid population increases during the critical growth period. Aphids are a sucking pest; they suck sap from the phloem for their amino-acid needs and secrete carbohydrates as honeydew on cotton leaves and lint. Researchers in Arkansas studied the physiological effect of aphids on a single leaf and on the whole plant.

Studies were conducted in a greenhouse on one variety. Aphids were collected from the field and reared in the laboratory for release on cotton plants in the greenhouse. At twenty days after planting, 100 aphids (wingless adults + nymphs) were individually transferred to the selected leaves. Moreover 5 aphids per leaf were released on other leaves. Aphids were allowed to multiply and at the time of recording the data, the aphid number had increased to 363 aphids per tagged leaf. Net photosynthetic rate, stomatal resistance, transpiration rate and chlorophyll were measured at 20 days after planting, before transferring aphids, and nine days after releasing aphids. At the same time, nonstructural carbohydrate concentrations of the leaves were determined. Additionally, leaf length, leaf area, dry weight of leaves, stems and petioles were also measured.

Results showed that nine days of extensive exposure to aphids did not significantly affect leaf length and area of a single leaf. Similarly, there was no significant effect on the whole plant leaf area. Photosynthesis and transpiration rates were found to be higher in the aphid-affected leaves but the differences were again insignificant. Results also showed that stomatal resistance and chlorophyll index did not change after nine days of aphid feeding. Starch concentration in aphid-infested leaves was found to be lower compared to non-infested leaves. Aphids directly take up photoassimilates translocating in the sieve elements and researchers believe that plant compensation may have led to reduced starch accumulation in the aphid-infested leaves. Sucrose, glucose and fructose concentrations were found to be similar between aphid infested and non-infested leaves. Total leaf, stem and plant dry weight appeared to be lower in aphid-infested plants after nine days of insect attack. Petiole dry weight was not affected. Research will continue on the mea-

sured and additional effects in 2002/03. (Work has been published in the Proceedings of the 2001 Cotton Research Meeting and Summaries of Cotton Research in Progress, Special Report No. 204, November 2001, University of

Arkansas, USA. Available on line at <<http://www.uark.edu/dept/agripub/publications/specialreport/>>. For more information on the issue contact Dr. Derrick M. Oosterhuis at <[oosterhu@uark.edu](mailto:oosterhu@uark.edu)>.)

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