NEW SOURCES OF GENETIC RESISTANCE TO COTTON PESTS

Papers Presented at a Technical Seminar at the 54th Plenary Meeting of the

INTERNATIONAL COTTON ADVISORY COMMITTEE

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Introduction

The Committee on Cotton Production Research of the International Cotton Advisory Committees met in Manila, Philippines and conducted a Technical Seminar on the topic "New Sources of Genetic Resistance to Cotton Pests." The Committee also decided a topic for the 1996 Technical Seminar and considered organizing a second World Cotton Research Conference in the next 3-4 years. The meeting was chaired by Dr. Isagani G. Catedral, Executive Director of the Cotton Research and Development Institute, Mariano Marcos State University, Batac 2906, Ilocos Norte, Philippines. Eight papers were presented in the Technical Seminar.

Dr. Edison C. Riñen, Head Cotton Breeding Department of the Cotton Research and Development Institute in Philippines presented a paper on the need for genetic resistance to cotton pests. He stated that in the Philippines, the data computed from experimental plots showed a 41-47% loss in yield due to insects and diseases. In the Philippines, about 15% of the total cost of production is spent to control insects but it costs even more to control insects in many other countries. While chemical control is still the most efficient and reliable method of insect control, its ill effects on the environment and side effects particularly in the form of insecticide resistance and high cost of insecticides have resulted in legitimate concerns in society worldwide. Quoting the example of fusarium and verticillium wilt resistant varieties and multi-adversity resistance in a single genotype, Dr. Riñen stated that, if the need for pesticides is reduced/eliminated through genetic resistance in the plant, the need for elaborate techno-transfer activities to control pests is also reduced. He hoped that the recent developments in the field of biotechnology will permit more aggressive utilization of non-species genes in the future.

Dr. A. K. Basu of the Cotton Corporation of India Ltd. presented a paper on activities and achievements of breeding for resistance to bollwoms in India. In India, cotton is grown on 5% of arable land but consumes 53% of pesticides used in the country. Dr. Basu stated that host plant resistance is a relative term and should not be used in an absolute sense. He said all three mechanisms of resistance, i.e. non-preference, antibiosis and escape, should be utilized to minimize insecticide use. In India, elaborate screening of germplasm for the last 25 years showed no significant resistance to bollworms; however, cultivated diploid species have shown a fair degree of tolerance to bollworms. Short season cottons have been extensively utilized particularly in northern India. Moreover, a number of MECH hybrids with okra leaf and long staple characters have been released for commercial cultivation and now occupy a substantial area in central and southern India. According to Dr. Basu, genetic engineering technology has a potential to make use of genes from cowpeas (resistant to tobacco budworm), legumes (digestive enzyme inhibitors), spiders, wasps and scorpions (insecticidal activity of venom).

A joint paper was presented from Egypt by Dr. Ahmed El-Gohary and Dr. Galal M. Moawad of the Ministry of Agriculture. In Egypt, a multidimensional approach was adopted to induct an earliness character into cultivated commercial varieties without losing their fiber characteristics. Consequently, early maturing varieties like Giza 83, Giza 84, Giza 85 and Mobarak 93 have been released which mature in 160-170 days as against 190-200 days in the case of many other varieties. Work to make use of other morphological characters is also in advanced stages. In Egypt, the use of sex pheromones has increased significantly in the last five years. During 1995, about 200,000 ha were treated with different formulations of sex pheromones. Use of sex pheromones, coupled with the strategy of the Ministry of Agriculture to reduce insecticide use, resulted in a significant reduction in the import of pesticides.

Dr. Jingyuan Xia from China (Mainland) presented a paper on the research being done in his country to enhance plant resistance to insects. The paper categorized the phenomenon of self resistance in plants to identification, evaluation, mechanism of resistance, genetics of resistance, breeding and utilization of insect resistance. In China (Mainland), methods have been standardized to verify resistance to insects under natural field environment and controlled conditions. High yielding varieties with special morphological characters have been developed which require 2-3 fewer sprays thus saving significant amounts in insecticide applications.

The available morphological characters do not provide an adequate solution to the complex insect resistance problem. While strong sources of resistance to certain insects are not known, the available most special characters have shown negative effects on the non-target insects thus limiting their utilization. Perhaps, this is the reason that every paper presented in the seminar referred to genetic engineering and utilization of non-species genes. It was noted that the technology has potential and some major achievements are expected in the future. The prospects of utilizing non-cotton genes in developing countries were reviewed by Dr. Fred Gillham, a private cotton consultant from Australia. A major break-through has been achieved in the transformation process but there is a need to extend this process to insert more than one gene at a time. However, limited scientific infrastructure, human resources and technology delivery systems and even marketing may be important obstacles to utilization of biotechnology options in the developing countries. Patent issues may also limit or at least delay the use of transgenic cotton in developing countries.

A paper on field performance of Bt cotton, trademarked as Bollgard™ in the USA and Ingard™ in Australia, was presented by Mr. Thomas Luehder, Delta and Pine Land Company country representative for China (Mainland). Two varieties close to marketing are NuCOTN 33^B and NuCOTN 35^B . Wide scale field trials conducted during 1994 showed that Bt cotton was superior in yield by 109 kg/ha over the commercial spray program. The difference in yield increased with the increase in the lepidopteran pressure. Bt cotton with a gene resistant to lepidopteran insects will be available for commercial cultivation to the US cotton growers for the first time in 1996.

The Multi-Adversity Resistance (MAR) program of the Texas A&M University, USA, is one of the most successful host plant resistance programs in the world. A paper outlining the MAR program and its achievements was presented by Dr. Kamal El-Zik. In the last 30 years, since the initiation of the MAR program, 11 Tamcot varieties and over 300 elite breeding lines have been released from the program, in addition to 27 varieties selected/developed using MAR breeding material. MAR procedures involve extensive seed, seedling and plant screening in the laboratory and field against pathogens, insects and abiotic stresses (52 traits in total). It was realized that there is a need to transform MAR varieties and add novel genes from outside cotton into commercially cultivated and elite MAR genotypes.

Dr. Said Akbar Rakhmankulov, Director General of the Uzbek Scientific Research Institute of Cotton Production, Tashkent stated that cotton production in Uzbekistan is completely mechanized and requires considerable scientific support. He said that genotypic assessment for immunity to cotton pests is a very important step in Uzbekistan and best resistance has been achieved either from intraspecific hybrids or interspecific crosses involving distant parents. Dr. Rakhmankulov said that genus *Gossypium* is rich in highly valuable traits like resistance to wilts, bacterial blight, sucking insects, etc., and Uzbek breeders have developed a practical technique to produce hybrid seed from interspecific crosses among least related genotypes. In Uzbekistan, new physical, chemical and mutagenic methods including irradiation are utilized to acquire inbuilt genetic resistance to insect pests. Dr. Rakhmankulov advised using the plant's self defense system in combination with agronomic management of varieties.

Dr. Ahmed Zibdieh of the Cotton Bureau Syria; Dr. K. Venugopal of the Central Institute for Cotton Research, India; Dr. Ostan J. Jalilov of the Institute of Experimental Biology of Plants, Uzbekistan; and Dr. Barkat Ali Soomro of the Pakistan Central Cotton Committee prepared papers for the Technical Seminar. These papers are included in the publication.

The Committee on Cotton Production Research decided that the 1996 Technical Seminar will be held on the topic "Short Season Cotton: How Far Can It Go?" The Committee also recognized the need for continuous review of Common Fund funded projects of the ICAC and decided that progress reports on these projects will be the topic of the seminar for the 56th Plenary Meeting.

The Committee accepted an invitation from the Hellenic Cotton Board of Greece to host World Cotton Research Conference-2 in Greece in 1998.

The Need for Genetic Resistance to Pests in Cotton

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Pests are persistent threats to successful cotton growing. Substantial economic losses are incurred. Yield loss estimates due to insect pests and diseases in the Philippines (computed from experimental data between 1989 to 1994) ranged from 41 to 47 percent (CRDI, 1994). In the United States, available field data indicated an annual average yield loss of about 7 to 8 percent (Gazaway, 1986; King et al., 1986). Earlier, the National Academy of Sciences (1975) put it at 14 to 15 percent.

For cotton production to be profitable, pests must be controlled. This fact is evident from the amount of investments generally poured on pest management. A survey by the ICAC (1992) showed that about 15 percent of the total cost of producing raw cotton is generally set aside for pest control, particularly for insect pests. The share is just about that much in the Philippines (CRDI, 1993a), but in other countries the share can be higher, like that under irrigated regimes in Australia (ICAC, 1992). In Sudan, the use of insecticides alone constituted about 42 percent of the total expenditure (ICAC, 1994c).

Pest control investments are basically earmarked for chemicals, occasionally in tandem with cultural and biological approaches aside from host plant resistance (ICAC, 1993c). But, while the use of pesticides is still the dominant and most reliable means of achieving immediate relief from cotton pests, legitimate concerns regarding its ill-effects on health and environment have been constantly echoed worldwide. Even in the area of pest control, problems regarding its incessant use have not been wanting. Thus, it is the pesticides' adverse side-effects, in addition to their becoming more and more expensive, that lead production research workers continuously to search for novel pest management approaches — environmentally sound and economically viable ways that could dependably deliver the kill and, at the same time, reduce, if not totally eliminate, dependence on chemicals. Individual as well as integrated approaches are purposefully explored and applied with considerable success. In the quest for new pest control strategies, the use or inclusion of built-in resistance is always a primary consideration (NAS, 1975; Russell, 1978; Ortman and Peters, 1980; Jenkins, 1981; Hooker, 1983; Huffakker and Smith, 1983; Phillips et al., 1983; Hansen, n.d.). In fact, it is regarded as the cornerstone for any successful integrated pest control system (El-Zik and Thaxton, 1989).

The effectiveness of resistant varieties in containing the destructive effects of various cotton pests is unquestionable. The most satisfactory control for major cotton diseases, for example, particularly for fusarium and verticillium wilts, is still obtained only from resistant cultivars (NAS, 1975; ICAC, 1993a). Several varieties developed through the MAR system of Bird (1982) have also been reported to provide satisfactory resistance to various cotton pests. Likewise, progress has been continuously achieved from breeding work for earliness providing for escapes from late season pests, and for morphological characteristics conferring resistance primarily to sucking insects. Gains within this area have been well-documented, and reviews were given by Niles (1980); Bell, 1984; Ridgway (1984) and El-Zik and Thaxton (1989). Similarly, the use of biotechnology to develop cotton resistant to lepidopterous insects is making a headway (Perlak et al., 1990; Wilson et al., 1992), in particular the transgenic cotton with the Bt gene reviewed by Buehler (1993) and Wofford (1993). We understand updates of all these things shall be the subject of succeeding papers during this seminar.

Nevertheless, the attendant driving force for the continuing pursuit of genetic resistance development in cotton may be worth emphasizing. Setting aside efficiency in reducing damage from pests depending on the level of resistance present, the principal factor in always considering it with long-term solutions to cotton pest management problems is its amiability to the environment. This feature makes it outstandingly agreeable to advocates of chemical-free organic cotton production (ICAC, 1993b). This could be because complaints on health hazards and environment-related problems arising from pesticide handling, application and waste disposal are reduced or eliminated outright. In addition, worries of detrimental collateral effects to beneficials, including natural enemies of target pests, are obliterated.

With pest resistance integrated into the plant, growers are also freed from rigorous requirements on pesticide dosages as well as on method and timing of applications. In effect, elaborate techno-transfer activities on pest control are no longer required.

Expected with the use of built-in resistance is the subsequent decrease of expenditures for pest control. Resistant cultivars may require minimal, if any, application of additional control measures, and thus their associated costs, to achieve adequate control (Martin and Woodcock, 1983). The use of a hairy variety against leafhopper, *Amrasca biguttula*, for instance, reduces the number of chemical sprayings at early stages of the crop, consequently decreasing leafhopper control expenses (CRDI, 1993b; Pascua, 1993). This development is eagerly anticipated with Bt cotton as major cotton pests, where most of the control costs are channeled, are targeted.

Particularly for insect pest management, the employment of resistant cultivars may also subsequently help arrest the development of insecticide resistance of particular pests resulting from the recurrent usage of the same chemicals. Resistance development by major cotton pests to frequently used chemicals can be extremely damaging. Resistance was suspected to be one of the factors for the outbreak of the American bollworm, *Helicoverpa armigera*, in Pakistan in recent years causing substantial losses in cotton production (ICAC, 1994a, b). The use of genetic resistance, in concert with other strategies, is expected to play a contributory role in the management of such problems against pests (Xia, 1993).

With these matters in view, and in light of the continuing complexities of cotton pest management, research efforts geared towards improving the cotton plant’s capability to resist insect pests and diseases shall carry on. With the aid of biotechnology, which is increasingly in the forefront of plant genetic transformations, genetic resistance sources extending beyond the cotton species to other organisms will likely be more aggressively explored and utilized for the development of pest control strategies.

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Breeding for Resistance to Bollworms in Cotton with Particular Reference to India

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Introduction

Cotton is an important commercial crop of Australia and many countries of Asia, Africa, America and Europe. Production and productivity of cotton have increased over time in most of these countries. There is a need not only to sustain productivity but to improve it further to meet the demands of the future. The biggest constraint to productivity is the menace of pests and particularly bollworms. Sole reliance on chemical pesticides for management of bollworms has made some of them resistant, escalated production costs and adversely affected the environment. A search for alternatives is thus called for. Host plant resistance is one alternative. Host plant resistance offers the most important component in pest management strategy for sustainable cotton production (Jenkins, 1994).

Bollworms and their Distribution

The most important bollworms of cotton and their distribution in the world cotton growing countries are listed in Table 1. American bollworm/cotton bollworm/tobacco budworm which are found in all cotton growing countries are presently the most serious pests. Pink bollworm (*Pectinophora gossypiella*) is also distributed in most of the countries growing cotton. Spotted and spiny bollworms (*Earias* spp.) are limited to Asia and Africa. In India the bollworms which affect cotton are American bollworm, pink bollworm and spotted bollworm.

Losses Caused by Bollworms

Pests particularly bollworms, cause considerable damage to the cotton crop. While statistics of actual loss in global cotton production due to bollworms are not readily available, it is estimated to be in the range of 30 to 40 per cent. According to Basu (1988), data from three growing regions for two seasons showed that bollworms caused 49 per cent loss in yield in India.

Management of Bollworms

Chemical pesticides play a key role in management of bollworms today. Cotton growers of many countries spray pesticides 11-15 times to control bollworms. 30 per cent of the pesticides used in agriculture globally are used in cotton alone against *Helicoverpa* spp. in cotton (Lukefahr, 1981). This proportion must be much higher now because the damage due to *Helicoverpa* and pesticide consumption have increased since then.

World Pesticide Consumption (000 Tons)

1980	**1995**
1,590	2,235

In India, cotton, occupying only 5 per cent of the area, consumes 53 per cent of the pesticides used in the country. The indiscriminate use of pesticides has escalated the cost of cultivation, polluted the environment and most importantly made the insects resistant to insecticides in cotton growing niches of the world. Integrated pest management (IPM) which integrates varietal, cultural, biological and chemical methods of pest management is therefore gaining ground world over.

Host Plant Resistance

Host plant resistance (resistant variety) is the most important component of the integrated pest management strategy. Mechanisms conferring resistance are non-preference, antibiosis and escape potential (Painter, 1951).

Many morphological traits such as glabrous leaves, stem and bracts, red plant, fregobract, nectariless, yellow pollen and thick boll rind make plants less preferred (non-preference) by insects.

High gossypol, high condensed tannins and heliocides (H_1, H_2, H_3, H_4) impart resistance/tolerance to bollworms (antibiosis).

Plants with quick rate of flowering, short flowering period and quick rate of boll development leading to earliness escape the later bollworm attack (escape mechanism).

Table 1. Important Bollworms of Cotton and their Distribution

Common Name	Species	Countries
American Bollworm	*Helicoverpa armigera* (Hubney)	Africa, S. Europe, Turkey, Israel, Egypt, Sudan, India, Pakistan, S. E. Asia, Uzbekistan, Australia
Cotton Bollworm	*Helicoverpa zea* ((Boddie)	America
	Helicoverpa punctigera (Wallengren)	Australia
	Helicoverpa geletopoen (Dyar)	South America
Tobacco Budworm	*Heliothis virescens* (Fabricius)	America
Spotted Bollworm	*E. vitella*	India, China, S. E. Asia
Spiny Bollworm	*Earias insulana* (Boisd)	Egypt, Sudan, Madagascar, Arabian Peninsula, India, Pakistan, China, S.E. Asia
Pink Bollworm	*Pectinophora gossypiella*	All cotton growing countries, except Uzbekistan, Central America (Honduras, Guatemala, Nicaragua, Salvador, Costa Rica), parts of South America (Ecuador, Guyana and Queensland of Australia)

Gene Sources and their Utilization

A large number of accessions having one or more of the above attributes have been identified in the cultivated species and their wild races. Many more have been developed by hybridization or mutation. The effects of some of these lines on bollworm incidence are shown in Tables 2, 3 and 4.

A MAR and MIR genepool has been established in the USA

Table 2. Role of Morphological Characters Singly or in Combination on Incidence of Bollworms

Lines	Morphological Character(s)	Bolls Locule Infested, in %
1035	OL, FB	30.2
957	NL, FB	38.2
Roil 3	OL	58.2
1014	OL, NE	36.3
1001	NL, NE	44.4
Check	NL, NB	50.8
CD at 5%		7.8

Source: Singh, 1982
OL: Okra leaf; FB: Frego bract; NL: Nectariless
NL: Normal leaf; NB: Normal bract

Table 3. Role of Morphological Characters on Incidence of Bollworm

Character	Percent Bollworm Incidence	Character	Percent Bollworm Incidence
Red stem	32.5	Nectariless	28.8
Green stem	44.3	Nectaried	39.7
Okra leaf	38.2	Frego bract	32.8
Broad leaf	32.6	Normal bract	32.8

Source: Bhat and Basu, 1984

Table 4. Role of Morphological Characters on Egg Laying of *Helicoverpa*

Morphological Character	Percent Reduction in Egg Laying
Nectariless	64
Glabrous, nectariless	80

(El-Zik and Thaxton, 1989; Jenkins, 1989), India and other countries (Narayanan et al., 1990), AET-5 (Pedigree: *G. arboreum* x *G. thurberi* x *G. hirsutum*) isogenic lines for nectariless, smooth leaf and okra leaf developed at Texas, (Bhat et al., 1988) and MAR, PEE DEE, La HG lines (Narayanan et al., 1990) for Heliothis resistance as well as FORN (frego, okra, red and nectariless) may also be cited.

Lines with high content of secondary metabolites (gossypol, tannin, helocides) have been identified/developed.

Table 5 shows the effect of condensed tannin on larval growth of *Heliothis virescens*.

Tannins also have been found to confer tolerance to cottons in experiments conducted in India (Chart below). *G. arboreum*, the diploid cotton, has in general more tannin and other phenols compared to tetraploids. G. 27 particularly has more tannin among arboreums.

Extensive screening of germplasm carried out for the last 25 years in India at the research centers under All India Co-ordinated Cotton Improvement Project of Indian Council of Agricultural Research, showed no resistance to bollworm. However, a fair amount of resistance was found in lines mostly belonging to

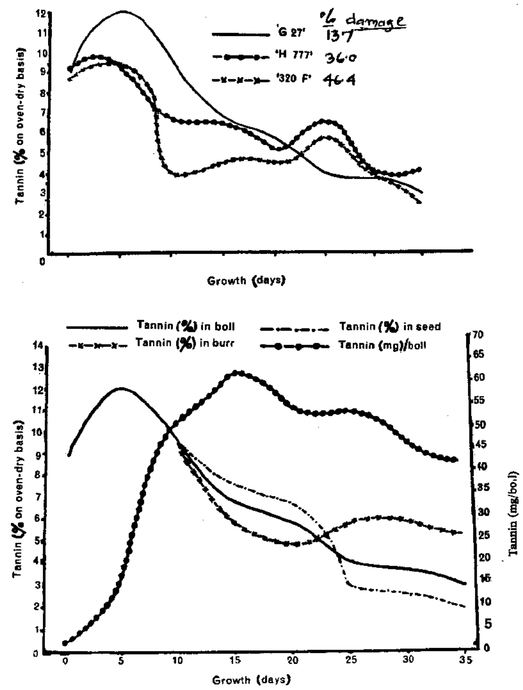

Fig. 1. *Top,* Changes in tannin content during growth of bolls in 3 cotton varieties; *bottom,* changes in tannin in boll, bur and seed in 'G 27' cotton along with tannin (mg)/boll at different stages of growth.
(Source Chakravarthy et al, 1981)

Gossypium arboreum (Jayswal and Sundaramurthy, 1992).

The diploid species *G. arboreum* and *G. herbaceum* have greater tolerance to bollworms compared to the tetraploid species *G. hirsutum* and *G. barbadense* and the change in species composition during the last two and a half decades in favor of tetraploids has aggravated the bollworm menace in India.

Wild Species

Several wild species have one or more of the morphological and biochemical attributes which confer resistance or tolerance to bollworms (Table 6).

The three wild species of Australia viz. *G. australe*, *G. sturtianum* and *G. bickii* have the unique characteristic of delayed morphogenesis of gossypol glands, having glands on the plant's body but none on the seed. This characteristic is being introduced from *G. sturtianum* into American upland using ovule culture (Altman et al., 1987). Commercial varieties in the USA have 0.3 to 1.2% gossypol which can be increased to 1.5%.

Table 5. Effect of Condensed Tannin on *H. virescens*

Tannin in Diet	Mean Larval Weight (mg.) After 7 days
0.0	266
0.1	207
0.2	45
0.3	20

Source: Lukefahr, 1981

Table 6. Wild Gene Sources for Resistance to Bollworms

Wild Gene Source	Contributing Characters
G. sturtianun, G. australe & *G. bickii*	Delayed morphogenesis of glands, high gossypol
G. anomalum	High gossypol
G. armourianum & *G. harkenesii*	Coducous bract
G. raimondii & *G. thurberi*	More gland
G. gossypiodes & *G. tomentosum*	Nectariless

A study conducted at Surat in India showed no bollworm damage (0%) in *G. aridum*, *G. anomalum* x *G. aridum*, *G. anomalum* x *G. somalense* (*G. thurberi* x *G. raimondii*) F_1 x *G. anomalum*.

The other wild species cross combinations recorded 20 to 30% bollworm damage as compared to 50% or more in Hybrid 6 and Hybrid 8 commercial hybrids (Anonymous, 1989). Successful introgression of genes has been made in some of the cross species and between wild species and cultivated species (Mehta et al., 1988):

G. arboreum x *G. anomalum* (A_2 x B_1)
x
G. tomentosum (AD)3
x
G. hirsutum (AD)1

Evolution of Commercial Cultivars

Early Maturing Cottons

Short season cottons have a distinct advantage as they escape the onslaught of the later and perhaps most damaging broods of bollworms, reflected in higher yield and better quality. Short season commercial varieties of cotton have been developed in many countries and some of them are listed in Table 7. They are very popular with farmers because they need comparatively fewer inputs and vacate the land early for a second crop, apart from reduced incidence of bollworms resulting in higher production. Texas A & M University in the USA has succeeded in developing early varieties known as Tamcots (Bird et al., 1968). In northern India, which is entirely irrigated, development of short season cottons in the early seventies totally replaced longer duration cottons and made it possible for farmers to grow wheat in winter. The advent of these varieties changed the entire cotton-wheat scenario of the region.

Table 8 shows the bollworm infestation in early commercial varieties of northern and central India vis-à-vis the long duration checks. The lower incidence of bollworm in early types is attributed to escape from late season pink bollworm and later broods of American bollworms. NHH-44, a medium staple hybrid, is now the most popular hybrid of central and south India by virtue of its earliness and tolerance to sucking pests and bollworms.

The morphological characters which contribute to bollworm resistance/tolerance have been incorporated in many commercial cotton varieties of the world (Table 9).

Table 7. Early Commercial Cultivars of a Few Cotton Growing Countries

Early Genotypes	Country	Reference
Tamcot Cottons, Stoneville 506, 586, etc.	USA	Lukefahr, 1981 and Jenkins et al., 1986
Siokras	Australia	Thompson, 1994
Tashkent 6, C4 727, Yulduz, C 90-70 etc.	Uzbekistan	Nazarov, 1995
LH 900, F 846, F414, LK 861, NHH 44, etc.	India	Basu, 1994

Table 8. Mean Bollworm Infestation (%) in Early and Long Duration (Check) Genotypes in India

Genotype	% Bollworm Infestation
F-414	32.7
J 205 (check)	37.6
Lh 900	40.8
F 286 (check)	52.2
SH 131	16.3
Pramukh (check)	23.6
NHH 44	16.0
H4 (check)	20.1

Source: Basu, 1988

Siokra, an okra leaf cotton, now accounts for 40-50 per cent of Australian production (Fitt, 1994). It is felt that incorporation of a glabrous gene in okra cottons will further increase resistance to *Helicoverpa* in Australia (Thompson, 1994). MECH hybrids with okra leaf and long staple cotton now occupy a substantial area in central and southern India. Abadhita, a derivative of a multi species cross is endowed with both antibiosis and antixenosis (non-preference) characteristics and is becoming popular with farmers for its lower bollworm damage. G 27, a diploid cotton of north India having a red plant body and high tannin confers resistance to bollworms (Fig. 1). Two nectariless Stoneville varieties occupy some acreage in the USA (Jenkins, 1989).

It is apparent that all the above three mechanisms of resistance, viz. non-preference, antibiosis and escape, have been utilized by cotton breeders the world over for the development of lines for further breeding as well as in commercial varieties conferring a fair amount of resistance/tolerance to bollworms.

It is, however, common knowledge that we are yet to achieve complete resistance to bollworms. Again some traits particularly morphological traits, while contributing to resistance to bollworms make the plant susceptible to other insect pests. An example is glabrous leaves which, while providing resistance to American bollworm, make it susceptible to leafhopper in India, Pakistan and many other countries. Many of the characters are also associated with low yield.

New Gene Sources

There is therefore a need to look for new gene sources. Two alien sources of bollworm control genes have been identified and are

Table 9. Commercial Cultivars with Morphological Traits Conferring Resistance to Bollworms

	Country	Morphological Trait
Stoneville 825	USA	Nectariless
Siokra	Australia	Okra
MECH Series	India	Okra
G 27 (*G. Arboreum*)	India	Red plant
Abadhita	India	Thick boll rind, small bract

being exploited, insect pathogenic microorganisms and plants and animal systems. The toxin gene of *Bacillus thuringenesis*, a soil bacterium which is toxic to all bollworms, has been incorporated into cotton through genetic engineering. The toxin gene is found in all the cells of the transformed cotton and the bollworm feeding on it is killed by the toxin's action on the midgut of the insect's stomach. Field tests of Bt cottons are in progress in the USA, South Africa and Australia. Delta and Pine Land Company and Monsanto conducted more than 100 field trials of several insect resistant Bt cotton varieties from Arizona to North Carolina during 1994 (Anonymous, 1995). Dr. Jenkins of Mississippi State University has been conducting Bt cotton trials for six years. Monsanto, USDA and Mississippi State University are satisfied with the effectiveness of Bt cotton in controlling bollworms particularly budworm and cotton bollworm. Fiber properties of transgenic lines are at par with the check (Table 10). Insect resistant lines derived from DP 5415 and DP 5690 will now be tested throughout the US cotton belt.

A large number of D & PL Bt cotton lines tested at Goondiwindi, Australia, were found to be highly effective. During 1993/94 a New South Wales Crop Test site showed very little damage to the transgenics, whereas conventional cotton suffered severe damage according to Dr. Henry Collins of D & PL (Anonymous 1995). In collaboration with Monsanto, CSIRO is introducing a array of Bt genes (Monsanto) into elite Australian varieties by conventional breeding and by direct transformation of Australian cultivars using *Agrobacterium* mediated transformation (Llewellyn et al. 1994). Bt cotton harvested in Marble Hall, South Africa, is said to be excellent (Anonymous, 1995). However, Bt cottons cannot be released for commercial cultivation unless they are cleared by all concerned federal agencies. In India progress has been made in cotton transformation with a Bt gene and regeneration of cotton from cell culture (Dongre et al., 1994). It is expected that resistance will develop to Bt genes sooner or later. It is essential therefore to develop strategies to delay development of resistance in insects to Bt transgenic cottons. The most promising strategy for resistance management is to maintain some percentage of completely susceptible plants within the cropping system (Roush, 1994).

A gene from cowpea encoding a protease inhibitor (Cowpea Trypsin Inhibitor or CPTI) when introduced into tobacco imparted resistance to tobacco budworm (*Helicoverpa virescens*). Laboratory tests indicated that CPTI is antimetabolic to a variety of pests in addition to tobacco budworm (Gatehouse et al., 1990). Cotton transgenic with CPTI gene is said to have been created (Kranti, personal communication).

Table 10. Fiber Properties of Transgenic Cotton Grown under Field Conditions

Cotton Strain	Fiber Micronaire	Fiber Length 2.5% span (mm)	Fiber Strength KNM/kg
TS1	4.3	31.6	200.0
TS2	4.2	32.2	209.7
TS3	4.4	31.5	206.6
TS4	4.3	32.1	206.0
C312 (Check)	4.2	31.4	203.7
LSD 0.05	0.23	0.71	NS

Genes of the Future

Heliothis Stunt Virus

These are extremely small viruses containing only three genes and attack the midgut of *Helicoverpa* (Hanzlik et al., 1994) and *Amylase* inhibitors. These are digestive enzyme inhibitors present in most legume seeds.

Insecticidal Proteins

Spiders, wasps and scorpions pray on insects and can kill them with venom containing insecticidal proteins. Only a few of these are toxic to mammals and hence can be used against bollworms.

Insect Neuropeptides

A large number of neuropepdtides have been identified and characterized. In view of the small size of the genes, it is easy to use the genes for genetic engineering to transform cotton for imparting resistance to bollworms. Insect neuropeptides provide exciting new approaches to insect management (Mena and Borkovee, 1989).

Azadirictin

The gene that generates azadirictin in neem, if incorporated into cotton, will retard growth, development and fecundity leading to death by interfering with nervous, reproductivity and digestive systems.

Conclusion

Considerable progress has been made in breeding for resistance to bollworms by conventional breeding approaches. There is a need to tap the immense genetic diversity available in national gene banks of countries for identification of resistance sources for utilization in breeding programs.

Transgenic cottons with resistant genes from unrelated organisms being developed through genetic engineering hold great promise. International cooperation in both conventional breeding and genetic engineering may hasten our efforts to combat the biggest menace of cotton.

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Current Strategy for Non-chemical Control of Cotton Pests in Egypt

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(Presented by Dr. Ahmed El-Gohary and Dr. Galal M. Moawad)

Egypt is a major producer and exporter of fine cotton. It produces about 50% of the total world production of extrafine cotton and its share in the international trade of ELS cotton exceeds 30%. Such accomplishments that give Egypt a comparative advantage should be maintained and promoted. However, creating and maintaining genetic sources of resistance to cotton pests are considered the most important goal of the Egyptian cotton breeding program.

Genetic resistance is one of the oldest methods of pest control. In the nineteenth century, plant resistance to insects and diseases was recognized. However, the deliberate development of pest resistant cultivars was not possible until after the rediscovery of Mendel's laws of heredity in the year 1900.

Genetic resistance to pests is of critical importance in cotton production. Losses due to pests in cotton cause significant reduction in yield and quality of lint and seed and increase production costs.

The Main Contributions of Genetic Improvement for Resistance to Cotton Pests in Egypt

Short-duration Cotton Germplasm

Breeding early-maturing cotton varieties has been considered a main objective in the Egyptian cotton breeding program. Earliness means shortening the time between planting and harvesting. There are many criteria to define earliness. In Egypt, the criterion used for earliness is the" Earliness Index " which is the percentage of weight of seedcotton harvested in the first picking to the total weight of seedcotton.

Many attempts have been made to introduce earliness in the background of the Egyptian cotton varieties. In fact, cotton started as perennial but transferred to annual types under natural selection. Recently, the growth period of the Egyptian cotton varieties was minimized to about 6.5 months on average. However, breeders at the Cotton Research Institute (CRI) succeeded in introducing new early cotton varieties which remain in the field for not more than 5.5 months. This result was achieved through intensive selection for earliness. The available genetic sources of earliness were

- The old Egyptian cotton varieties: Ashmouni, Menoufi, Dandara and Giza 72.
- Some early-maturing strains introduced by mutageneses.
- Some imported varieties belonging to either *G. barbadense* or *G. hirsutum*.
- Some early strains derived from intra & interspecific crosses through intensive selection for earliness.

The breeding efforts in Egypt succeeded in producing three early commercial cotton varieties in the 90s namely Giza 83 (LS cotton variety grown in Upper Egypt), Giza 84 (ELS cotton variety grown in the Northern part of the Delta) and Giza 85-Mobarak 93 (LS cotton variety grown in the Southern part of the Delta). These three varieties are characterized by a short duration growth period requiring only about 160-170 days from planting to harvesting in comparison to 190-200 days required by other commercial varieties. In addition, the CRI succeeded in introducing new strains characterized by a very short growth period. However, all these strains can mature within 160-165 days.

Early maturity in cotton has many advantages. One of the main goals to introduce early-maturing cotton varieties is to reduce losses from diseases and insects to its minimum. It is a source of escape from late season infestations, especially bollworms, whitefly, aphids and fungi.

Morphological Characteristics

Cotton geneticists and entomologists are increasing their efforts to find resistance to cotton pests through some morphological characters. In Egypt, basic and applied research carried out at several research stations, including diverse morphological genotypes, has contributed to our understanding the complex interrelationships of the cotton plant morphological characteristics and its most serious pests. However, the main morphological characters affecting cotton pests are okra-leaf, frego bract, smooth leaf, nectariless, high gossypol content and compact plant type which lead to pest resistance in various cases.

Recently, the CRI is carrying a breeding program to induce resistance to cotton pests from these morphological types to commercial cotton varieties while maintaining their economical characters through backcrossing. To reach this goal, various crosses between resistant plant types to cotton pests and commercial varieties were attempted followed by intensive selection.

Disease Resistance

Fusarium wilt was one of the earliest recognized cotton diseases in Egypt causing great damage to the cotton crop. This serious disease was the main cause for deterioration of many famous cotton cultivars in Egypt which were removed from cultivation. However, the CRI had succeeded in inducing resistance to fusarium wilt into the Egyptian commercial varieties.

The Egyptian resistant commercial cotton varieties were originated from crossing the high quality susceptible cotton varieties to the highly resistant varieties and strains to fusarium wilt organism. Strict selection was followed to maintain the economic value of the Egyptian commercial cotton varieties characterized by high resistance to such a disease. Such a significant contribution was due to the integrated research program carried out by both the CRI and the Plant Pathology Research Institute. However, growing resistant cultivars is the most effective defense against this disease.

Biotechnology and Cotton Pest Resistance

The Genetic Engineering Research Institute is carrying out a comprehensive study concerning cotton insect resistance, specially pink bollworm and leaf worm through genetic engineering.

This study is based on isolating the gene responsible for producing the toxic protein from *Bacillus thuringiensis* bacteria. The researchers were able to isolate such gene from Egyptian types of bacteria characterized with high toxicity against insects belonging to lepidoptera. There is a considerable integration between the CRI and Genetic Engineering Research Institute in this regard.

Pesticide Use and a New Strategy

Cotton leafworm, pink (PBW) and spiny (SBW) bollworms, whiteflies and aphids are considered the key pests of cotton in Egypt. The wide spread use of pesticides during the last few decades resulted in the occurrence of many problems which could be summarized in the following points:

- Increasing environmental pollution which affects humans, animals and plants in agricultural and urban ecosystems.
- Harmful and deleterious effects on natural enemies and beneficial organisms, which contribute in the occurrence of new pest species.
- Rapid development of resistant strains of pests to recommended pesticide types and dosages, which leads to the shortening of the life and duration of commonly used pesticides.

The Egyptian Ministry of Agriculture (MOA) succeeded, during the last 5 years, in controlling early season cotton pests through different approaches, with minimum use of the recommended pesticides. These approaches contribute much in increasing the population of natural enemies and reducing the infestation level of sucking pests, especially whitefly and aphids. Several encouraging phenomena in cotton's agroecosystem have been observed in the last few years. Among these phenomena are the following:

- Cotton leafworm infestations are coming under control, with a gradual decline in early season infestations within the last few years.
- Loss of cotton yield caused by bollworm infestations also has declined from about 20% to about 5-8%.
- The average number of insecticidal sprays per season were reduced to 2-3 sprays.
- The average yield of cotton per acre fluctuates, but in general it follows a higher level.

The Role of Bollworm Sex Pheromones

An economic injury level was established, for infestation of the cotton bolls by bollworms, at 5% infestation of green bolls in fields close to the villages. In early July, trained teams from the Ministry of Agriculture periodically inspect cotton fields for the level of bollworm infestation. Chemical control is not allowed in any village unless the economic injury level is attained. This procedure has helped minimizing pesticide sprays by delaying unnecessary earlier spraying. In general it has reduced the total cotton area treated with pesticides. Wherever possible, pesticides with selective action are chosen including hormone analogues and pheromones.

There are many techniques of using pheromones as control agents but the one which has been employed most is the disruption or confusion technique. This technique relies on the application of pheromones to a wide area, using one of the commercially available formulations. Different methods of pheromone application are

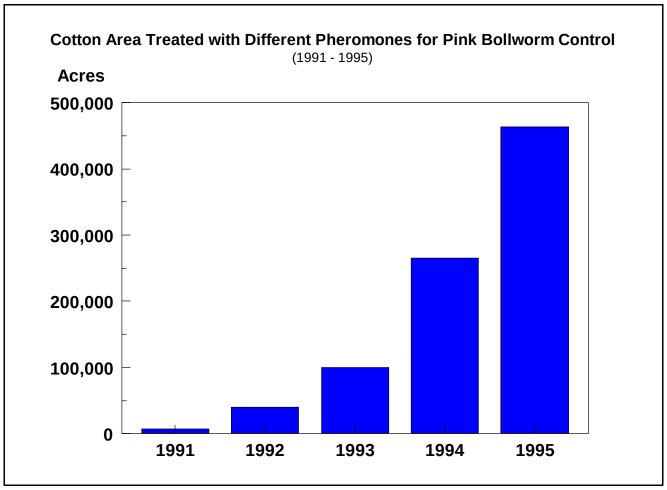
- Pheromones are dispensed into the atmosphere as a "fog", in which male insects cannot locate the plumes of natural pheromone being produced by females. Hence, they cannot find the females and mate with them.
- Pheromones can also be dispensed as thousands of discrete point sources which outnumber the females and attract the males, thus preventing them from finding the females.
- The mass trapping technique in which sufficient pheromone baited traps are placed in the crop to catch a very high number of available males and hence prevent females from being mated.
- A more recent technique is the lure and kill (or attracticide strategy) in which the pheromone, mixed with a very small quantity of conventional pesticide, is dispensed in the field. The pheromone then attracts the insect to the insecticide which kills it or makes it incapable of mating.

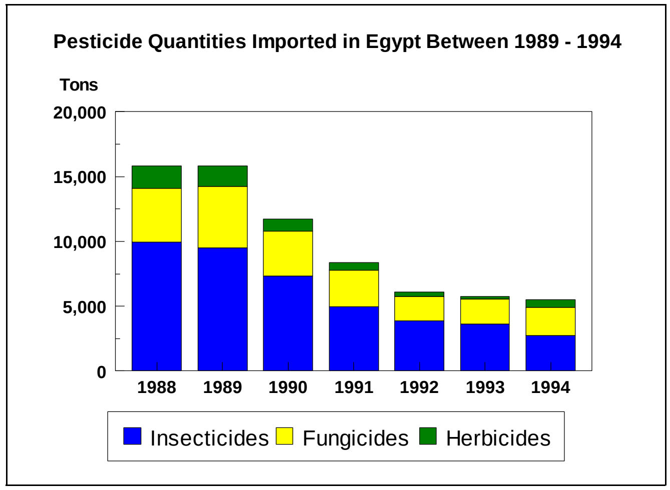
However, all these techniques can only be effective when applied as a protective control. They must be applied early in the season when insect numbers are low and thus prevent a build-up of the population. However, when insect numbers rise to economically important levels, conventional pesticides must then be used to control the insect. Trials using all these techniques have been carried out in Egypt in an attempt to find alternative methods to conventional pesticides to control cotton insect pests. The Ministry of Agriculture concentrated on distributing sex pheromone traps for leafworm, pink and spiny bollworms all over the year to reduce the population of these pests.

The results of a major research program on the control of bollworms have shown that 4-5 pheromone sprays can successfully control the pink bollworm. This represents the most advanced of the current new developments and could be used to supersede chemical control of this pest in the near future. Figure 1 shows the increase in cotton areas treated with different pheromome formulations to control pink bollworm during the last 5 years.

In general, the use of non-chemical control techniques, specially sex pheromones, proved to be a major success in the control of cotton pests and in reducing the use of chemical pesticides in Egyptian agriculture. Figure 2 shows the decrease in the amount of imported pesticides during the last 5 year period (1989-1994). The advantages of this new approach are as follows:

- Avoid the well known problem of pest resistance to chemicals already encountered in many parts of the world.
- Prevent the occurrence of new pests which can arise through pesticide misuse.
- Take maximum advantage of beneficial insects and para-





sites in pest control which can in turn reduce the need for pest control practices.

- Protect farmers and the environment from the toxic effects of pesticide use.

Research in Cotton Resistance to Insects in China

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Cotton is the most important cash crop in China, though it suffers serious damage from different kinds of insect pests throughout its growing period. More than 300 species of insect pests have been recorded to attack cotton in China, of which around 30 are economically important with four being the key insect pests: cotton aphid *(Aphis gossypii* Glover), cotton bollworm *(Helicoverpa armigera* Hubner), pink bollworm *(Pectinophora gossypiella* Saunders) and spider mites *(Tetranychus* spp.*)*. Annual losses caused by damage from insect pests are estimated to be over 15% of potential cotton yield.

Breeding and growing insect-resistant cultivars are considered to be the most economic and effective approach to control cotton insect pests. Since the founding of the People's Republic of China in 1949, significant achievements have been made in studies and utilization of resistance of cotton to insect pests in China, particularly during the past 15 years (1981-1995). In the present paper, advances in studies on resistance of cotton to insect pests during the past 15 years are reviewed, covering six aspects: (1) identification of sources with insect-resistance, (2) techniques for evaluation of insect-resistance, (3) mechanisms of insect-resistance, (4) genetics of insect-resistance, (5) breeding for insect-resistance, and (6) utilization of insect-resistance.

Identification of Sources with Insect-resistance

Identifying sources of insect-resistance is the basis for breeding resistance to cotton insects and collection of germplasm is the first step. So far, 6,190 accessions of cotton germplasm have been collected, including 5,826 accessions of cultivated species, 350 accessions of semi-wild species and 15 accessions of wild species.

1,044 accessions of germplasm resistant to cotton aphids have been identified with 306 reaching the level of resistant and highly resistant (accounting for 29.3%), 1,020 accessions were found resistant to cotton bollworm with 392 at the level of resistant and highly resistant (38.4%), 1,010 accessions were found resistant to pink bollworm with 85 at the level of resistant and highly resistant (8.4%), and 490 accessions were found resistant to spider mites with 88 at the level of resistant and highly resistant (18.0%). In addition, 989 accessions of germplasm have been simultaneously evaluated for resistance to the three insect species (cotton aphid, cotton bollworm and pink bollworm), and 490 accessions tried for four insect species (cotton aphid, cotton bollworm, pink bollworm and spider mites).

Techniques for Evaluation of Insect-resistance

Techniques for evaluation of insect-resistance, being quick in speed, high in efficiency and easy in operation, are crucial not only for identification but also for evaluating insect-resistant materials. Standard biological methods for identification of cotton materials with insect-resistance have been established for the four key insect pests. Thus, the field and the petri-dish methods are commonly applied for cotton aphid; the field and the cage methods for cotton and pink bollworms; and the field, the cage and the life table methods for spider mites. Besides, some biochemical methods for identification of cotton material with insect-resistance have also been studied in recent years. Thus, sugars and nitrogen content and the ratio of carbohydrate to nitrogen in the cotton leaf at seedling are negatively correlated with the level of resistance to cotton aphid and spider mites, while the amount of proteinase inhibitors is positively correlated with the level of resistance to both species.

Mechanisms of Insect-resistance

Mechanisms of cotton resistance to the four key insect pests have been extensively studied; the two major groupings are morphological and biochemical resistance.

Morphological Resistance

It was found that nectariless is resistant to cotton aphid, cotton bollworm and pink bollworm, but susceptible to spider mites; hairiness is resistant to cotton aphid and cotton bollworm, but susceptible to pink bollworm and spider mites; glabrous is resistant to cotton and pink bollworm, but susceptible to cotton aphid; and frego bract and okra leaf are resistant to cotton and pink bollworms.

Biochemical Mechanisms

High content of gossypol and tannin shows resistance to all four key insect pests, and high content of flavonoid and sugars exerts resistance to spider mites.

Genetics of Insect-resistance

The genetic nature of resistance of cotton to all four key insects, has been researched to some extent. Thus, the hairiness in leaf veins related to cotton aphid-resistance is controlled by additive gene effects. The density of nectar related to pink bollworm-resistance is regulated by additive and dominant gene effects; the content of water in boll shell and that of starch in seed related to pink bollworm-resistance are also governed by additive gene effects; and the content of tannin in seed related to pink bollworm-resistance is controlled by non-additive gene effects. Most traits related to spider mite-resistance are exerted by additive gene effects.

Breeding for Insect-resistance

Conventional Breeding

During the period of 1981-1985, breeding for resistance dealt with only one single insect through morphological resistance, and brought about only 6 resistant lines. In 1986-1990, breeding for resistance expanded to multiple insect pests with morphological or biochemical resistance, and brought about more than 25 resistant lines. Since 1991, breeding for resistance has covered multiple resistance (insect pests and diseases) with combination of morphological and biochemical resistance, and brought about 3 widely-released resistant cultivars, CRI21, HM101 and CM99.

Genetic Engineering

With the rapid development of biotechnology, genetic engineering has been applied for breeding resistance to cotton insect pests (particularly to cotton and pink bollworm) since the early 1990s. Thus, the toxin protein gene of *Bacillus thuringiensis* (Bt) was successfully inserted and expressed into a single cotton plant in 1991. The Bt gene was transferred into commercial cultivars with several transgenic Bt lines developed during 1992-1994. Two foreign genes of Bt and cowpeas trypsin inhibitor (CPTI) were inserted into a single cotton plant during 1995. Five transgenic Bt lines were tested in some major cotton-producing provinces for evaluation of their efficacy for control of cotton bollworm and their role in cotton IPM during 1994-1995.

Utilization of Insect-resistance

During 1981-1992, several lines resistant to the key insect pests were released for production. Since 1993 three cultivars resistant to the key insect pests have been widely utilized in production, CRI21, HM101 and CM99. The main characters of CRI21 are thick leaf, high content of gossypol and tannin, and resistance to fusarium wilt, cotton aphid and cotton bollworm with lint yield of 1,029kg/ha (higher by 8% in comparison to its check cultivar), fiber length 29.9mm, fiber strength 20.5g/tex and micronaire 4.6. The main characters of HM101 are glabrous leaf, high content of gossypol and tannin and resistance to pink bollworm, cotton bollworm and cotton aphid with lint yield of 1,055kg/ha (higher by 15% in comparison to its check cultivar), fiber length 29.6mm, fiber strength 20.1g/tex and micronaire 4.4. The main characters of CM99 are hairiness, high gossypol and tannin content and resistance to fusarium wilt and cotton aphid with lint yield of 1,125kg/ha (higher by 12% in comparison to the check cultivar), fiber length 29.3mm, fiber strength 21.7g/tex and micronaire 4.5.

During 1993-1995, the accumulated area planted with those three insect-resistant cultivars reached 0.3 million hectares with 2-3 sprays saved. The net economic return from cultivation of those insect-resistant cultivars was estimated to be 400 million yuan (RMB) or US$ 50 million.

In conclusion, significant progress has been made in studies and utilization of host plant resistance to the four key cotton insect pests in China during the past 15 years. It is well recognized that breeding for insect-resistance is essential for sustainable cotton production in China and development of insect-resistant cultivars is a great challenge for our cotton production in the next decade. For prospective research, multiple resistance is needed. Towards such end, multi-disciplines, biotechnology and basic research must be strengthened. For prospective application, host plant resistance should be fitted with cotton IPM systems in order for multi-profits to be gained.

Prospects of Utilizing Non-Species Genes in Cotton in Developing Countries

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Introduction

World Bank projections suggest that there will be a continuing increase in demand for cotton but there is unlikely to be any major increase in the area planted to cotton, which has not changed substantially since 1955. Cotton faces increasing competition for land, labor and other inputs as a result of economic factors and of the demand for increased food production. There is also increasing concern regarding the environmental impact of excessive use of chemicals in cotton production. Thus the increase in production required to meet the increased demand will have to come mainly from increased yields. This will entail the development of higher yielding, earlier maturing varieties with a high water utilization efficiency and reduced dependence on pesticides while maintaining the quality required by the textile industry.

There are three fundamental requirements for successful variety improvement: clearly defined objectives, adequate genetic variability and appropriate means of testing and evaluation. The development of new varieties also takes time and is stimulated by financial rewards in the form of higher returns from the new variety. More breeding programs have failed as a result of poorly defined objectives than from inadequate genetic variability. Nonetheless, plant breeders are continually seeking new sources of variability, traditionally from intra or interspecies hybridization. Biotechnology is a new tool which provides a means for plant breeders to expand genetic variability in ways never before possible and of tailoring varieties to their environment with less dependence on agro-chemicals in a more sustainable agricultural system. However, it is no more than a tool for plant breeders and needs to be considered in terms of its possible contribution to traditional plant breeding and not as a breeding system in itself.

There is a widespread perception in developing countries that competitiveness in agricultural markets and the solution to specific problems such as the development of resistance to diseases and insect pests in crop plants will depend on the incorporation of advanced technology. This could be an important factor in ensuring continued yield increases, in improving the economic competitiveness of cotton and in minimizing the environmental impact of cotton production. Consequently many countries have established policies and programs in biotechnology, often with the support of international development agencies such as the World Bank. However, the introduction of biotechnology to developing countries needs to be viewed in relation to constraints which may exist in resources, institutions and policies which may be acute in specific cases. Short term benefits will be limited by several factors which will be considered in this paper.

Agricultural Biotechnology

Biotechnology includes everything from mutation breeding, the long established commercial utilization of microbes such as nitrogen fixing rhizobia in legumes, and the relatively straightforward and inexpensive procedures of tissue culture to advanced applications of molecular biology, including genetic engineering. These techniques provide powerful tools for agricultural research in general and for variety improvement in particular.

Genes, which are composed of DNA, determine the appearance and performance of all living organisms. Each gene comes in more than one form, thus determining differences between individuals of the same species. They are arranged along DNA strands called chromosomes and during sexual reproduction, each parent contributes half of these chromosomes. New world cotton species have 52 chromosomes in 26 pairs, half from the male parent and half from the female parent. Sexual reproduction enables the reordering of the complement of genes that is necessary for adaptation of the species to changes in its environment (Trollinder et al., 1995).

Sexual reproduction in cotton occurs in flowers that contain both the male and female reproductive organs. Thus unless pollen from an outside source is introduced into the flower by insect or human intervention, the extent of reordering of the genes is strictly limited. Plant breeders attempt to widen the variability in order to develop improved varieties by cross pollination, using the male portion of one plant to fertilize a second plant. However, the chromosome complements of the two parents may be so different that fertilization is impossible, leading to infertile offspring. The trait desired may not be present in the variety being improved or its compatible relatives, thus limiting the ability of the breeder to achieve the desired improvements (Op. Cit.).

Scientists have discovered that genes are divided into two sections, the first, known as the coding sequence, determines what product is to be made and the second, known as the promoter, determines when, where and how much to make. Scientists have also developed techniques for cutting genes from one DNA strand in one organism and pasting them to a DNA strand in another organism. The product of this operation is known as a transgenic organism. They have also developed techniques to transfer the coding sequence from one gene and the promoter sequence from another. This enables molecular scientists not only to shuffle genes between incompatible organisms but also to determine when and where the new product is to be made in the transgenic organism and to enhance how much is made. In the case of Bt cotton, the bacterial gene from *Bacillus thuringiensis* was isolated and a promoter from a virus attached to the coding sequence. The promoter acts in all parts of the plant. In cotton, an intermediary, *Agrobacterium tumefaciens*, is used to facilitate the transfer of the DNA into the cotton cells (Op. Cit.).

New techniques in recombinant DNA technology, monoclonal antibody production and cell tissue culture are the components of modern biotechnology and, in combination, form the basis of genetic engineering in microbes, plants and animals (Persley, 1991). Recombinant DNA technology comprises the series of techniques indicated above which permit the manipulation of DNA. Monoclonal antibodies permit rapid detection of specific proteins produced by the cells and form the basis of specific diagnostic tools which could be of value in developing countries in such areas as quarantine of living organisms and diagnosis of plant and animal diseases. Cell and tissue culture permit the rapid propagation of genetically engineered cells.

The key components of genetic engineering are the identification and isolation of suitable genes to transfer, delivery systems to introduce the desired genes into the recipient cells and expression of the new genetic information in the recipient cells. Rapid progress is being made in developing and refining techniques for genes to be transferred from one species to another and for them to express themselves in the new species. However, there are major limitations in the identification of genes that will confer agriculturally useful traits when transferred between species with appropriate molecular controls.

Historical Background

During the 1950s, high hopes were held out for mutation breeding as a means of widening genetic variability in order to improve crop varieties. Programs were developed in a number of countries with the support of the Food and Agricultural Organization and the International Atomic Energy Authority for work on crops such as rice. The results of these efforts were disappointing. In North Carolina, an extensive program was initiated on mutation breeding in groundnuts. This resulted in the development of a rosette virus resistant groundnut variety which was one of the few successes in mutation breeding but similar results could probably have been achieved with far less effort through the exploitation of available natural variability.

A great deal of the pioneering work on tissue culture was conducted on tobacco, *Nicotiana tabacum*, which lends itself to this technology and has the added advantage that it can be propagated either vegetatively or sexually. Tissue culture was used successfully by Wauk in Australia in developing blue mould resistant tobacco cultivars. Haploid plants were produced from pollen mother cells of interspecific hybrids which had undergone several generations of backcrossing to *N. tabacum*. Homozygous lines were then developed for field testing without a protracted period of self fertilization, through chromosome doubling with colchicine. This technique was combined with mutation breeding in an effort to develop black shank resistance, using irradiated pollen mother cells to produce the haploid pollenlings. Large numbers of plantlets could be screened rapidly for resistance and promising individuals could undergo chromosome doubling and further testing. However, although this program had promise, it was terminated before it yielded any practical results.

A great deal of the pioneering work on tissue culture on cotton has been conducted in public institutions but the commercial application of these and more advanced techniques of genetic engineering have been largely in the hands of private, commercial companies. The results have been the development of transgenic cotton varieties which carry the genes from *Bacillus thuringiensis*, enabling them to produce their own endotoxins against certain lepidopterous insects, notable the cotton bollworm, *Heliothis/Helicoverpa* spp. and transgenic varieties that are resistant to a wider range of herbicides, facilitating chemical control of weeds that are resistant to the range of herbicides traditionally used on cotton. These transgenic cotton varieties have undergone extensive testing and are being increased for imminent release.

A number of countries, including Argentina, Brazil, China, Congo, India, Indonesia, Kenya, Korea, Malaysia, Mexico, Niger, Nigeria, Pakistan, Portugal, Russia, Rwanda, Senegal, Sri Lanka, Turkey, Uzbekistan and Zaire have biotechnology programs, in many cases supported by the World Bank. However, many developing countries have limited scientific infrastructure, human resources, technology delivery systems and markets, limiting their capacity to translate the development or importation of biotechnology products to farm level benefits (Anon, 1995). Data on field trials of genetically engineered crops show that while the first release experiments were conducted in Europe, releases are currently dominated by Canada and the USA (Beck and Ulrich, 1993).

Biotechnology Applications in Cotton

The main lines of research being followed are the development of insect and/or herbicide resistant, genetically transformed varieties. It is unlikely that herbicide resistant varieties will play any role in countries that rely mainly on mechanical and hand cultivation for weed control but Bt cotton could play an important role in cotton production in many countries, particularly those with pyrethroid resistant *Heliothis/Helicoverpa* populations, provided the technical, financial and legal problems associated with these varieties can be resolved.

Several varieties are being increased in the USA, pending registration that carry the Bt gene from *Bacillus thuringiensis*, rendering them toxic to Lepidopterous insects. Good results have been obtained with these varieties in minimizing the damage caused by cotton bollworm, *Helicoverpa zea*, tobacco budworm, *Heliothis virescens* and pink bollworm, *Pectinophora gossypiella* (Herzog, 1995). Currently, additional Bt genes are being added or "Pyramided" to increase the number of endotoxins produced by the plant in order to delay the development of Bt resistant insect populations. These varieties with improved resistance are in an advanced stage of development and are expected to be released during the next few years.

Varieties are available with tolerance to Glyphosate (Roundup) and to Bromoxynil (Buctril) and varieties with tolerance to sulfonylurea based herbicides (Grooms, 1992) are likely to be available in the USA within the next few years. In areas where the standard cotton herbicides have been used for many years, the spectrum of weed species has changed as more resistant, previously minor species have become dominant. The herbicide resistant, transgenic cotton varieties make it possible to use over-the-top applications of a wider range of herbicides that were previously unavailable for cotton, to control these persistent weeds (Wilcut, 1995).

Other areas where biotechnology could play a role in cotton improvement are virus control, the development of cottons with naturally colored lint, salt tolerance and in the development of male sterility for hybrid varieties. There are also conjectures that fiber properties could be modified to more nearly meet spinning mill requirements or to introduce new properties such as a plastic core. However, so far the only successes with transgenic varieties have been in the transfer of individual genes. The technology for the transfer of blocks of genes that would be necessary to modify fiber properties has not been developed yet.

As early as 1986, tobacco mosaic virus resistance was conferred on tobacco and tomato plants by introducing a gene encoding the coat protein of the virus, by transformation. Later tests demonstrated that the transgenic tomato varieties had a high level of resistance under field conditions (Beachy and Fauquet, 1989). Subsequently other research groups used a similar approach to protect plants from alfalfa mosaic virus, potato virus X, cucumber mosaic virus and tobacco rattle virus. Apparently, resistance against a range of viruses could be achieved by introducing a gene that encodes a coat protein identical to or related to that of the virus against which resistance is desired. In some way, the presence of the coat protein gene prevents the virus from infecting the plant (Persley, 1991). The implications of this for the possible control of leaf curl virus in Pakistan and India and of blue leaf virus in Argentina are worthy of further study.

Naturally pigmented cottons have been developed and selected over thousands of years by indigenous farming communities in Peru. These lines have been used in the development of colored varieties in green, brown, red, and ivory by BC Cotton Inc. in California. However, in 1991, the non-government organization Sociedad de Investigación de la Ciencia, Cultura y Arte Norteño led a move to prevent the export of native cottons without the consent of the regional native cotton peasant farmer organizations. Calgene, Agrecetus and the Russian Academy of Technological Science are applying genetic engineering to develop blue cotton (Fox, 1993).

Leemans et al. (1992) described a technique for targeting the expression of chimaeric ribonuclease genes such as barnase from *Bacillus amyloliquefaciens* to the tapetum of immature anthers of transgenic plants, using promoter sequences with extreme specificity to the tapetal cells of immature anthers from the tobacco gene TA29. Ribonuclease activity destroys the tapetal layer and stable male sterile plants are produced. Fertility is restored in crosses with female pollinators, expressing a chimaeric construct containing the ribonuclease inhibitor gene (barstar) fused to the tapetum specific TA29 promoter. This technique has been applied to several crop plants including cotton. Future applications of this hybrid system could reduce costs, increase stability of hybrid seed production, increase speed of hybrid breeding and extend the germplasm pool.

Scientists in China have developed techniques for the selection of salt tolerance involving tissue culture. Repeated selection and sub-culture of cotyledon, hypocotyl and radicle explants from the two varieties Lumian 6 and Lumian 1024 resulted in the selection of subcultures with increased salt tolerance (Wang et al., 1991). Techniques have been developed in Uzbekistan to utilize proteins as indicators of salt tolerance (Shadmanov and Igamberdieva, 1991).

Technical Implications

Effective utilization of biotechnology is dependent on strong conventional research, in particular, a strong conventional plant breeding program. The number of varieties that will accept transformation is very limited. Thus having developed a transgenic variety, it is necessary to transfer the desired character into otherwise adapted commercial varieties. As an example, Verticillium wilt is one of the major problems in Central Asia but the available transgenic Bt cotton varieties are susceptible. An extended period of conventional breeding would be necessary to transfer the Bt gene into a locally adapted, wilt tolerant variety.

Similarly, Bt cotton can only be successfully grown as part of an effective integrated pest management program. Varieties that produce their own endotoxins against Lepidoptera exert continuous selection pressure for resistance in the insect population and unless precautions are taken, resistance will develop within two to three years. In laboratory feeding trials with pink bollworm, *Pectinophora gossypiella* resistance began to become apparent after only three generations (Bartlett, 1995). This renders not only the transgenic variety ineffective but also insecticidal sprays based on *Bacillus thuringiensis*. The technical problems related to prolonging the life of Bt cotton call for diligent application of other IPM strategies and of strategies specific to Bt cotton (Deaton, 1955). Successful introduction of Bt cotton into the agricultural system would, therefore, depend on a region wide approach and on active involvement of the pesticide industry, the seed companies, the research institutes, the extension agents, the crop consultants (where applicable) and the growers.

Limitations in the research services and in the extension services in developing countries, in particular with regard to IPM among smallholder producers, are likely to be major obstacles to the effective utilization of biotechnology in many countries.

Financial Implications

Financial returns from the development and introduction of transgenic cotton would be expected by (Meredith, 1995):

- The originating company;
- The plant breeders who develop adapted commercial varieties from the Bt cotton breeding lines;
- The organization responsible for seed multiplication;
- The organization responsible for seed distribution.

Ultimately these costs would come into the cost of seed to the grower. How this could be accomplished in developing countries remains a difficult question since the cost of seed is likely to be too high for smallholders unless the technology is routed through government breeding programs under an aid program (Gillham et al., 1995).

In the USA, Monsanto, the developers of Bt cotton, has proposed that growers pay a registration fee of $30 per acre. A certificate would then be issued for the purchase of the appropriate quantity of seed from the seed dealer to plant the registered area. Safeguards would be necessary in the event of replanting and to ensure that growers do not retain their own seed for the following year. Under this proposal, in developing crop budgets, the cost of seed would become a fixed cost and no longer a variable cost. Thus in addition to training the players in the pesticide industry, the seed companies, the research institutes, extension agents, the crop consultants (where applicable) and growers in the complexities of growing Bt cotton, it would also be necessary to train the bankers who provide the growers with credit (Op. Cit.).

Patent Issues

Private bioscience companies are the main investors in research and development on biotechnology. The incentive for this investment, mainly in industrialized countries but also in some developing countries, has come from the extension of legislation to protect intellectual property rights to include living organisms, leading to the expectation of product benefits in the knowledge that competitiveness in agricultural markets will increasingly depend on advanced technology. The main disadvantage of this legislation is that it gives proprietary protection to living organisms and also to biotechnology procedures.

In the USA, broad patents have been taken out on all transgenic cotton varieties and of the procedures required for their development. There are concerns that academics will not work on genetic engineering in cotton for fear of transgressing this broad patent (Mestel, 1994). Developing countries may also be unwilling to allow access to cotton genetic resources if a US company is seen to have a monopoly on the use of cotton in biotechnology (Shand, 1993). Broad patents could have a negative impact on crop improvement in the future and could lead to worldwide monopolies. Some of these patents have been appealed on the grounds that they are disincentives to continued research on biotechnolgy by other organizations, including universities. The matter will take time to resolve and until it is, the patents will remain valid.

The absence of legislation to protect intellectual property in many developing countries is a disincentive for private investment in research and development and for transfer of technology agreements.

Biosafety

Consideration has been given to the fate and effect of introduced genes and gene products in transgenic plants, particularly with regard to the transfer to weedy relatives and the health risks associated with products such as *Bacillus thuringiensis* delta endotoxin. Tests provided valuable information on which to evaluate the potential environmental impacts of more extensive field testing and commercial introduction of genetically engineered plants (Fuchs et al., 1991). The outcome of these studies suggests that valuable agricultural products that are being generated through genetic engineering can and will be safely and successfully incorporated into agricultural systems, that the products will receive fair and reasonable regulatory scrutiny generated and that the products derived from these plants will be accepted by the public. Concern has also been expressed that large scale introduction of transgenic varieties of African crops could have adverse effects on the biological diversity of these crops and their relatives and could carry risks of ecological damage (Visser et al., 1993).

The regulatory climate governing the release of the products of biotechnology is a major policy issue (Persley, 1991). Many countries have existing legislation that is sufficient to regulate the use of most agricultural products likely to emerge from biotechnology. However, new guidelines need to be framed to cover the handling of genetically engineered organisms at the experimental stage and for the assessment of any risk associated with the release of genetically engineered organisms into the environment. Several reports suggest that the benefits of new technologies outweigh the risks but all countries need to establish functioning national review bodies and institutional biosafety committees and to develop guidelines to monitor and regulate the application of biotechnology (Op. Cit.).

Biotechnology Policy Options

Specific measures are required by individual countries to assess the opportunities for advancement through biotechnology and to incorporate this analysis into their research priorities and strategies, resource allocation and enabling policies. Several countries have already established fora to bring together universities, non government organizations, consumer groups and the private sector in order to promote a broad institutional base to the research effort and widespread acceptance of the products of biotechnology (Anon, 1995).

International development agencies have three policy options in facilitating the application of biotechnology in developing countries (Persley 1991):

- Public sector support would support biotechnology in association with other support for agricultural research, including training and research facilities. Little change would be necessary from the current practices in support of agricultural research (Op. Cit.).
- Public/private sector support would provide support for biotechnology at public sector institutions, including support for collaborative activities in either the public or private sector. Private companies would have the opportunity to participate on a contract research basis or on the basis that the project may confer commercial advantages that are not directly linked to the project (Op. Cit.).
- Commercial joint ventures which would facilitate the establishment of commercially viable joint ventures between public and private sector agencies in both industrialized and developing countries. Joint ventures could facilitate both the development of new products and processes and the business system necessary for delivery of these products to end users in the Third World (Op. Cit.).

Technical assistance and special facilities may be required for the development and implementation of biosafety procedures (Persley et al., 1992). Effective regulatory processes are essential in countries where projects support the use of molecular biology or the import and field testing of transgenic products (Anon, 1995).

Biotechnology Advice and Information

Individual countries need to have access to impartial advice on how to integrate biotechnology into existing research programs. The first priority is to identify current problems where biotechnology could be helpful and where significant time or other resource savings could be achieved over more conventional approaches. A case in point could be the use of tissue culture in inter-specific hybridization programs such as the tobacco breeding described above. Information is also required on institutional and management arrangements, policy issues such as regulatory requirements and intellectual property rights, and early warning systems on potential negative effects of biotechnology, either on individual commodities or countries. Research networks could make significant contributions to the solution of regional problems through biotechnology.

The recent international cotton workshop in Egypt that wound up the World Bank study of cotton production prospects for the next decade discussed the need for increased communication between scientists in different countries in such fields as plant breeding and integrated crop management. The development of modern scientific technology could benefit from the exchange of information on a regional basis through the utilization of the modern communications technology of electronic mail. The World Bank is investigating the establishment of such a system in Central Asia in connection with water conservation and the Aral Sea.

The Technical Information Section of the ICAC and the French research organization CIRAD-CA are ideally placed to aid in the development of these networks, using existing facilities. However, in order to be successful, they need strong, ongoing support from individual countries and regions. The information they disseminate is only as good as the information they receive. Every encouragement should be given to these organizations from individual countries, from the existing regional networks and from the organizers of the World Cotton Research Conferences to identify and develop the requirements for information services.

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Field Performance of Bt Cotton

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(Presented by Thomas Luehder, Delta and Pine Land, China (Mainland))

Delta and Pine Land Company has been working with Bt cotton since 1991. We are working collaboratively with Monsanto on a gene that has been trademarked as Bollgard™ in the U.S. and as Ingard™ in Australia. The gene encodes for the protein *Bacillus thuringiensis.* Cotton with the Bollgard™ gene provides in-plant, season-long control of many lepidopterous pests. The primary target insects of this gene include: American bollworm *(Helicoverpa armigera)*, tobacco budworm *(Heliothis virescens*), cotton bollworm *(Helicoverpa zea)* and pink bollworm (*Pectinophora gossypiella*).

Delta and Pine Land Company has backcrossed the Bollgard™ gene into commercially popular Deltapine varieties. Two cultivars are close to market in the U.S. The varieties are designated as NuCOTN 33^B and NuCOTN 35^B. DP5415 was the recurrent parent used in developing NuCOTN 33^B, while DP 5690 was used to develop NuCOTN 35^B.

Deltapine conducted its first wide scale field tests with these two NuCOTN cultivars in 1994. The two cultivars were compared against their recurrent parental varieties in 32 strip trials across the U.S. cotton belt from North Carolina to Texas. The number of trials per state were as follows: North Carolina 3, South Carolina 4, Georgia 5, Alabama 4, Tennessee 1, Missouri 1, Mississippi 5, Arkansas 2, Louisiana 5, and Texas 2.

The experimental unit ranged in size from 4 to 6 rows with the rows being between 180 to 365 meters in length. For statistical analysis, locations were used as replicates. There were three subplot treatments of interest for this presentation:

- The recurrent parent without the Bollgard™ gene with conventional lepidopteran control;
- The recurrent parent without Bollgard™ with no sprayed lepidopteran control;
- NuCONT 33^B and NuCOTN 35^B with no lepidopteran control.

All plots received the same pest management applications except for lepidopteran control.

Planting dates varied according to region, but were slightly later than normal because the planting seed was generated at a winter nursery. Planting dates averaged May 4 with a range from April 17 to May 16. Seedling vigor ratings were taken within 10 days of emergence. Plant map data were collected. Varieties without the Bollgard™ gene were scouted and treated with insecticides for worms as needed. Yields were taken from either the center two or four rows and weighed with field scales. A minimum of 5.5 kg of seed cotton was shipped from each plot to Scott MS where it was ginned on a research gin with one lint cleaner to determine gin turnout percentage. Fiber samples were sent to the USDA Dumas Cotton Classing Office for HVI testing.

NuCOTN 33^B and NuCOTN 35^B have greatly improved seedling vigor compared to their recurrent parents. This is especially true of NuCOTN 33^B which has 9% larger seed size and 15 percent more seedling vigor than DP 5415.

Plant map data were collected at 28 locations early in the 1994 season when the plants averaged 10.7 nodes. The number of vegetative nodes prior to the first fruiting branch was not significantly different for any treatment.

The NuCOTN cultivars are slightly taller than the recurrent parents they were derived from. Node number, when 95 percent of the harvestable bolls were set, as well as Nodes Above Cracked Boll (NACB), suggests there were no maturity differences between the NuCOTN cultivars and the recurrent parents.

Fiber quality data were taken at 22 locations (Table 1). The two NuCOTN varieties are very similar to their recurrent parents. The NuCOTN cultivars had reduced micronaire and decreased lint percentage compared to the recurrent parents. Values for fiber strength, fiber length, and lint grade were similar. Cultivars derived from DP 5690 had fiber strength and length that was slightly greater than cultivars derived from DP 5415.

Lint yield data for the different treatments are given by lepidopteran pressure in Table 2. Averaged over all trials commercial lepidopteran sprays increased the yield over no sprays by an average of 149 kg/ha of lint. NuCOTN with the Bollgard™ gene increased yield (fiber) an average of 109 kg/ha over the commercial spray program. The increase was similar for both cultivar comparisons (i.e., there was no significant cultivar by treatment interaction). Fields with the highest pressure averaged 313 kg/ha more lint than the commercial lepidopteran spray program when no sprays were used. Yield of NuCOTN averaged 108 kg/ha more lint than the commercial spray program. The NuCOTN cultivars had equal improvement in yield over commercial spray treatments at both moderate and high lepidopteran pressure. The NuCOTN cultivars had higher yield than the conventional cottons even in areas with low insect pressure. This was not expected. The improvement could be due to benefits derived from sub-threshold lepidopteran levels, to improved agronomic performance of the NuCOTN cultivars, or a combination of the two factors.

Other researchers have conducted trials in the U.S. with NuCOTN cultivars. Davis et al. (1995) designed trials to evaluate large plot comparisons of Bt transgenic cottons (NuCOTN) under "on-farm" situations. They found that "the non-Bt cotton received an average of 5.5 more treatments for the budworm\bollworm complex compared to no applications for the Bt cotton." In these trials the Bt (NuCOTN) cultivars averaged 222 kg/ha more fiber than the conventional cottons sprayed with insecticides. Dr. Johnie Jenkins, at Mississippi State University, conducted trials where plots were artificially infested with tobacco budworm neonates. He found that the Bt cottons provide "very effective levels of control of Heliothines." The Bt cotton cultivars averaged 1,901 kg of lint per hectare while the non-Bt counterparts averaged 732 kg of lint per hectare. Similarly, Dr. Theo Watson reported that "Bt transgenic cotton has proven extremely effective against the pink bollworm, tobacco budworm, cotton leafperforator and

Table 1. Fiber quality comparisons averaged over 22 locations.

	Fiber	Fiber †			Grade		Loan††
	Strength	Length	Mike	Lint%	Leaf	Color*	Value
DP5415 Spray	29.2	28.6	4.46	38.5	2.8	93.1	1.18
DP5415 No Spray	29.1	28.6	4.47	38.1	2.7	93.8	1.17
NuCOTN 33^B	29.0	28.5	4.28	38.2	2.8	93.8	1.19
DP5690 Spray	30.7	28.5	4.42	37.1	2.8	94.1	1.18
DP5690 No Spray	30.9	28.6	4.34	37.0	2.9	94.3	1.20
NuCOTN 35^B	30.6	28.8	4.29	36.6	2.9	93.7	1.20
LSD 0.05 Treatment	NS	NS	0.09	0.3	NS	NS	NS
LSD 0.05 Cultivar	0.3	0.13	NS	0.4	NS	NS	.01
LSD 0.05 Interaction	NS	NS	NS	NS	NS	NS	NS

* Color Grade is an index that combines color and preparation into an index where middling white (31) equals 100. Higher values have better grades while lower values have poorer grades.

†† U.S. dollars per kg of cotton

† Length in millimeters

Table 2. Lint yield (kg/ha) comparisons according to lepidopteran pressure.

	Lepidopteran Pressure			
	Average	Low	Moderate	High*
	25 Loc	11 Loc	7 Loc	7 Loc
DP5415 Spray	1196	1139	1316	1197
DP5415 No Spray	1046	1080	1289	821
NuCOTN 33^B	1331	1268	1497	1309
DP5690 Spray	1144	1149	1315	1007
DP5690 No Spray	1006	1053	1254	756
NuCOTN 35^B	1225	1210	1382	1139
LSD 0.05 Treatment	66	83	NS	106
LSD 0.05 Cultivar	62	NS	NS	109
LSD 0.05 Interaction	NS	NS	NS	NS

* Lepidopteran pressure estimated as the average difference in retention of first position fruit between the non-sprayed recurrent parent and cultivars containing the Bollgard™ gene (i.e., NuCOTN). Differences averaged 0.2% for low, 9.0% for moderate, 19.1% for high and 7.9% for average.

salt-marsh caterpillar in small-plot field trials in Arizona."

Delta and Pine Land Company is very excited about the prospects of delivering more value to the farmers of the world through seed. The Bollgard™ gene in the Deltapine NuCOTN varieties is the first offering of some new and exciting technologies that are coming to farmers.

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Breeding For Multi-adversity Resistance (MAR) to Cotton Pests

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Introduction

Cotton (*Gossypium* spp.) is a major cash crop in the United States and in many countries around the world. About 16 million acres of cotton are planted in the USA cotton belt which extends from the Atlantic to the Pacific Oceans, with an annual production of 20 to 22 million bales. Texas is the leading cotton producing state with about 5.5 to 6 million bales annually followed by California with 2.5 million bales.

Host plant resistance to pests is of critical importance in cotton production. Losses due to pests and stresses in cotton cause significant reduction in yield and quality of lint and seed, and increase production costs. The need to develop and implement alternative pest control strategies has been documented globally.

The primary objective of programs for genetic improvement of resistance to pests and stresses in cotton is to develop cultivars that are resistant to one or more pests, while improving or maintaining their basic agronomic characteristics, yield, fiber and seed quality. Pest management is an important part of the overall crop production scheme in cotton. The availability of adapted cultivars and gene pools with high levels of heritable resistance to multiple pests and abiotic stresses is essential. Progress in the breeding of improved cotton cultivars has been particularly rapid during the past 15 to 20 years. Remarkable advances have also been made in breeding concepts and methodologies for developing cottons resistant to pests.

The Value of Resistant Cultivars in IPM and Sustainable Agriculture

A key consideration in sustainable agriculture is maintaining the health of plants throughout the growing season, so that they may approach their full genetic potential for both quality and quantity. Crop health describes the relative freedom of plants from biotic and abiotic stresses. We have not yet realized a totally healthy plant and its potential. It is estimated that we are achieving about 60% of the cotton plant's genetic potential because of losses caused by pests and environmental stresses (El-Zik and Thaxton, 1989). The main constraints affecting cotton yield and quality are insects (aphids, boll weevil, tobacco budworm, bollworm, pink bollworm, jassid, whitefly, spider mites), pathogens causing diseases (seedling, bacterial blight, leaf spots, fusarium and verticillium wilts, menatodes, viruses), moisture, nutrients, and environmental stresses. Whenever plants are attacked by pathogen or insect pests, one or more of their functions is altered. The prevention of epidemics and ultimately the reduction of losses in yield and quality has been of great concern. Successful crop management minimizes unfavorable effects caused by pests and environmental stresses and optimizes profits.

Historically, new cotton cultivars have been developed to meet the ever-changing demands and needs of the cotton producer and industry, including the textile mills. A wide range of cultivar types have been developed through breeding, and this process is continuous because of changing textile requirements, cultural practices, mechanization, environmental conditions, and pest pressures. Yield and fiber quality are the top priorities in most breeding programs, but there are constraints on both.

The choice of cultivar probably is the single most important management decision the grower makes in an integrated crop management system (ICMS). El-Zik (1985) pointed out that the cultivar sets the framework for the level of susceptibility to pests, the tactics applied to manage the crop, and production costs. Resistant cultivars provide the cornerstone for a successful IPM system (El-Zik and Frisbie, 1985). Genetic resistance is most likely to be used in concert with other pest control measures, which include cultural, biological, and chemical approaches. Resistant cultivars may not require as many treatments or as high rates of pesticide application to achieve adequate pest control. This results in reduced production cost and risk, and increased profits.

Levels of Resistance

Resistance to pests is a relative plant trait rather than an absolute quality. Genetic resistance is a heritable characteristic which suppresses pest populations or reduces pest damage. Defense of host plants against pests may be due to avoidance, non-preference, antibiosis, or to resistance mechanisms. The magnitude of resistance can range from very small to very large; plant reaction ranges from highly susceptible to immune.

Breeding for Resistance

Breeding for pest resistance begins with identifying sources of resistance. The probability of resistance occurring in the host populations is directly related to the diversity of germplasm available. A wide range of resistance levels to pests exists among cultivars within a species. There usually is greater variation among nonrelated species. More importantly, techniques for screening and evaluating germplasm and segregating populations, and for identifying plants resistant to many pests, have been developed.

Jenkins (1982, 1986) reviewed his research for the past 25 years which identified *G. hirsutum* race accessions with varying levels of resistance to tobacco budworm, pink bollworm, plant bugs, and the boll weevil. He reported that 65 accessions have been identified as resistant to boll weevil, 61 resistant to *Heliothis* spp., 98 resistant to pink bollworm, 11 resistant to plant bugs, and 6 resistant to spider mites. Resistance to more than one pest is common in these resistant accessions (Jenkins, 1986). Resistance in cotton germplasm to pink bollworm recently was discussed by Wilson (1987). Significant progress is being made by several researchers in improving *Heliothis* spp. resistant cotton germplasm for yield and adaptation. Some breeders in developing fast-fruiting, short-season cotton strains also have obtained a useful level of resistance to tobacco budworm.

Cotton cytoplasm from different species have been shown to affect resistance to insects. The cytoplasms from *G. arboreum*, *G. herbaceum*, *G. anomalum*, *G. harknessii*, and the *G. hirsutum* cytoplasmic male sterile strains have a negative effect on boll weevil populations (Bowman et al., 1981). However, these cytoplasms also have an adverse effect on agronomic performance. Cytoplasm from *G. tomentosum* has a slightly negative effect on *Heliothis* larval development (Meredith et al., 1979). *G. anomalum* cytoplasm has a small negative effect on tarnish plant bug.

Since the 1900's, researchers have successfully developed cotton germplasm and cultivars resistant to pathogens. Many adapted cultivars have resistance to the fusarium wilt/root-knot nematode complex; few are resistant to either bacterial blight or verticillium wilt (Bird, 1973, 1982; Brinkerhoff et al., 1984; El-Zik, 1985; El-Zik and Frisbie, 1985; El-Zik and Thaxton, 1989; Sappenfield, 1963; Sappenfield et al., 1980). Hyer et al. (1979) and Shepherd (1987) developed cotton germplasms highly resistant to root-knot nematodes.

Comprehensive reviews on the genetics, mechanisms, and sources of resistance, and on breeding cotton for resistance to pathogens and insects were provided by Bird (1973, 1982), Dahms (1943), El-Zik and Frisbie (1985), El-Zik and Thaxton (1989), Jenkins (1982, 1986), Jones (1972, 1982), Maxwell et al. (1972), Maxwell (1980), Niles (1980), and Russel (1978).

Breeding Approaches

Methods to improve crops genetically for resistance to pests are numerous when one considers the various aspects of host variability and sources of resistance, pest variability, genetic stability and elasticity, environmental influence, economic threshold, and injury level of pests. Genetic improvement includes breeding for resistance to one pest (i.e., a single insect or plant pathogen), multiple species of pests, or multi-adversity (insects, pathogens, and environmental stresses). Achieving higher levels of resistance to pests and stresses should be paralleled by improvement in yield, and fiber and seed quality. Improvement of cotton for resistance to only a few pests or adversities is not sufficient for sustainable agriculture.

Presently, pedigree selection is utilized in most cotton breeding programs. The bulk breeding method is used under certain circumstances, in which plant selection is delayed for a few generations to allow for natural selection to act on successive generations. The backcross method is effective for transferring genes controlling resistance to one pest or a trait from known sources of resistance to adapted cultivars. Short-cycle recurrent selection has been used successfully by El-Zik and Thaxton (1989) to pyramid genes for broad resistance to pests and abiotic stresses.

Multi-adversity Resistance (MAR)

The MAR concepts, procedures, and techniques which are based on extensive basic research from the past 30 years are used to develop MAR germplasm (Bird, 1982; El-Zik and Thaxton, 1989). It is a monumental task to select directly for resistance to every pest and environmental stress affecting cotton. A system of selecting directly for few traits (four to six) which indirectly improves levels of the remaining traits, would simplify genetic improvement. A key element in the MAR program is to determine which traits are positively linked or correlated. MAR utilizes direct seed, seedling and plant selection procedures and techniques in cotton for the simultaneous genetic improvement of resistance to pests and abiotic stresses in addition to higher yield potential, earliness, agronomic characteristics, drought tolerance, and higher fiber and seed quality. Fifty-two traits are considered simultaneously in the MAR program, from stand establishment, resistance to pests, to yield and its components and fiber quality.

MAR Procedures

Direct selection in the laboratory and greenhouse is practiced for the following four traits:

- Seed coat resistance to mold.
- Slow rate of radicle elongation (when acid-delinted, non treated seed are held at 13.3°C for 8 days in an incubator).
- Resistance to the bacterial blight pathogen.
- Resistance to seedling pathogens caused by *Rhizoctonia solani* and *Pythium ultimum*.

Direct selection for the four traits indirectly provides genetic gains for resistance to major diseases and insects, in addition to higher yield potential, earliness, and improved fiber and seed quality. Application of the MAR system has been very effective and successful in developing new superior cottons.

The MAR genetic improvement procedures involve extensive seed, seedling and plant screening and selection in the laboratory and greenhouse, followed by a four stage field testing and evaluation at ten locations. These procedures make it possible to identify cotton strains with the many desired traits. The ten field testing locations include the major Texas cotton growing regions from the Rio Grande Valley in South Texas to as far north as Lubbock (1,368 kilometers), and represent a wide range of diverse environments including moderate to severe water stress, and insect and disease pressures. Advanced strains are also included in regional tests throughout the USA cotton belt.

The MAR cotton breeding scheme is presented in Figure 1. Each year about 400 crosses representing 60 to 80 cross combinations are made in the field among the elite strains of the MAR germplasm and parental types. Crosses are planted in the greenhouse, screened for resistance to the bacterial blight pathogen, and seed from the F_1 generation are planted in the field.

Each year about 80,000 seeds representing 500 F_2 individual plant and progeny row field selections are processed with the MAR procedures in the laboratory and greenhouse. About 15,000 clean seeds with no mold growth and slow rate of radicle elongation are selected, planted in cups, and placed in the greenhouse. About 10,000 seedlings are inoculated with a mixture of four races of the bacterial blight pathogen (*Xanthomonas campestris* pv. *malvacearum*). Immune and susceptible reactions to the bacterial blight pathogen are identified. Seedlings resistant to bacterial blight are transplanted into pots (3,000). About 2,000 plants are selected and produce seed in the greenhouse during the winter. The other 1,000 plants are eliminated because of susceptibility to insects, fruit shedding, lateness in maturity, and undesirable agronomic characteristics (El-Zik and Thaxton, 1989).

Seed from the laboratory-greenhouse MAR selections are planted in single progeny rows in the field in the spring the same year

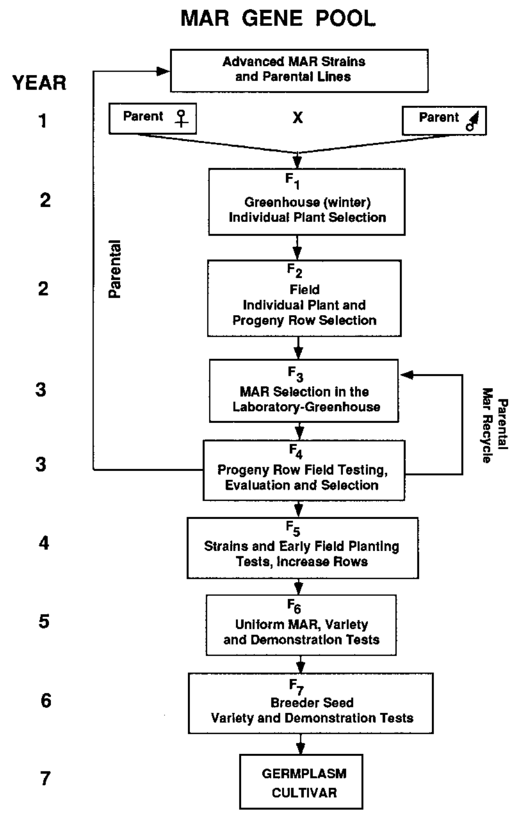

Figure 1. Multi-Adversity Resistance (MAR) cotton breeding scheme. The MAR procedures involve extensive laboratory and greenhouse screening followed by a four stage field testing at 10 locations throughout Texas.

(2,000 progeny rows). Seedlings are inoculated with a mixture of four races of the bacterial blight pathogen. Field evaluations are made for seedling establishment, resistance to bacterial blight, resistance to insects, fruiting pattern and plant type. We select for several characteristics of agronomic value such as close fruiting, short internodes and high boll retention, productivity, earliness, and higher fiber and seed quality. Seed produced by the selected F_4 progenies (50 to 60 strains) are adequate for planting MAR nurseries and increase rows the following season.

The MAR field testing procedure has four stages (Figure 1). The new strains are grown in 10 nurseries throughout Texas (Strain Test) for extensive insect, disease, and environmental stress performance evaluation. Selected promising strains are also evaluated in replicated Early Field Planting tests (EFP) at five locations. The following year, the best strains from the Strains Test and Early Field Planting test are included in a Uniform MAR (UMAR) test at ten locations. The elite strains of this group are also entered in cultivar and demonstration tests in growers' fields. Strains are evaluated for their performance in each of the tests for: stand establishment, plant type, fruit load, boll type, resistance to plant pathogens and insects, drought tolerance, earliness, yield, fiber and seed quality, and stability over environments. Elite MAR strains are quantified for photosynthetic capacity and partitioning of biomass. Strains with a higher allocation of assimilate to fruiting organs and high lint conversion efficiency (harvest index) are identified and selected.

Final selection of the best MAR strains is made based on this extensive performance evaluation over years, and periodically released as new elite MAR breeding lines. When a strain shows exceptional performance for all traits, it is increased as breeder seed and released as a MAR Tamcot cultivar (Figure 1). A new Tamcot cultivar will have the desired characteristics to all 52 traits. It takes 7 to 8 years from the time a cross is made to releasing germplasm in the MAR program. This is in contrast to 11 to 12 years in conventional breeding programs.

Genetic Gains and Performance of MAR Germplasm

Since 1963, the MAR program has created, processed and evaluated seven gene pools; the MAR procedures were used to make selections from each pool. Gene pools were established by making crosses between parental breeding lines and advanced MAR strains to provide genetic variability, followed by evaluation and selection. The sequential gene pools are referred to as MAR-1 to MAR-7. Progress is indicated by whether each new gene pool gives higher average levels of multi-adversity resistance in addition to higher yield potential, earliness, and improved fiber quality in comparison to previous ones.

Achievements in the MAR-1 gene pool were seedling disease escape, seed-seedling cold tolerance, preservation of seed quality, resistance to pathogens causing bacterial blight, Fusarium and Verticillium wilts, high yield potential, and earliness. The MAR-1 gene pool produced three commercial cultivars: Tamcots SP21, SP23, and SP37. In MAR-2, the achievements included resistance to *Heliothis* spp., fleahoppers, boll weevils, Phymatotrichum root rot, and seedling pathogens. Products of the MAR-2 pool were Tamcots SP21S, SP37H, and CAMD-E. In MAR-3, the genetic base was broadened, and this led to improving fiber length and strength and the addition of resistance to Southwestern cotton rust (*Puccinia cacabata*). The MAR-3 pool produced breeding lines with higher levels of multi-adversity resistance and improved fiber quality. In the MAR-4 pool, levels of resistance to spider mites, plant bugs, thrips, aphids, leaf spots, and new isolates of the bacterial blight pathogen from Africa were increased. A new glabrous MAR cultivar, Tamcot CAB-CS, and nine elite lines were released in 1984 from MAR-4. In 1986, Tamcot CD3H and Tamcot GCNH (glandless) also were released from this gene pool.

The MAR-5 and MAR-6 gene pools represent higher levels of resistance to all plant pathogens, insects, and abiotic stresses. In the MAR-6 and MAR-7, genes are being further intensified to insure a broader genetic base for resistance to pests and abiotic stresses, drought tolerance, in addition to higher yield potential, improved fiber and seed quality. Tamcot HQ95 and eight breeding lines were released from the MAR-5 gene pool. Releases from the MAR-6 gene pool are the Tamcot Sphinx cultivar and 12 breeding lines.

Several morphological mutant traits and their combinations have been incorporated into the MAR germplasm. These traits are glabrous, glandless, nectariless, okra-shaped leaf, frego bract, and red plant color (Thaxton and El-Zik, 1995).

Resistance to Plant Pathogens

Cotton seed having the inherent ability to germinate, to resist seed-seedling pathogens, and to produce healthy seedlings, when planted early in the season under cool moist conditions, is essential for efficient cotton production. The ability of seed and seedlings to perform in cool soil with minimal damage from soil fungi is a key trait of MAR cottons. The MAR-1 germplasm was susceptible to seedling pathogens and partially resistant to seed deterioration, the MAR-2 and MAR-4 germplasm had intermediate resistance, and the MAR-5 and MAR-6 were resistant. Generally, levels of resistance to pests and stresses in the MAR-3 germplasm (1980-81) were intermediate between the MAR-2 and MAR-4. Wallace (1985), Poswal (1986), and Hernández (1987) documented a progressive improvement in resistance to the seed-seedling pathogens, stand establishment, and seedling vigor in the MAR germplasm. The MAR program has maintained high levels of resistance in its germplasm to all 19 USA races of the bacterial blight pathogen (*Xanthomonas campestris* pv. *malvacearum*).

Progressive improvements in resistance to vascular wilt pathogens, Phymatotrichum root rot, and nematodes have been made, even though no direct selection for these pests was practiced. However, the rate of genetic gain in resistance to these pathogens was slower than the rate for the four traits for which direct selection was employed. The MAR-1 germplasm had intermediate resistance to the fusarium wilt/root-knot nematode complex, and the MAR-2 to MAR-6 strains were resistant. New MAR-6 and MAR-7 strains have higher levels of resistance to Verticillium wilt than the MAR-1 (partial resistance) and MAR-2 or MAR-4 (intermediate resistance) strains. Progressive gains in resistance to *Phymatotrichum omnivorum* have been made in each gene pool of the MAR program (Thaxton et al., 1991). Six new MAR-5 strains have been identified with higher levels of resistance than the MAR-1 to MAR-4 cultivars and non-MAR commercial cultivar checks. Of special note is the association of high yield potential with reduced cotton root rot for the MAR-5 and MAR-6 strains (El-Zik et al., 1988; Thaxton et al., 1991; Thaxton and El-Zik, 1994). The MAR-6 Tamcot Sphinx is resistant to both root-knot and reniform nematodes.

Resistance to Insects

The initial objective of the MAR program dealt with diseases; however, there was a simultaneous gain in resistance to insects. The MAR-1 germplasm was susceptible to most insects. Partial and intermediate levels of resistance to certain insects were obtained in the MAR-2 to the MAR-4 germplasm. In the MAR-5 to MAR-7 germplasm resistance levels increased to intermediate resistance and resistance (El-Zik and Thaxton, 1989, 1990, 1995; El-Zik et al., 1991; Thaxton and El-Zik, 1994).

Bird (1979) provided evidence that Tamcot CAMD-E (MAR-2) has intermediate resistance to the boll weevil. It was later confirmed that Tamcot CAMD-E has intermediate resistance to *Heliothis* spp. (McCarty et al., 1983; Zummo et al., 1983) and fleahopper (Lidell et al., 1986). Tamcot CAMD-E was the first Upland cultivar with significant levels of resistance to five plant pathogens and three insects (Bird, 1979). Tamcot HQ95 (MAR-5) and Tamcot Sphinx (MAR-6) have the highest levels of resistance to insects and water stress; they are resistant to six plant pathogens and four insects (El-Zik and Thaxton, 1990, 1995). New MAR-5 and MAR-6 strains with higher levels of resistance to early and late season insects subsequently have been identified and released (El-Zik et al., 1988, 1991; El-Zik and Thaxton, 1989; Thaxton and El-Zik, 1994). An important point to emphasize is that the MAR system is making progress with genetic variability provided by adapted cottons.

Drought Tolerance

Water stress is the most important adversity affecting fruit production, square and boll shedding, lint yield and fiber quality in cotton. Cotton cultivars and strains have been found to differ significantly in their response to mid-season water stress. Results obtained by Cook and El-Zik (1993) demonstrated that genetic variability exists among currently available cotton germplasm sources in the USA for flower and boll production, lint yield, earliness, and water use efficiency. Tamcot CD3H (MAR-4) was identified as a drought tolerant cultivar with greater water use efficiency and high flowering potential which has a major role in drought tolerance, than other cultivars studied. Selection for and incorporation of increased seedling vigor, rapid root system establishment and lower root-to-shoot ratios into future cotton germplasm could improve drought tolerance and lint yields in regions subjected to limited water sources or poorly distributed rainfall conditions (Cook and El-Zik, 1992).

Lint Yield

The main objective of cotton genetic improvement programs is to develop cultivars with high yielding ability and superior fiber and seed quality. Incorporating high levels of resistance to pests and abiotic stresses into adapted high yielding cultivars is a major challenge.

The superior performance of MAR germplasm and cultivars has been documented (Bird, 1975, 1982; El-Zik et al., 1988, 1991; El-Zik and Thaxton, 1989; Thaxton et al., 1991; Thaxton and El-Zik, 1994). Data collected over 30 years indicate that the MAR cultivars have high yield potential in either the presence or absence of adversities. The MAR germplasm also has a distinct advantage in early fruit set and crop maturity.

Average first harvest (early yield) and total lint yield of MAR and non-MAR cultivars, grown in four county tests in the Texas Coastal Bend region near Corpus Christi, Texas, over a three year period, 1984-1986, are presented in Figure 2. All MAR cottons produced higher first harvest and total lint yields than the non-MAR McNair 220 and Deltapine Acala 90 cultivars. The results clearly show the progressive genetic gains in earliness and total lint yield from the MAR-1 to the MAR-4 gene pools. The incremental increases in earliness and yield paralleled the increases in levels of resistance to insects, plant pathogens, and abiotic stresses. The MAR-4 Tamcot CD3H matured 806 lbs of lint per acre in 125 days from planting compared to 505 lbs for the MAR-1 Tamcot SP37, and a total of 1,122 lbs compared to 873 for SP37 in 140 days. Tamcot CD3H has been reported to have high levels of resistance to plant pathogens and insects (Bird et al., 1987).

Earliness

Since the 1970s, most breeding programs have emphasized early crop maturity. The percentage of acreage planted in early maturing cultivars in the USA mid-south increased by an average of 19% in 1978 and 90% in 1986 (Bridge and McDonald, 1987). In contrast, only small increases in the percentage of early maturing cultivars occurred in the Southeast and Western cotton growing regions during the same period. The Southwest (Texas and Oklahoma) grows predominantly early short-season cultivars. El-Zik and Frisbie (1985) and Bridge and McDonald (1987) reviewed earliness and discussed the advantages of early crop maturity and the short season production system.

Earliness which is expressed as the ratio of first harvest to total lint yield, ranged from 19% for the non-MAR NcNair 220 and

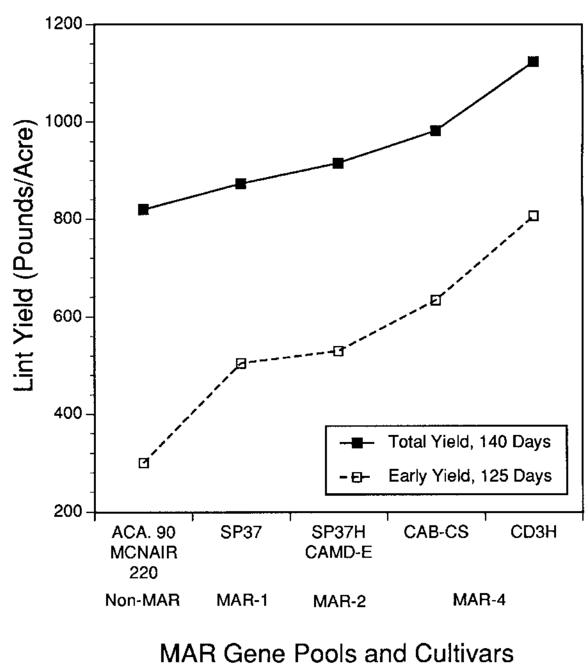

Figure 2. Average first harvest (early yield) and total lint yield for non-MAR and MAR cotton cultivars representing the sequential gene pools MAR-1, MAR-2, and MAR-4. Data were obtained from tests conducted in four counties in the Coastal Bend region of Texas over three years.

Deltapine Acala 90 to 73% for Tamcot CD3H (Figure 2). This represents a three week earlier maturity for the MAR cultivars, reducing losses and costs due to late season insects, diseases, and adverse weather.

Fiber Quality

Generally, as yield increased from the MAR-1 to MAR-7, fiber quality traits also improved. In the MAR-6 germplasm, fiber length ranges from 1.10 to 1.25 inches, uniformity 83.0 to 88.2, strength 27.6 to 35.0 g/tex, elongation 5.8 to 7.1, and micronaire ranges from 3.7 to 4.6.

A substantial improvement in fiber strength has been achieved in the MAR germplasm. Averaged over six years and 35 tests, there has been a progressive increase in fiber strength (Figure 3). The incremental increases in fiber strength were 1.3% (0.3 g/tex) from the MAR-1 to the MAR-2, 4.6% (1.1 g/tex) from MAR-2 to MAR-4, 5.2% (1.3 g/tex) from MAR-4 to MAR-5, and 14% (3.7 g/tex) from the MAR-5 to MAR-6 cultivars. The overall increase in fiber strength from MAR-1 to MAR-6 was 6.4 g/tex (27%).

The progressive improvement in yield and fiber strength in the MAR germplasm from the MAR-1 to MAR-7 gene pools is shown in Figure 4. The MAR-5 Tamcot HQ95, averaging 27.6 g/tex is 3 g/tex higher than the MAR-1 and MAR-2 germplasm. The MAR-6 Tamcot Sphinx cultivar with high yielding ability and fiber strength of 30 g/tex averages 5 g/tex higher than MAR-1 and MAR-2 releases and 2.5 g/tex higher than Tamcot HQ95. A new MAR-7 genotype with the highest fiber strength (32.1 g/tex) and yielding ability is CD3HGCBU8H-1-91. It averages 7 g/tex higher than MAR-1 and 5 g/tex higher than the MAR-5 germplasm.

Figure 5 summarizes the progressive simultaneous improvement in lint yield, fiber strength, and resistance to insects and plant pathogens from the MAR-1 to the MAR-6 gene pools. Disease and insect indices are an average of the levels of resistance to six pathogens and four insects (El-Zik and Thaxton, 1989; El-Zik, et al., 1991; Thaxton et al., 1991; Thaxton and El-Zik, 1994). Disease index progressed from 30 for the MAR-1 to 80 for the MAR-6. The MAR-6 germplasm has high resistance to pathogens causing bacterial blight and seed-seedling diseases, and intermediate resistance to root pathogens (verticillium wilt, Phymatotrichum root rot, and fusarium wilt/root knot nematode). The MAR-1 germplasm was susceptible to insects (index of 10), whereas MAR-6 has increased levels of resistance (index of 72). The MAR-6 germplasm has resistance to aphids, thrips, fleahopper, tobacco budworm, bollworm, and the boll weevil. The MAR

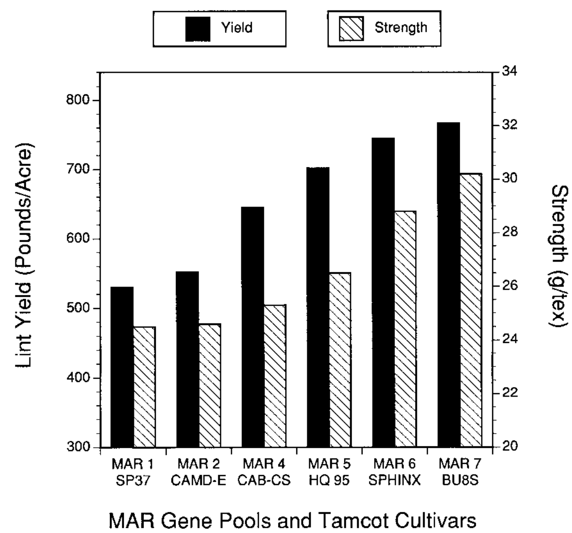

Figure 4. Mean lint yield and fiber strength of Tamcot cultivars representing MAR-1 to MAR-7 gene pools over 24 tests and two years.

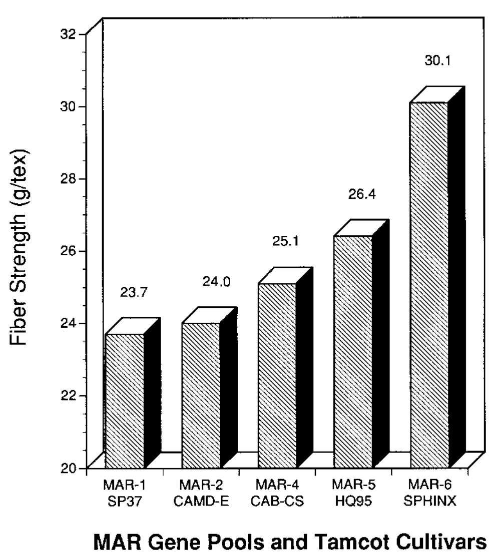

Figure 3. Mean fiber strength of Tamcot cultivars representing MAR-1 to MAR-6 gene pools over six years and 35 tests.

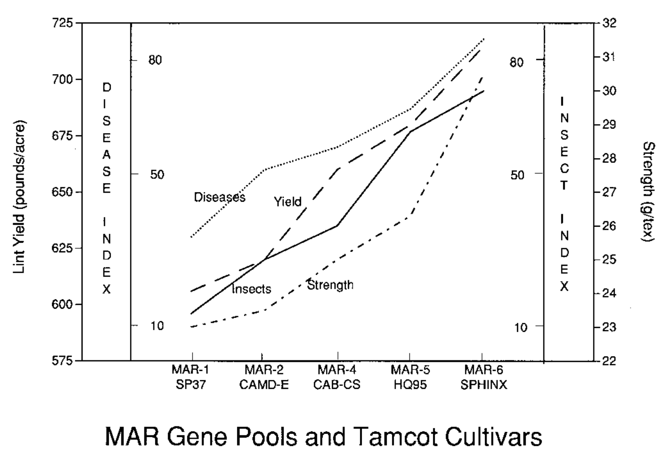

Figure 5. The progressive improvement in lint yield, fiber strength, resistance to plant pathogens and to insects from the MAR-1 to MAR-6 gene pools. Disease and insect indices are an average of the levels of resistance to six pathogens and four insects.

system is pyramiding and identifying favorable recombinations of genes for resistance to insects, pathogens, and abiotic stresses.

Impact of the MAR Germplasm

Application of the MAR breeding system has been very effective and successful in developing new superior cotton germplasm and cultivars that minimize growers' risk, reduce production costs, and increase profit. Eleven Tamcot cultivars and more than 300 elite MAR breeding lines have been released from the program. Commercial seed companies and breeders have released 27 cultivars by selecting from released MAR germplasm. For the last five years, the MAR germplasm and Tamcot cultivars were grown in 40 to 50% of the cotton acreage in Texas and Oklahoma. This represents four million acres or 25% of the total USA cotton acreage. The MAR germplasm is also being used extensively in many countries around the world. Narrowing the gap between actual and potential yield is due to host-plant resistance to pests and abiotic stresses in the MAR germplasm.

Biotechnology

Despite the breeding progress already achieved, additional gains in cotton productivity and quality, and resistance to pests are needed. The field of molecular biology offers opportunities to enhance our efforts in developing new cottons with higher levels of resistance to multi-pests and other important traits. In my view, there are four components to cotton biotechnology: genome mapping will facilitate both DNA marker-assisted selection, map-based gene cloning, and fingerprinting cotton cultivars. Agriculturally important genes in cotton need to be identified and isolated. Gene transformation will be a valuable tool in inserting pest and herbicide resistance genes, and other valuable traits from other genera, families and even kingdoms. Regeneration of transformed cells, through somatic embryogenesis, shoot tip, or other means, is a must to recover transgenic cells and plants. In cotton, somatic embryogenesis is cultivar specific, and it is difficult to obtain plants with the inserted genes, except in Coker 310 and 312. Transgenic plants should carry the gene or genes inserted, in addition to the many genes controlling crop productivity, quality, stability and adaptability. Transformation technology includes the use of *Agrobacterium tumefaciens*, particle bombardment, and DNA injection. There are advantages and disadvantages for each method. Biotechnology will not replace or be used independently from conventional breeding. It adds new tools for use.

The Future

A considerable number of advances have been made within the past 15 years in breeding cotton and other crops for resistance to pests. Resistant sources have been identified for most pests of cotton. Of particular significance is the development and release of adapted multi-pest and multi-adversity resistant cottons. However, higher levels of resistance to all adversities affecting cotton production (insects, plant pathogens, nematodes, abiotic stresses) are needed in future cultivars. Improved resistance will make it possible for growers to realize a higher proportion of the genetic potential of their cultivars, to reduce production cost and risk, and to increase profit.

Maintaining crop health is essential for profitable and sustainable cotton production. Breeding programs must focus on developing adapted cottons with multi-adversity resistance, in addition to high yielding ability, earliness, and improved fiber and seed quality. In addition, the cotton plant of the future must be highly efficient in fruit retention and its use of solar radiation, water, and nutrients in both irrigated and dryland production. The MAR system, which is different from conventional breeding procedures, has proven to be efficient in simultaneously pyramiding favorable and compatible genes for resistance to pests and abiotic stresses, earliness, yield, and quality. We believe that continued progress will be possible with this genetic improvement system.

As we approach the 21st century, we face many challenges and opportunities. The challenges are to mold and adapt current and new technologies in order to create and engineer future crop cultivars that will produce healthy and efficient plants. These cultivars will be the cornerstone and foundation of IPM and sustainable agriculture. New cultivars with higher levels of resistance to multi-pests and abiotic stresses are essential for the survival and future progress of cotton growers and agriculture worldwide, and also for preserving our natural resources and the environment, ... and planet earth.

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Current Status and Scientific Support of Cotton Production in Uzbekistan

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Cotton production is one of the most important branches of agriculture and Uzbekistan production of seed cotton is a mainstay of our well-being, especially as our republic is moving to the market economy. For this reason, cotton farming is regarded as a high priority by the Government of Uzbekistan.

Cotton is grown in Uzbekistan in a broad band between 37 and 43 N. latitude, primarily in river valleys on serozem and meadow soils. In terms of climatic conditions, the cotton-growing areas are divided into three zones: northern, central, and southern. The average duration of the frost-free season varies widely—from 195-205 days in the northern zone to 210-220 days in the central zone to 220-250 days in the southern zone. The sum of effective temperatures varies from one zone to another in the range of 1900-2900 deg. In 1994, 1.4 million metric tons of lint were harvested from a total area of 1.5 million hectares.

Modern cotton farming requires considerable scientific support. Cotton is grown in a system of scientifically proven, accelerated cotton-field crop and cotton-cereal crop rotation, preserving and even enhancing soil fertility. Such a system provides for highly effective land use, improved labor productivity and mechanization of cotton and crop rotation. All cotton farming processes, from planting to harvesting, are mechanized. Fields are plowed in the fall to a depth of 30 cm, using two-depth and combined plowing techniques; cultivation is done to a depth of 40 cm.

For each cotton producing zone, an optimum planting regime (planting time, methods and rates) has been established such as to lead to a reduction in seed consumption by 1.5 to 2 times. Thus, it is recommended to place a counted number of delinted seeds in each hole.

Also mechanized is furrow irrigation, the most popular method of bringing water to the fields by use of flexible, rigid or semi-rigid siphon tubes. Mechanized furrow irrigation boosts labor productivity by 1.5-3 times, reduces irrigation water consumption by 10-15 percent, and increases the land utilization factor by 10-15 percent. Advanced irrigation techniques, such as sprinkler irrigation, are also making inroads into Uzbek cotton farming. These methods help save 45-50 percent of irrigation water, boost productivity by a factor of 15 to 20, and increase the yield by 4-7 hundredweight (one hundredweight = 100 kg) per hectare.

Researchers have determined the optimum nutrient ratios for each stage of the growing season depending on the soil type and fertility. The recommended rates of fertilizer application to produce a seed cotton yield of 25-30 hundredweight/ha are 200 kg/ha of nitrogen, 100 kg/ha of phosphorus and 100 kg/ha of potassium (assuming average levels of these elements in the soil).

The leading enemies of cotton crops are insects (spider mite, thrips, aphids, bollworm, cutworm, etc.) and diseases (verticillium and fusarium wilts, root rot, and bacterial blight). The crop is protected against pests by an integrated pest management system, including organizational, agricultural, biological and chemical techniques.

Defoliation of cotton plants, speeding up the boll opening process, is an important precondition of mechanized harvesting. Defoliation is carried out with magnesium chlorate, calcium chlorate-chloride, and Dropp. Defoliation starts when at least 60-65 percent of bolls have opened. Seed cotton of medium varieties is harvested by means of domestically designed and manufactured vertical-spindle pickers. Usually cotton is harvested twice, the second time after another 20-30 percent of bolls have opened.

Genotypic assessment of the immune properties of cotton is an important stage in breeding for high yield and resistance to diseases and pests. The best results in developing highly valuable, wilt resistant cotton varieties have been achieved by means of intra-specific and distant inter-specific hybridization.

It is well known that the genus *Gossypium* is rich in valuable breeding attributes. It consists of 35 species, including 4 cultivars and 30 wild and semi-wild forms exhibiting a high level of resistance to the pathogens of wilt and bacterial blight, sucking pests, etc.

At present, the following species are widely used in our cotton breeding programs: *G. aridium*, *G. thurberi*, *G. sturtii*, and *G. triphyllium*. It has been established that the wild species *G. aridum* and *G. thurberi* have wilt resistant genes; *G. triphyllium* possesses resistance to sucking pests; the wild subspecies *G. punctatum* exhibits properties in terms of earliness, lint yield and wilt resistance; while *G. punctatum* and *G. morilli* have lint yield enhancing genes.

If high-yield, disease resistant cotton varieties are to be developed, it is important to thoroughly study the world collection and utilize it as starting material in hybridization.

Uzbek breeders have developed the theory and practical techniques of distant inter-specific hybridization of cotton.

It has been experimentally proved that it is feasible to cross different species of the genus *Gossypium*, including cultivars, wild and semi-wild, 26- and 52-chromosome species, as well as to obtain fertile 1st-generation inter-specific hybrids. This technique was used to develop a number of cotton varieties (e.g. C-8802, C-9621) possessing a new, highly valuable trait, viz. high immunity to bacterial blight.

Using the distant intra-specific hybridization method, a new, wilt resistant variety Tashkent-1 and a number of other varieties have been developed. Tashkent-1 is a product of crossing the wilt-susceptible variety C-4727 with a wild variety of Mexican cotton and subsequent back crossing of the 3rd-generation progeny with the parent variety C-4727.

Another technique used in our breeding programs is a gradual, step-by-step hybridization of the most wilt resistant cotton varieties. Such an approach yields progeny (F_3 and F_4) with up to 5 percent of the families exhibiting an even higher wilt resistance. Subsequently, this trait is further enhanced by selecting particularly resistant families in a highly wilt-infected environment.

However, the distant hybridization technique used to breed new, high-yield, wilt resistant varieties takes a lot of time; the breeding process typically runs for 10 to 15 years or even longer. For this reason, researchers at the "Phytotron" breeding-greenhouse center are looking for ways to speed up the breeding process and harvest two or three crops a year. New physical, chemical and other mutagenic methods are being tried out with a view to developing still more productive and wilt resistant cotton varieties.

One of such new methods involves irradiation of cotton plants. It has been found out that irradiation causes the emergence of mutants with different requirements to the environment. Radiation treatment of the semi-wild subspecies *G. punctatum* has brought forth a new variety, AN-401, possessing excellent wilt resistance, earliness and productivity while producing fiber with superior processibility.

Uzbek breeders have developed and offered for commercialization a range of wilt resistant, productive cotton varieties, such as C-6524, C=6530, Namangan-77, 175-F, Bokhara-6, AN Bayaut-2, Ferghana-3, Yulduz, Termez-24, and some others distinguished by virtue of high lint yield and quality. All of these varieties are widely used by Uzbek cotton farmers.

Breeding is not the only method used to improve the ability of cotton to resist wilt; agricultural techniques also play an important part in attempts to control this harmful disease. Special agricultural methods of growing cotton on soils infected with wilt pathogens are instrumental in curbing the proliferation of the fungus in the soil and creating a hostile environment for it.

Crop rotation with an accelerated alternation of the rotating crops is a highly effective method of wilt control and improved tolerance to this disease.

In a cotton-alfalfa crop rotation system, wheat is grown the 1st year; alfalfa the 2nd and 3rd years; cotton the 4th, 5th and 6th years; fodder and other crops the 7th year; wheat the 8th year; and cotton the 9th and 10th years. Thus, cotton is grown 50 percent of the time. In a cotton-cereal rotation system with a frequent crop alternation, 1:1 and 1:2 patterns are used, with wheat and fodder or other crops being grown the 1st year followed by one or two years of cotton. The cotton-cereal crop rotation permits cutting down the wilt susceptibility of cotton by 20-30 percent.

Thus breeding of new wilt resistant varieties combined with appropriate agricultural techniques are conducive to high yields of seed cotton with desired fiber properties. As for the future, we plan to step up the breeding effort to develop new, high-yield, wilt resistant cotton varieties by means of modern breeding, genetic and biotechnological methods capable of enriching the cotton gene pool with new, valuable traits.

Morphological Characters and their Role in Controlling Sucking Insects—A Review

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Genetic resistance to plant pests is an important breeding objective because pest susceptibility will generally result in decreased yield and quality. Resistant cultivars are used for pest suppression in integrated pest management. These cultivars would reduce the need for insecticidal control measures and conserve beneficial insects that help suppress the target as well as other insect pests.

Mechanisms of host plant resistance were grouped into 3 categories: nonpreference (antixenosis), antibiosis, and tolerance (Painter, 1951).

Nonpreference is the insect's response to a plant that lacks characteristics to serve as a host, resulting in reaction or total avoidance during search for food, oviposition sites, or shelter. The morphological resistance factors are physical barriers or deterrents to insects such as trichomes, nectariless, glandless, frego bract etc. which are expressions of genetically regulated biochemical processes.

Nonpreference is not considered a true resistance by some scientists. This is because preference presupposes that a choice is available. In modern agriculture, choice is not available except in case where trap crops are used; therefore, proposed antixenosis and Schuster (1980) used non-acceptance to substitute for the term nonpreference. It implies that the plant is avoided as a bad host because of negative stimuli. Antixenosis or non-acceptance is a characteristic of the plant, whereas Painter's nonpreference is an insect attribute. Antixenosis may be either physical (e.g. hairy stems) or chemical (e.g. noxious taste, oviposition deterrence, or another allomone).

Different factors for resistance are observed in cotton. Breeders were able to transfer and combine several morphological traits in one cultivar. Resistance in various combinations of okra leaf, glabrous leaf and nectariless enhances varietal yield and stability (Thomson et al., 1987). In general, morphological features confer a low or moderate level of resistance which can be used for pest suppression in integrated pest systems. In order to enhance resistance provided by morphological traits, resistance factors froma diverse gene pool should be identified and incorporated in the breeding material.

Resistance factors have complex complementary, synergistic, or antagonistic interactions with other pest and environmental factors. Where resistance is conditioned by quantitative changes in physical or chemical plant factors, researchers should attempt to determine the effect of incremental changes in the resistance factor on the magnitude of resistance; such knowledge may allow for selection of genotypes with effective levels of resistance, but with minimum interference to non-target organisms. Reduced pubescence on aerial parts of the cotton plant leads to smaller populations of the cotton fleahopper, but greater damage by leafhoppers of the genus *Empoasca* (Welsh) (Pamell et al., 1949; Lukefahr et al., 1971). Knowledge of the quantitative relationship of pubescence and pest infestation may allow for selection of genotypes with compromised trichome expression but to both pests.

Mutant forms have little effect on lint percentage and fiber quality (Thomson et al., 1987; Lee, 1984; Meredith et al., 1973).

The main morphological traits in cotton related to resistance to sucking insect pests will be discussed in this review.

Trichome Density

Trichomes are unicellular or pluricellular outgrowth from epidermis of leaves, shoots, and roots. The collective trichome cover of a plant surface is called pubescence. The species *Gossypium hirsutum* L., which includes American upland cotton, displays great variation in trichome density of fruiting and vegetative parts. Three phenotypes have been designated, smooth leaf (glabrous, no hairs), hirsute (medium length, normal hair density), and pilose (short, dense hairs), based on genetic studies.

The alleles were identified and designated as Sm for smooth leaf (Meyer, 1957), H_1 for hirsute, and H_2 for pilose (Knight, 1957). Lee (1968) found that pubescence alleles at three loci, either singly or in combination, imparted varying degrees of smoothness to plant leaves and stem. Two alleles, Sm_1SL and Sm_2, removed virtually all trichomes from stem and leaves except leaf margins; whereas the third allele, Sm_3, removed trichomes from the leaf surface between the principal veins. Various combinations of these alleles rendered the plant entirely glabrorus. Kohel (1973) proposed standard symbols and phenotypic names as follows: H_1, pubescent; H_2, pilose; and Sm_1 (Sm_1SL), Sm_2, and Sm_3, smooth leaf.

Lint yields of glabrous cottons are frequently lower than those of pubescent cottons. Glabrous types at the Sm_2 locus were associated with as slightly lower lint percentage and a slightly longer fiber than glabrous types with the Sm_1 locus. Smith (1964) observed glabrous cottons were late in fruiting, possessed longer internodes, and had bigger leaves; in general they were more vegetative than were pubescent cottons.

Trichomes serve many critical physiological and ecological functions, particularly those associated with water conservation. Lavin (1973) discussed the ecological functions as defense against herbivores.

Insect species respond differently to the presence of plant hairs. Pubescence as a resistance factor interferes with insect oviposition, attachment to the plant, feeding, and ingestion. However, glabrous forms of plants may be more resistant to some species. In general, the mechanical effect of the pubescence depends on four main characteristics of the trichomes; density, erectness, length and shape.

Trichome density is an important factor in *Lygus hesperus* oviposition site selection, regardless of the presence of other resistance mechanisms. Female oviposited 28% and 31% fewer eggs on normally hirsute and smooth leaf lines, respectively, than on the pilose lines. The pilose reduced nymphal weight by 37% compared with the smooth leaf isolines. Differences in number of eggs laid, nymphal emergence, growth, and survival were not significantly different between the smooth leaf and normally hirsute lines. The correlation coefficients between trichome density and number of nymphs hatching per female were highly significant (Benedict et al., 1983). Pilose can be considered tolerant to mired bugs (Meredith and Schuster, 1979). The number of both tarnished plant bugs and cotton fleahoppers are greater on pilose lines but damage is reduced depending on the degree of pilosity. The documented examples of crop resistance to mirids are those that lead to reduction in populations (Tingey et al., 1975b; Schuster et al., 1976b). Pilose controlled by H_2 gene confers resistance to plant bugs and thrips. Parnell et al. (1949) showed that plant pubescence is a key trait for resistance to Jassids. Leafhopper populations decreased but whitefly populations increased as the number of trichomes increased up to 70 trichomes per 13.7 mm., and then decreased as the trichomes became more dense (Butler and Wilson, 1988). Smith and Meagher (1994) reported that the genetic background influences the expression of resistance or susceptibility to potato whitefly *Bemisia tabaci* (Gennadius) conferred by trichome density. Density of cotton aphids was affected by cotton genotype and the apparent mechanism of resistance was associated with reduced leaf trichome densities. Cotton aphid density increased linearly with trichome density (Weathersbee and Hardee, 1994, 1995). Cotton aphids differentially impacted the yield of DES119 cotton and a smooth-leaf isoline. Yield of DES119 increased with aphicide treatments while those for the smooth-leaf isoline were not affected (Weathersbee and Hardee, 1995).

The glabrous character appears to be a desirable host plant resistance to certain insects. Field tests have shown that the glabrous character imparted hypersensitivity to plant bug feeding, resulting in increased feeding damage (Jones 1976; Schuster and Frazier, 1976; Meredith and Schuster, 1979). However, the multi-adversity resistance program has broken the association of susceptible glabrous cottons with plant bugs and fleahoppers (Bird et al., 1983). Glabrous cotton plants have high leafhopper (*Empoasca* spp.) numbers, but fewer whiteflies.

In order to alleviate glabrous cotton susceptibility to tarnished plant bugs, a nectariless trait is added to these cottons so as to increase their resistance to plant bugs, but they are still less resistant than normal cultivars. Bailey et al. (1980) concluded that frego or glabrous cottons, even after the addition of the nectariless characters, may still have serious problems with plant bugs and it has been shown from the study of the interacting effects of L^0, fg, and Sm_2 damages by early season insect pests mainly attributed to tarnished plant bugs, Sm_2 to be moderately sensitive, fg to be severely sensitive, and the combination of Sm_2 and fg to be more sensitive than either trait alone.

Performance of most mutant strains was similar for all measured traits in either glabrous or pubescent backgrounds. Jones et al. (1975) reported that glabrous isolines were frequently late in maturity and lower in yield than their pubescent isolines. The glabrous trait reduces the trash content in fibers.

Nectariless Trait

The presence of two recessive genes, ne_1 and ne_2, results in the absence of extrafloral nectaries on the leaf midrib, bracts and calyx. The floral nectaries located at the base of the inside of the leaf calyx are not removed by these two genes.

Nectariless plants, which may be an inadequate source of food for certain insects (Lukefahr and Ryne, 1960; Lukerfahr et al., 1965; 1971), have shown resistance to plant bugs (cotton fleahopper, tarnished plant bug, and lygus bug complex). However, extrafloral nectaries have shown to be import in the maintenance of beneficial insects (Lukefahr and Rhyne, 1960; Buttler, 1968; Benchoster and Leal, 1974; Schuster et al., 1976) but provide a port of entry to some pathogens.

Several studies have shown that nectariless is a host plant resistance factor and has no detrimental effects (Adjei-Maafo et al., 1983; Meredith et al., 1973). Schuster and Frazier (1976) suggested that nectariless confers resistance through nonpreference and antibiosis, the latter a result of nutritional deficiency in the absence of nectar. The impact of this trait is most effective to the invading insects before flowering begins and floral nectar is available. Furthermore, honeydew produced by whiteflies and aphids may negate the effect of nectariless (Schuster, 1979).

Laster and Meredith (1974) reported lower plant bug populations, earlier maturity, and higher yield of nectariless cotton than their nectaries isolines. Meredith et al. (1973) found that nectariless strains reduced populations of plant bugs about 60% and these results were confirmed by Schuster et al. (1976), who also found reduced populations of fleahopper (*Pseudatomecelis seriatus*) (Reuter) on nectariless. This nonpreference reflects the fact that 45% of the feeding time of tarnished plant bugs on cotton is spent on squares and that an additional 25% is spent at leaf nectaries (Lastson et al., 1977).

Many adult insects prefer to feed and lay eggs on cotton that have extrafloral nectaries (Adjei- Maafo and Wilson, 1983; Henneberry et al., 1977; Lukefahr and Rhyne, 1960; Schuster et al., 1976). However, additional sources of resistance to plant bugs are needed because the nectariless trait alone does not give adequate protection when populations of this insects are huge. Timok (Jenkins et al., 1977), a cross involving *G. hirsutum* stocks (Jenkins and Parrot, 1976), and cottons from Bulgaria (Jenkins and Parrot, 1976; Lambert et al., 1970) have been reported to show resistance, and these germplasm sources have been released for use by breeders.

Okra Leaf Shape

Okra leaf and super okra leaf are leaf-shape mutants characterized by palmately lobed leaves which have the effect of opening the plant canopy.

Studies of near-isogenic cottons with contrasting leaf shapes indicate that mature plant canopies of okra leaf (L^0) and super-okra leaf (L^8) have about 40 percent and 60 percent less foliage and allow 70 percent and 190 percent more sunlight to penetrate their respective canopies than normal leaf cotton (Andries et al., 1969).

Furthermore, the open canopies of L^0 and L^8 cottons result in significantly lower relative humidity within their canopies, prompted faster drying of soil surface and of plant parts, and significantly increased temperature at or near the soil surface in comparison with normal leaf cotton. Agronomic studies of the open canopy traits indicated that L^0 and L^8 were associated with a significantly higher flowering rate during the first 6 weeks of fruiting and were ready for harvest about 1 to 2 weeks earlier than genetically comparable lines with normal leaves (Andries et al., 1969; Jones, 1982). Plants with okra-shaped leaves produced fiber with less trash.

The open-canopy traits have conferred distinct yield advantages over normal leaf under growing conditions conducive to rank growth where excessive shade, boll rot, and late maturity limit productions. Where these yield factors are relatively less important, lint yield and quality of open-canopied cotton have been equal or slightly below that of comparable normal-leaf types. Major disadvantages of open-canopy are increased need for herbicides for late-season weed control and increased attractiveness to the plant-bug complex.

The effect of okra leaf on productivity in the USA has been variable. Meredith (1982) concluded that regardless of genetic background, okra leaf isolines developed by backcrossing usually result in a 5% yield reduction. However, Meredith (1983) reported no significant differences between normal and okra leaf backcross isolines; whereas Jones (1982) in a review concluded that okra leaf compared to normal leaf either had a neutral or a positive effect on yield, this positive effect usually being atritutable to a reduction of boll rot. However, contrary to Thomson and Williams' results, Meredith did not find any beneficial effect of okra leaf with insect control.

Okra leaf confers resistance to banded-wing whitefly, *Trialeurodes abutilonea* (Holdeman) (Jones et al., 1975), but makes the crop more attractive to plant bug complex (Burris et al., 1981). A yield advantage of 12% was obtained by incorporation of hairiness and open plant canopy to control jassids and whitefly, as well as improved fiber quality, full resistance against jassids and hence delayed early sprayings, and better overall tolerance against the insect pest complex (Mursal, 1994). Jones (1982) noted that high fruiting rate of okra leaf lines confers a degree of tolerance to plant bugs but this insect nevertheless can negate the earliness of the open canopy cottons and cause yield losses if large numbers of plant bugs persist for a long period.

Frego Bract

Frego is a mutant of cotton in which the floral bracts are narrow, twisted and flared, compared to the normally flattened bracts. Frego bracts do not enclose the flower bud or boll but leave it exposed (Green, 1955).

Frego bract in glabrous and hairy types, and in both normal and okra-shaped leaf type, has been reported to reduce yield. If plant bugs were controlled or failed to develop, lines with frego bract gene (fg) produced yield comparable to or greater than normal-bract isolines, with acceptable levels of fiber and maturity.

Frego bract is associated with an increase in susceptibility to plant bugs and to fleahoppers damage, causing delayed flowering and maturity in addition to reducing yield (Jones, 1982; Thaxton et al., 1985; Jenkins et al., 1973; Waddle, 1972). Lincolin et al. (1971) reported that *Lygus hesperus* was generally more abundant on frego bract than normal-bract cotton. There was no significant difference on *Empoasca* spp. and *Trichoplusia ni*. The nectariless trait was found to reduce the severity of the plant bug damage associated with frego bract trait. The specific mechanism for enhanced susceptibility of frego bract cotton should be explored. If susceptibility is conditioned by chemical factors genetically distinct but linked to the frego bract trait, it may be possible to sever the linkage and select frego bract genotypes of normal or reduce susceptibility. Alternatively, combinations of several resistance factors such as large levels of gossypol and reduced densities of nectaries might be used to counter susceptibility (Schuster et al., 1976a).

Color

Red vein, red stem, and red margins were found to be allelic to each other (R_2) and allelic to R_1 and R_1dar. Agronomic studies of red types indicated that red vein, red stem, and red margins are comparable with green isolines in yield and quality of fiber, but red leaf and red darwinii are negatively associated with yield in a high-yield potential environment. The performance of some hirsutum varieties with red, brown, green and pale green colored lint were as good as other cultivated varieties with white lint in yield of seed cotton.

Some of the colored cottons were found to be as highly tolerant to sucking insects as some of the cotton varieties with white colored lint (Thaxton and El-Zik, 1994). An upland cotton strain

Table 1: Morphological Characters Resistant to Sucking Insects in Cotton

Morphological Character	Gene Symbol	*Empoasca* spp.	Mirids	Whiteflies	Thrips	Aphids
Frego bract	fg	N	S	N	N	?
Nectariless	ne_1, ne_2	?	R	N	N	?
Glabrous	Sm_1, Sm_2, Sm_3	S	S	R	S	R
Hirsute	H_1	R	R	N	N	R
Pilose	H_2	R	R	S	R	S
Okra leaf	L^0, L^8	N	N	R	N	R
Red color	R_1, R_1^{dar}, R_2	?	?	R	?	R
Bractedness		?	?	?	?	S
High gossypol	Gl_2, Gl_3	R	N	S	S	?

Source: Modified after Schuster (1980)

Table 2. Advantages and Disadvantages Resulting from the Incorporation of Resistance Characters into Cultivars

Advantage	Disadvantage
Pilosity	
1. 50% increase of mirid tolerance to feeding.	1. Gin and mill dust increase. 2. Slight yield and quality reduction. 3. Bollworm oviposition 2x on hirsute, 4x on pilose. 4. Increased cotton aphids density.
Glabrous	
1. Less gin and mill dust. 2. Reduced cotton aphids density.	1. 40% increase in mirid damage. 2. Increased *Empoasca* numbers. 3. Slight yield reduction.
High Gossypol	
1. Significant mirid reduction.	1. High seed gossypol results in reduced seed value for food. 2. Slight increase in thrips and spider mites susceptibility.
Red Color	
1. Slight boll weevil non-preference. Useful only as trap-crop. 2. Resistance to potato whitefly. 3. Resistance to aphids.	1. Yield reduction, greater intensity results in greater reduction.
Frego Bract	
1. Increased insecticide coverage. 2. Reduced boll rot.	1. Increased mirid damage 2-3 fold.
Nectariless	
1.60% reduction in mirid. 2. Reduced boll rot.	1. Removal of nectaries reduces food of certain parasites and predators.
Okra Leaf	
1. Confers resistance to whiteflies and aphids. 2. Produces fiber with less trash.	1. More attractiveness to plant bug complex. 2. Increased need to herbicides for late-season weed control.

Source: Modified after Schuster (1980)

carrying all three genes for red color was most resistant to potato whitefly, *Bemisia tabaci*, and strains carrying only one of the three were intermediate in resistance. (Smith and Meagher, 1994). Red plant color confers resistance to cotton aphids.

Alate aphids are attracted to leaves reflecting about 500 nm regardless of the species of plant because they seem to be attracted to plants at physiologically suitable stage of growth. Yellow is usually most attractive to aphids (Orter, 1966). Therefore, breeding plants that germinate rapidly and grow fast so as to form a continuous green surface might be advantageous.

Mutant strains generally were similar in fiber uniformity, elongation and micronaire, but red plant strains produced a higher micronaire value.

Bractedness

Effect of bractedness on shedding of squares due to thrips, *Frankiniella accidentalis* was significant greater in abnormal squares which have more than 3-bract squares. Thrips were the primary cause of shedding of abnormal squares and a secondary cause of shedding in 3-bract squares (Hollis and Curtice, 1988).

Gossypol Content

Plants of the genus *Gossypium* have epidermal pigment bearing glands; the principal component of the glands is gossypol, a polyphenol yellow pigment, an allelochemical in cotton, is toxic to many cotton pests (Lukefahr and Houghtaling, 1969). Gossypol is toxic to non-ruminant mammals. Some gossypol free cotton varieties have been developed. Chemical and physical process for removing glands and associated pigments from cotton seed oil and meal are expensive. Hence, the decision for glanded cotton should be taken on a cost benefit basis.

Dominant alleles at the GL_2 and GL_3rai loci are responsible for the presence of glands. The relative potency for pigment gland density and gossypol content among three $G1_3$ alleles was Gl_3' >gl_3rai>Gl_3. Gossypol content can be decreased from a normal 5% to 1.5% genetically. Probably the most useful of five visual selection methods for high gossypol content in high x low F_2 populations was a rating of the number and size of glands on the calyx lobes.

Previously the glanded condition was believed to be resistant to *Lygus* bugs (Bottger et al., 1964; Tingey and Pillmar, 1977). Glandless cotton strains with level of resistance to *Lygus* similar to glanded ones are known. The insect can feed between glands. However, high gossypol content increases plant susceptibility to thrips and whiteflies (Schuster, 1979).

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Exploring New Cotton Genes for Resistance to Cotton Insect Pests and Diseases

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Introduction

Cotton, the crop of commerce, history, industry and civilization, attracts renewed global interest. Cotton is noted for its vulnerability to many insect pests and diseases like bollworms, jassids, whitefly, bacterial blight and wilts. To maintain the desirable yield levels, these pests and diseases are to be managed with frequent application of large amounts of insecticides. Major insects developing pesticide resistance, frequent resurgence of certain pests and diseases, disruption of natural enemies/predators and environmental and health hazards are the major concerns encountered. Of late, cotton production performance in major cotton growing countries, notably in China and Pakistan, has been reported to be affected significantly due to insurmountable insects and disease problems.

In order to overcome the foregoing problems, the world's cotton industry is now focussing its attention to reduce dependence on chemical pesticides to a substantial level by way of introducing integrated pest management strategies focusing on biocontrol agents, botanical pesticides, pheromones, moulting inhibitors, resistant cultivars, etc. Of the various strategies contemplated in controlling pests and diseases without disturbing the ecosystem, manipulation of gene and evolving transgenic cotton and utilization of a rich gene pool for increasing host plant resistance are now considered important.

India is bestowed with a vast collection of cotton germplasm numbering around 6000 accessions of the four cultivated cotton species and their wild relatives. This offers a tremendous scope for exploiting the hitherto untapped novel genes for host plant resistance. Introduction of pest resistant cultivars both by conventional and genetic engineering technologies could result in higher crop production, reduced pesticide usage and thereby ensuring ecological safety.

The present paper reviews the prospects of exploring the use of novel genes for resistance to cotton pests and diseases.

Genepool Evaluation for Host Plant Resistance

Bollworms Resistance

Out of one thousand germplasm accessions screened for resistance to bollworms *(Earias vittella, Helicoverpa armigera* and *Pectinophora gossypiella)* under field infestation, 25 entries (Table 1) were found to have moderate level of resistance and less than 10% boll damage (Anon, 1986; 1990). Bhat and Jayaswal (1988) reported that eight isogenic lines of AET 5 with nectariless, smooth and okra type leaves either singly or in combination reduced the square damage by 33 to 61% by bollworms as compared to their counterparts. An okra genotype Stoneville 7A Okra and AET 5 have been reported as source of resistance to pink bollworm *(Pectinophora gossypiella)* under field conditions (Wilson, 1990). *Gossypium hirsutum* lines possessing nectariless, smooth leaf, frego bract and high flower bud gland density yielded more than the commercial cultivar "Deltapine 41" under high insect pest pressure (Calhoun, 1992).

Densely haired culture L92 was the most preferred one for oviposition by *E. vittella* (52 eggs/plant) and a glabrous, pigmented, nectariless and frego bract culture, FBRN 2-6, was the least preferred one with 3 eggs/plant (Surulivelu and Sundaramurthy, 1988). Further, the eggs were not firmly attached to the plant parts (leaf, square and bud) in the resistant culture as compared to the susceptible culture.

Table 1: Field Tolerance/Resistance to Bollworms

Sr. No.	Accession/Cultivar	% Boll Damage	No. of Bolls per Plant
1.	IC 1021	7.9	10
2.	IC 1028	3.6	6
3.	IC 1029	9.8	12
4.	IC 1030	10.2	10
5.	IC 1053	8.3	12
6.	IC 1059	8.8	18
7.	IC 1064	10.4	10
8.	IC 1066	8.0	10
9.	IC 1073	5.0	10
10.	IC 1078	9.1	7
11.	IC 1085	6.7	8
12.	IC 1111	8.3	12
13.	IC 1146	9.1	11
14.	IC 1572	9.4	6
15.	KG 1128	4.3	18
16.	KG 5-29	6.6	17
17.	FBRN 2-6	9.1	7
18.	HG IPS 625	8.9	12
19.	JK 270-6	8.2	15
20.	AET 5 NSL	5.7	11
21.	DF 61-4	6.4	11
22.	JK 345	9.3	11
23.	BCS 1-9	7.9	11
24.	BCS 9-67	8.5	12
25.	G 27	5.4	6
26.	IC 2013 (Susceptible)	86.9	6
27.	IC 2005	85.7	4
28.	IC 2018	100.0	2

Interestingly, FBRN 2-6 was less preferred for feeding by *H. armigera* (Surulivelu and Sundaramurthy, 1992). The cultures of *G. hirsutum* viz., 8/3 and MESR 17 were also less preferred by *E. vittella* for oviposition (9 eggs/plant) as compared to the hairy commercial cultivar MCU 5 VT (21 eggs/plant). The larval growth of *E. vittella* was significantly retarded in the resistant genotypes like MESR, JK 345, FBRN 2-6 (3.50-5.01 mg/day) as compared to LRA 5166 (14.08 mg/day).

Disease Resistance

A horizontal, polygenic type of tolerance is preferred. This is relatively more stable than absolute resistance to verticillium wilt which is liable to break when a new physiologic race of the pathogens emerge. Several sources of such multiple genes for resistance are available in *G. barbadense* for verticillium wilt *(Verticillium dahliae)*. On the other hand, resistance in Hopicala, Aleppo 40 *(G. hirsutum)* and semi wild species of *G. hirsutum* ssp. *mexicanum* var *nervosum* of Leningrad strain is due to a single dominant gene. In India, Hopicala a hirsutum cotton from USA has been found resistant and is used in breeding as a gene source for verticillium resistance to breeding.

Bacterial blight, a disease of Indian origin is of serious concern wherever cotton is grown. This is because of a number of virulant physiologic races of the pathogen (*Xanthomonas campestris* pv. *malvacearum)* and the limited range of the sources of resistance involving around 19-20 genes and gene complexes. The Indian cottons (*G. arboreum* and *G. herbaceum)* are highly resistant. In India, *G. hirsutum* genotypes like Reba B50, BJA 592, 101-102B and Badnawar 1 have been used in the breeding program for development of bacterial blight tolerant and high yielding varieties. At this Institute, RKR 4145, Culture 1412, Glx 2/1, KG 5-29 and KG 9-18 have been found resistant.

A few *G. hirsutum* germplasm lines viz., 45/1, RA 33-12 479/25, Kunjit White, 2196-4 and PK 860 with favorable biochemical characteristics and resistance to areolate mildew *(Ramularia areola)* have been deployed in resistant breeding programs. *G. hirsutum* germplasm collections viz., HB 55, 0494-4, EL 500, CTI 4-25-28, 2196-4 and PK 860 have been found resistant to alternaria leaf blight caused by *A. macrospora.* These resistant sources are utilized in the development of the alternaria resistant lines and several progenies combining resistance and yield have been selected.

Breeding Resistant Cotton Cultivars

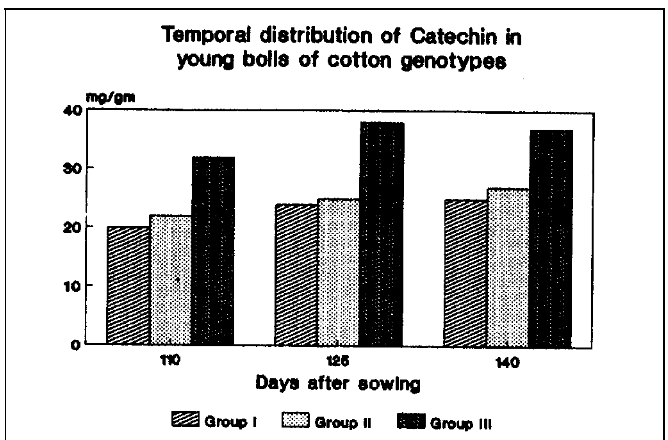
A glabrous cultivar possessing resistance to whitefly was developed and released as "Kanchana" (Henry et al., 1990). An early maturing and moderately glabrous culture was developed and released as bollworm resistant cultivar "Abadhita" for commercial cultivation in south India. Two cultures viz., 29-20-22-1 and 19-9-6-2 possessing bollworm resistant characteristics like okra leaf, fregobract, tough bolls, moderately glabrous and early maturing have been developed at this station and are in advanced stage of testing.

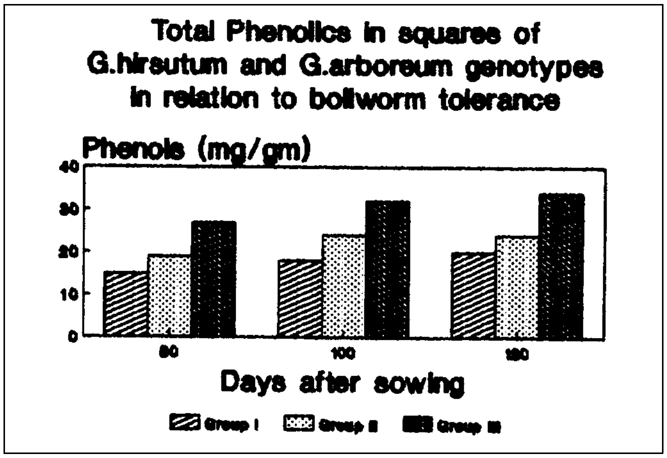
By reselection of the rarely occurring resistant mutants or segregants from a population of elite and susceptible variety, a verticillium tolerant genotype MCU 5 VT was evolved (Srinivasan et al.,1976). By adopting a series of back crosses, the resistance found in the semi wild *G. hirsutum mexicanum* has been transferred to an MCU 5 background and a series of highly resistant cultures have been developed, including the high yielding VRS 7 (Kannan and Gururajan, 1992). Promising bacterial blight resistant selections have been obtained from crosses involving LRA 5166 (a high yielding but susceptible cultivar) and MCU 10 (resistant). Of these, CBR 1 has given significantly higher yield than LRA 5166 (Kannan et al.,1994).

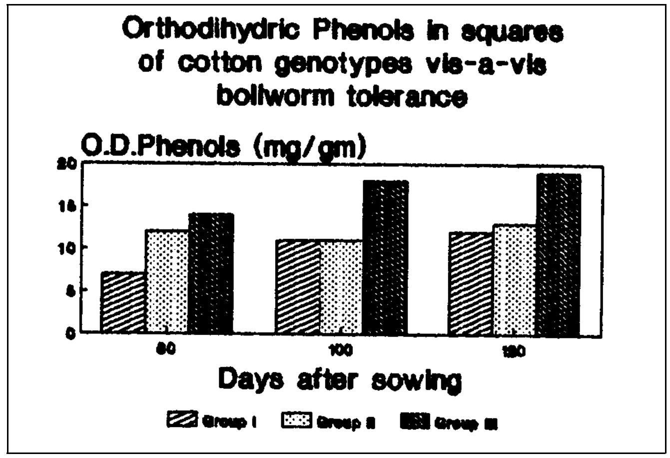
Identification and Utilization of Biochemical Traits for Pest and Disease Resistance

Plants are endowed with certain biochemical factors for resisting numerous pests and diseases. Certain constitutive and infection-induced secondary metabolites of cotton act as antibiotics for inhibiting pests and diseases (McColl and Noble, 1992). Among the cotton species, *G. barbadense* is comparatively more resistant than *G. hirsutum* to several soil borne fungal pathogens and certain insects. It is documented that around 40-60% of constitutive and induced terpenoids in *G. barbadense* exist as methyl ethers, enhancing their antibiosis to pests, as compared to 10% in *G. hirsutum*. In addition, crosses between *G. hirsutum* and *G. barbadense* gave F_1 progeny that had no terpenoid methylation in pigment glands, while F_2 progeny segregated approximately into 1 methylated: 3 nonmethylated plants indicating that a dominant gene, perhaps a regulator gene, inhibits methylation in *G. hirsutum* (Bell and Stipanovic, 1978). Similarly, the formation of the ocimene and myrcene precursors of the heliocides are under the control of separate dominant genes. Therefore, the possibility exists in developing cottons having heliocides from ocimene or myrcene or both.

In *G. arboreum,* two flavanoids, namely gossypetin 8-o-rhamnoside and gossypetin-8-o-glucoside which were absent in *G. hirsutum,* were the contributing factors for resistance to feeding







by *Heliothis virescens*. In a study conducted at this Institute, it was observed that most of *G. arboreum* genotypes possessed higher levels of phenolics in squares and young bolls as compared to *G. hirsutum* (as shown in graphs). A combination of a moderate level of gossypol along with higher level of condensed tannin and catechin, accompanied by less nutritive substances for bollworms in fruiting parts during the reproductive phase, dispose the plants to lesser damage by bollworms (Gopalakrishnan et al., 1993). The number of gossypol glands in calyx and bract tissues of squares of resistant lines was significantly greater in susceptible lines (Hedin et al., 1992). With regard to host-pathogen interaction, the speed with which desoxyhemigossypol is synthesized may determine the resistance of cotton plants to *V. dahliae*. Phytoalexins produced quickly and in higher concentrations may determine disease resistance against fusarium wilt. Phenolics and terpenoids may induce resistance against root rot, while asparagine induces susceptibility against anthracnose. Cuticle thickness and peroxidase activity confer resistance against boll rot pathogens (Vidhyasekaran, 1990; Srinivasan,1994).

The speed, quantity and structure of metabolites are important variables in determining how quickly an antibiotic environment is developed. It is, therefore, felt that biochemical engineering for improved pest and disease resistance is now possible in cotton and it should allow development of resistant cottons not attainable by classical methods of screening for resistance.

Prospects of Exploring New Genes for Cotton Pest and Disease Resistance

Pest management strategies continue to receive utmost priority both through conventional and biotechnological research due to its obvious implications in increasing cotton production. Prospects of inserting desirable foreign genes have opened up new vistas for achieving the goal in a shorter span of time. The agrobacterium mediated transformation method continues to be the choice for several institutions in the world. With further advancement of biotechnology, encapsulation of DNA within microdroplets of polar lipids, microinjection, electroporation, particle bombardment techniques, ovule and cell culture, meristem culture etc. are now paving the way for easy and quick transformation of transgenic cotton plants with desirable traits.

Nearly all biotechnogy laboratories working with cotton transformation have included Bt toxin genes as a part of their program. Scientists at Agracetus Inc. in collaboration with USDA scientists field tested the cotton plants engineered with a Bt toxin gene (Jenkins et al., 1993; Umbeck et al., 1990). In an effort to increase plant expression of a Bt gene, Monsanto scientists reported success in the modification of a triplet code of a Bt gene to make it more compatible with the genetic codes preferred by the plant protein synthesizing system (Perlak et al., 1990). High levels of expression of this Bt toxin have been obtained in cotton. With the current active search for novel *Bacillus* strains, the range of insects to which toxins are effective is likely to increase.

Other peptides, lesser known than Bt toxins, have a potential as insecticides or as feeding deterrents. The protease inhibitors as a class are receiving considerable attention (Ryan, 1990). When the insect diet contains a protease inhibitor in addition to another insecticidal toxin, the efficacy of the toxin can sometimes be increased, because the toxin is not broken down quickly. The genes for three protease inhibitors from the tobacco hornworm itself have been isolated and transformed into cotton tissue and plants regenerated with inhibition to those insects with an alkaline gut. Transforming plant cells or tissues with aprotinin (a serine protease inhibitor) encoding DNA and regenerating insect resistant plants holds much promise.

Lectins are a class of proteins that also act on the digestive system of insects. Transformation of cotton with lectin-like genes from monocotyledons and *in vitro* feeding studies are in progress to determine the efficacy of the lectins on selected cotton insect pests (Stewart, 1981). Another class of proteins being examined for use in genetic engineering for host plant resistance is the insecticidal venoms of the arachnid group. Scorpions and spiders prey on insects and kill or paralyze them with a venom that contains a variety of insecticidal peptides. There is great possibility for engineering some of these genes into plants without causing toxicity to human beings (Quicke and Usherwood, 1991).

The potential of engineering plants to produce insect repellents is another realm of definite possibility. A new source of genes that will be effective in insect control is the target insect itself. Insect development and behavior is controlled by an array of neuropeptides acting on the neural system. These peptides have low molecular weight and are effective in minute quantities; properties that make them ideal for genetic engineering. A cotton plant that can produce any number of these peptides could drastically alter the behavior or development of the insect pest. Identification of the responsible genes and ensuring the presence of these peptides from the cotton plant to the site of activity of the insect are the two major tasks ahead for accomplishing this feat; the reality is not far behind.

A new method for controlling lepidopteran insect infestation involves providing a 3-hydroxysteriod oxidase for ingestion by the insect larva. The possibility of a transgenic cotton plant expressing this gene has become brighter (Corbin et al., 1995). Use of cholesterol oxidase gene for control of *Heliothis virescens* in tobacco has also been indicated. Transgenic plants containing a recombinant cDNA comprising ds cDNA fragment derived from a shark virus RNA(Plum-pox virus) have been developed. It is used to increase the resistance of plants against pests and diseases like tomato spotted wilt virus, fungi and certain nematodes. Such an effort may also be useful in the case of cotton.

Promising results have been obtained using chitinase gene product in the biological control of *R. solani* in cotton (Shapira et al., 1989) and the enzyme may have a more suitable role in engineered host plant resistance. Humans and other mammals produce families of protective proteins called "defensins" (Ganz et al., 1990) and cecropins (Lee et al., 1989). A variety of different defensive proteins has been found in insects (Lambert et al., 1989) and magainins protect amphibian skin from infection. Future research strategies shall identify and involve these antimicrobial peptides through genetic engineering in host plant resistance of cotton to pathogens.

The foregoing research strategies suggest that transgenic cottons will occupy a pivotal status in cotton pest management in the world cotton scenario. However, development of any resistance in insect pests to Bt toxins and other such envisaged transgenic traits has to be continuously monitored. To obviate this eventuality, gene pyramiding of many insecticidal genes in selected genotypes shall provide a more stable system. It is appropriate to mention that any change conceived through genetical and biochemical mechanisms in the plant to make it less attractive to pests shall enhance further the capability of transgenic cotton plants.

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Reliable Bases for Resistance to Fusarium and Verticillium Wilts

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Fusarium and verticillium wilts are the greatest enemies of cotton. These fungal diseases reduce cotton yield and productivity, and negatively affect fiber and seed quality.

Over the years, researchers in many disciplines, including phytopathologists, mycologists, geneticists, breeders, etc., have spent much time and efforts trying to develop wilt resistant varieties of cotton by using all sorts of techniques (intra-specific and inter-specific hybridization as well as chemical and radiation mutagenesis). The massive endeavor has brought a measure of success, particularly in the breeding area, but resistance to fusarium and verticillium wilts to this day remains a major problem presenting unique challenges. For one thing, in trying to select plants for wilt resistance the breeder has to deal with two distinct problems: the tremendous intra-specific diversity of the host plants in terms of resistance, and the equally enormous diversity of the verticillium and fusarium fungi in terms of virulence. It is known that the phenotypic manifestation of a disease depends not only on the genotypes of the host and the pathogen, but also on the combination of environmental conditions, above all, temperature and humidity. The difficulty in developing wilt resistant cotton varieties and maintaining their resistance under field conditions is attributable to the fact that the pathogens undergo continuous morphological changes and persistently expand their geographic range, while the host plants constantly broaden their genotypic diversity. Still, breeding highly productive, wilt resistant cotton varieties constitutes one of the most radical, economically advantageous and environmentally sound methods of achieving the foregoing objective. It has been observed that no investment will bring a greater return in terms of improved living standards worldwide than continued attempts to breed new varieties of agricultural crops.

Constant screening for new, reliable sources of resistance is a necessary stage and a major requirement of any program of breeding for wilt resistance. In the late 1950s - early 1960s, S.M. Mirakhmedov from the Academy of Sciences of Uzbek Republic IEBP (Institute of Experimental Biology of Plants), discovered a wild, wilt resistant Mexican variety (*G. hirsutum* L. ssp. *mexicanum* var. *nervosum*) and crossed it with certain cultivars to produce wilt resistant, high-yield cotton varieties, Tashkent-1, Tashkent-2 and Tashkent-3, which have long dominated Uzbekistan's cotton sector and been widely used in cotton breeding programs run by other research centers. A number of other cotton varieties were also developed. However, wide commercial utilization of genetically identical or close cotton varieties resulted in the loss of their wilt resistance and the emergence of even more virulent strains of the fungus adapted to overcome plant resistance. Clearly, new, reliable sources of resistance to the pathogens were needed. The search for wilt resistance donors has focused on the cotton collections of a number of Uzbek research institutions, such as the SPO "Biologist" (Institute of Experimental Biology of Plants), USRIP (Uzbek Scientific Research Institute of Plant), and USRIPBSC (Uzbek Scientific Research Institute of Plant Breeding and Seed Production of Cotton).

A major research program to discover new sources of wilt resistance is under way at the Uzbek Republic Academy of Sciences SPO "Biologist" (Institute of Experimental Biology of Plants). The effort spearheaded by the Systematics and Species Formation Laboratory (A.A. Abdullayev) makes particular emphasis on wild varieties and their forms as well as various cultivars of *G. hirsutum* and *G. barbadense* L.

A. G. Rakhimzhanov and M. Bolbekov from the USRIP have established that the form *Ucatanense* and the species *G. thurberi* and *G. trilobum* show resistance to two strains of the verticillium fungus, while *G. aridum* and *G. davidsonii* are resistant to the 2nd race of verticillium.

S. S. Alihojayeva from the USRIPBSC reports that *G. tricuspidatum* ssp. *purpurascens* is an excellent donor of wilt resistant genes. U.. Ikramova from the same institution proposes conducting preliminary testing and evaluation of the breeding material for wilt resistance at the stage of 3 to 4 true leaves for 20 to 24 days in an artificial-climate chamber at a temperature between 27° C and 29° C, and selecting resistant plants at a temperature of 24° C to 26° C. This procedure permits identifying and rejecting poorly resistant seeds in winter, prior to planting.

Fusarium wilt is every bit as pernicious as the verticillium form. Up until recently, many experts believed that fusarium wilt attacked only cotton varieties belonging to the *G. barbadense* L. species. But in recent years not only fine cotton, but also medium staple cotton varieties have been affected with fusarium wilt. The annual growth of fusarium wilt on *G. hirsutum* L. cotton poses additional challenges for breeders to develop new, wilt resistant varieties, hence, necessitates to look for donors conferring resistance to fusarium as well as verticillium wilts.

V. P. Senoyedov from the Cotton Wilt Resistance Genetics Laboratory of the Uzbek Republic Academy of Sciences "Biologist," studying strains of various geographic origins and belonging to various species from the collections of the USRIP, USRIPBSC and SPO "Biologist" Research/Production Association in artificial, race-differentiated infectious environments, has identified a number of *G. hirsutum* L. and *G. barbadense* L. strains exhibiting group- or race-specific resistance to races 1, 2 and 3 of the verticillium fungus and races 1 and 2 of the fusarium fungus. The greatest promise for breeding purposes has been shown by the strains of the species *G. barbadense* L. from Peru (Pais-1), the US (Pima-5) and Galapagos Islands (*G. darwinii*) resistant to verticillium and fusarium wilts and amenable to crossing with *G. hirsutum* L. cottons. Besides, some forms of *G. hirsutum* spp. *mexicanum* var. *microcat* from Mexico and *G. barbadense* spp. *vitifolium* from Colombia have been found to be resistant to verticillium race 2. The researchers have further identified varieties of the diploid species (*G. herbaceum* L. and *G. arboreum* L.) highly resistant to fusarium races 1 and 2: C-7058, C-7060, Jai-Dar and Malle. They do not lend themselves readily to hybridization with tetraploid varieties; however, they can be used as repollinators, in genetic engineering, and also after pretreatment with colchicine. Among the resistance sources identified, the varieties Pima-5, Pais-1 and *G. barbadense-darwinii* have been successfully used for breeding purposes.

Analysis of the search for wilt pathogen resistance donors suggests that while there is no dearth of proven sources of resistance, they are containing very few factors reliably conferring general and specific resistance. The field is dominated by identical genes imparting specific protection against pathogens, which is the reason why modern strains are by and large based on a very narrow pool of resistance genes whose genetic homogeneity makes them potentially vulnerable to pathogens. There is an acute shortage of resistance genes which threatens to grow into the number one problem in breeding for immunity. Considering modern breeding requirements, this fact is decisive as far as donor selection is concerned.

Breeders exhibit considerable interest in plant forms with durable traits of moderate resistance or tolerance as well as varieties capable of slowing down or pushing back the development of the disease. The success of the breeding effort will depend by large on our ability to choose the right approach to the selection for resistance. It is our belief that research of this nature should be conducted in differentiated, controlled, artificial infectious environments with subsequent verification in a natural wilt-infected environment to ascertain the true resistance of the variety being tested.

Whether or not the wilt resistance trait is genetically determined is hotly debated in our country and elsewhere. The results and the conclusions drawn on their basis are far from unambiguous; some researchers believe that wilt resistance is inherited by a simple monogenic trait, others put their faith in polygenic heritability, and both schools of thought marshal convincing evidence in support of their arguments. Neither has it been settled which type of resistance to be selected for: race-specific (vertical) or general (horizontal).

At the dawn of cotton research in Uzbekistan, the preferred method was one of intra-specific, inter-varietal hybridization, selection of the best plants. But as far back as the 1950s, this technique exhausted its potential for breeding wilt resistant varieties. New approaches to breeding for wilt resistance were needed.

Analysis of the literature and practical results suggests that the field of cotton breeding for resistance to wilt pathogens is currently dominated by traditional methods, viz. intra-specific geographically distant hybridization and inter-specific hybridization with or without back crossing. Experimental radiation and chemical mutagenesis also enjoy some popularity.

As noted above, hybridization on the basis of a wild form of Mexican cotton has led to a range of wilt resistant varieties, but they quickly lost their resistance, becoming susceptible to new, more virulent forms of pathogens. New, early varieties combining improved wilt resistance with valuable economic traits are needed. For this reason, we believe that breeding for wilt resistance should be a continuous program with a view to forestalling massive attacks of new, aggressive and virulent forms of pathogens.

Thus, V. A. Avtonomov from the USRIPBSC, using the subspecies *punctatum* for hybridization, has succeeded in developing a number of wilt tolerant varieties (C-6524, C-6030, Namangan-77) that have been introduced in commercial practice in Uzbekistan. More promising lines have been developed.

Recently, inter-specific crossing, using *G. hirsutum* L. and *G. barbadense* L. as parents, has gained acceptance alongside intra-specific distant hybridization. In a wilt-infected environment, inter-specific hybrids are less susceptible than their intra-specific counterparts. *G. barbadense* L. cotton varieties and forms have been known to be highly tolerant to verticillium wilt ever since phytopathologists and breeders had to study cotton strains for this trait. A.A. Avtonomov from the USRIPBSC has taken the lead in crossing *G. barbadense* L. with *G. hirsutum* L., developing a new variety, C-6501, but it has not been commercialized.

Researchers at the Uzbek Republic Academy of Sciences "Biologist" have used intra-specific distant hybridization and inter-specific hybridization of a range of *G. hirsutum* L. and *G. barbadense* L. varieties and forms to develop a number of early, high-yield cotton varieties and lines tolerant to fusarium and verticillium wilts.

Thus, the Cotton Heterosis Laboratory (O. Z. Zhalilov), using the geographically distant hybridization and inter-specific hybridization techniques and radiation mutants, has developed the following commercial varieties widely used in our Republic: Samarkand-3, Ok-Oltin, Farhad, and Yulduz. The early, high-yield Yulduz variety is particularly widespread. Two more varieties, Mehr and Duru-Gavkhar, are successfully undergoing state testing at this time. Several cotton lines possessing a combination of valuable economic traits have also been developed.

The Cotton Wilt Resistance Laboratory (V. P. Senoyedov), using artificial environments infected with the verticillium and fusarium races most widespread under field conditions, has developed early, high-yield, wilt tolerant cotton varieties. Particularly large areas are planted to Tashkent-6 cotton developed by means of intra-specific distant hybridization and tolerant to verticillium race 1. Being an early variety, Tashkent-6 is also used for reseeding in cases of crop failure. Another variety, Arzu, is successfully undergoing state testing. This cotton developed by means of inter-specific hybridization is tolerant to two verticillium races and has a superior lint yield. A new, wilt tolerant, high lint yield variety Parvaz has been cleared for testing and evaluation.

The Applied Genetics Laboratory (S. S. Sadykov) has split the Tashkent-1 population into separate biotypes followed by self-pollination to develop a new variety, AN-Bayayut-2, which has gained considerable popularity in the Uzbek cotton farming community.

The Radiobiology Laboratory (N. N. Nazirov and F. Janikulov) has developed wilt tolerant cotton varieties AN-402, AN-410 and AN-415 by radiation mutagenesis using radioactive phosphorus and subsequent selection in a wilt-infected environment.

Aside from the search for wilt resistance donors and breeding for that trait, other vital lines of research are development of cotton varieties maintaining their resistance to pathogens or less susceptible to diseases; controlling the pathogen population with a view to timely identifying new, virulent forms of the parasite before it is capable of mounting a massive attack on the crops; and developing other approaches to the task of controlling cotton wilts.

The Pathophysiological Laboratory (M. Kh. Avazhojayev) has developed a method of improving the wilt resistance of cotton by artificially enhancing its immunity and identified substances for physiological stimulation of wilt resistance which impart high antiwilt activity and, as an added benefit, improve the yield of seed cotton.

A determined antiwilt screening effort has resulted in the development of Bisol-2. Laboratory and field tests have demonstrated that this preparation is effective in reducing the susceptibility of cotton to wilts by 30 percent to 40 percent.

The immunodepressive effect (synthesis of phytoalexins) has been recommended as a physiological test for selecting cotton strains resistant to verticillium race 2.

The Molecular Genetics Laboratory (A. P. Ibragimov) has proved the feasibility of using the DNA for purposeful manipulation of genomes, specifically as a way to develop new, wilt resistant cotton forms. Research along this line has given an insight into the nature of virulence, permitted determining whether the pathogenic fungi undergo transformation, and helped to solve some other problems germane to wilt control.

Measures aimed at reducing soil infection and improving the productivity of wilt-infected cotton fields constitute an important reserve of cotton farming.

M. V. Mukhamedjanov has evaluated the effectiveness of agricultural techniques for wilt control on typical serozem and sasa meadow soils and found that three-year crop rotation of alfalfa-corn or joughara combined with special tillage practices provide for dramatically improved productivity, leading to an enormous increase in the population of cellulose-destroying fungi and highly active antagonists of *V. dahliae* paralleled by a reduction in the number and diversity of toxigenic fungi. As a result, the infectious potential of the pathogen is reduced by a factor of 2 or 3.

Thus, in spite of certain successes in wilt resistance studies, the problem has not lost its importance.

Even among the zoned cultivars, it is difficult to select a leader in terms of wilt resistance. Hence, we need new cotton varieties featuring diverse genotypes and possessing multiple or group resistance or tolerance to wilt pathogens.

Another priority, as we see it, is to find out why the newly developed varieties lose their wilt resistance. Unless we find the answer to this question, breeders will not be able to achieve their goals. Once the mechanism of wilt resistance fading is understood, wilt-infected fields can be cultivated in an appropriate manner, maintaining the resistance of new varieties for a longer time, and obtaining stable seed cotton yields with a high fiber quality.

New Sources of Genetic Resistance to Cotton Insects

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Introduction

It is well documented that cotton is native to the Indo-Pakistan subcontinent (Gulati and Turner, 1928) and that cotton fabrics dating back to 3000 BC were found in the excavations of Mohenjo Daro of Pakistan. Old specimens of Dacca muslin indicate not only cultivation but also the art and craft of cotton spinning and weaving well-known to this sub-continent and older than that of other continents. Cotton specimens dating 2500 BC were also found in excavations in Peru (Bird and Mahler, 1951). These findings provide ample evidence of an ancient cotton culture both in the Old and the New Worlds. The oldest written record of cotton by Marco Polo and his contemporaries, however, reveals that the aborigins of India had long known cotton both as a plant and as a fabric. The Indian sub-continent as a whole and particularly the Indus Valley can thus be presumed to be the oldest center of the cotton industry.

Cotton is a warm-weather-loving crop generally grown in the tropics and temperate zones. It is produced in the north between 37-47° latitudinal and in the southern hemisphere extending to 32° in Australia and South America. Cotton is generally a long-season crop requiring 180-200 days to mature, where at least 140 days are frost free. Different varieties show considerable flexibility in adapting to different soil, rainfall and cultural systems. This broad adaptation is due to its indeterminate fruiting habit which enables the plant to regulate its fruiting during stress and to resume when the conditions are favorable.

Among the major adversaries of the cotton crop, the most important stresses are temperature, moisture, pests and diseases. The major goal of cotton breeding programs has been yield, quality and adaptability; nevertheless, plant resistance to pests and diseases is also of prime importance.

Cotton is attacked by a large number of pests in almost all the cotton producing countries. Due to the crop's economic importance, much attention has been given to pest control research. Hargreaves (1948) published a list of 1,326 species of cotton insects, 15% of which were considered pests and less than half proved to be of economic importance. Aston and Winfield (1972) listed 46 groups of insects known to occur in cotton throughout the world, 42 classified as economically important in one or more cotton producing countries. Dahms (1943), Painter (1951) and Maxwell et al. (1972) reviewed the status of research on insect resistance in cotton. The occurrence of insect pests varies from place to place, some of which are considered to be the most destructive to cotton in most parts of the world. These insects attack different parts of the cotton plant in varying intensity at different stages of plant growth. Pakistan cottons are not an exception. About 145 species of insect pests are recorded on the cotton plant in Pakistan. However, only about a dozen of these are major pests and cause economic losses (Shafi, 1993). These are listed in Table 1.

Surface grasshopper *(Chrotogonus* spp*.),* black-headed cricket and termites attack the crop at seedling stage. Thrips, jassids,

Table 1. Major Insect Pests that Attack the Cotton Crop in Pakistan

Sr. No.	Common Name	Technical Name
1.	Indian cotton jassid	*Amrasca devastans* (Dist.)
2.	Sweet potato whitefly	*Bemisia tabaci* (Genn.)
3.	Black thrip	*Thrips tabaci* (Lind)
4.	Yellow thrip	*Scirtothips dorsalis*
5.	Cotton aphid	*Aphis gossypii* (Glover)
6.	Spider mite	*Tetranychus urticae*
7.	American bollworm	*Helicoverpa* (*Heliothis armigera*) (Hb.)
8.	Spotted bollworm	*Earias insulana* (Boisd.) & *E. vittella* (F.)
9.	Pink bollworm	*Pectinophora gossypiella* (Saund.)
10.	Armyworm	*Spodoptera littoralis* (Hb).
11.	Black headed cricket	*Gryllus bimaculatus* (Deg.)
12.	Termite (white ant)	*Microtermes obesi* (Hol.)
13.	Cotton leaf roller	*Syllepta derogata* (Fb.)

aphids and a complex of mite species *(Tetranychus* spp.*)* are the foliage sap suckers. Cotton leaf roller, armyworm and cotton greyweevil *(Myllocerus maculosus* Desv.) are the foliage eaters. The fruiting parts (squares, flowers and bolls) are attacked by pink bollworm, American bollworm and spotted/spiny bollworm. Jassid, whitefly, pink, spotted and American bollworms are major pests. Mites and aphids do not occur regularly. Armyworm is sporadic. Baloch et al. (1982) and Soomroo et al. (1983) have reported occurrence of different cotton insects in Sindh and their resistance to certain morphological genetic traits. The economic loss rendered by these insect pests not only depends on the population of the attacking insects but also varies with the plant reaction to infection, the stage of the plant growth and the duration of the insect attack. Abiotic (temperature, humidity and rain) and biotic (interspecific and intraspecific competition, parasites and predators population) factors regulate the population dynamics of insect pests. Abiotic factors are uncontrollable whereas biotic factors are very sensitive and are affected by injudicious and indiscriminate use of pesticides, making the problem more complex.

At present, researchers and growers heavily depend on pesticide use to get the highest seedcotton yield and quality lint. There has been a tremendous increase in pesticide application in the world during the last decade, especially in Pakistan where the economy largely depends on cotton. At the same time, insecticides have brought many problems such as resistance, resurgence and the emergence of new pests as well as environmental contamination. The development of insect-resistant germplasm has received special attention and excellent work has been reported on cotton by many researchers around the world.

The purpose of this paper is to discuss the morphological/biochemical characters in cotton that make the plant unpalatable as a host for insects, the source of such characters, their manipulation for transfer to commercial cultivars and evaluation of that resistant material developed in Pakistan.

Plant Genetic Resources of *Gossypium*

Cotton belongs to the genus *Gossypium,* which has about 50 species (Fryxell et al., 1992). Of these, four are cultivated and the remaining forty-six are wild. The former are distinguished by their spinnable fiber. The wild species are lintless or produce short, sparse (dirty white to brown) seed hairs that are not spinnable. Two of the cultivated species, *G. arboreum and G. herbaceum,* are distributed throughout Africa and Asia with 13 pairs of chromosomes (called diploids). The tetraploids (2n=52), G. *hirsutum* and *G. barbadense,* are native to the Western Hemisphere. The origin of tetraploids through hybridization between Asian diploids and American wild species has long been confirmed cytologically.

The genus *Gossypium* is widely distributed in tropic and subtropic regions of the world. Hutchinson (1962) reported that the present day commercial cottons are almost annual plants derived from perennial forms under selection pressure for early maturity and elimination of photoperiodism. The true wild species of *Gossypium* are mostly perennial shrubs found in the frost-free deserts of Asia, Africa, Australia and the United States.

Centers of variability have been established for the four cultivated species of *Gossypium. G. arboreum* was developed into annual types in India, Pakistan, S.E. Asia, China and Africa. *G. herbaceum* has been identified in India, Pakistan, Central and East Africa, Iran and Afghanistan. The centers of variability for *G. hirsutum* are Mexico and Central America; while *G. barbadense* is attributed to northern South America, the West Indies and Central America.

Except for Egypt, Sudan and Peru where *G. barbadense* predominates and some parts of Southeast Asia where *arboreum* and *herbaceum* are also grown, the great bulk of cottons grown in the world today is American *(G. hirsutum)* or its derivatives. Despite the apparent narrow germplasm base of *G. hirsutum* varieties grown worldwide, these varieties have a surprising degree of plasticity, which allures the breeders to collect a large number of germplasm within *G. hirsutum* both for acclimatization and use in the hybridization program. This increasing interest of breeders in utilizing the broad range of germplasm in the improvement program is of special significance where the major objectives are either to improve fiber properties or pest resistance.

In cotton, genetic variability for insect resistance is available both from cultivated as well as wild species. In cultivated *G. hirsutum,* several varieties have been developed in the early half of this century in India and Pakistan with remarkable fiber properties, especially length and strength, resistance to bacterial blight *(Xanthomonas malvacearum)* and resistance to jassids. (Pakistan Central Cotton Committee Annual Reports, 1970 to 1994).

The use of geographical races or primitive stocks of *G. hirsutum* has increased in recent years by breeders for incorporating insect resistance in commercial varieties. The majority of these types are short-day, which flower sparingly or not at all during the normal growing season in the temperature zone. Flowering and boll setting in these types can be facilitated by growing them in greenhouses or in tropic to sub-tropic regions during the winter.

Wild Species of *Gossypium*

The wild species of *Gossypium* are a rich source of genetic variability which can be effectively utilized, though with difficulty, for the improvement of cotton. These wild species have great potential as genetic resources for transferring stress tolerance and pest resistance into cultivated commercial cotton. Differences in chromosome complements between wild diploids and tetraploids, including structural differences among chromosomes of different species, make it difficult to use them in hybridization. Of the 50 species reported so far, about 36 are culturable and have been grown at the Central Cotton Research Institute, Multan, since 1980 (Appendix I). This is one of the world's largest species collection ever grown together in a field. (Mirza and Sheikh, 1995). Some distinguishing characters of different species of *Gossypium* are given in Appendix II.

Interspecific Hybridization

Our hybridization efforts involving these wild species have demonstrated variable fertility relationships. From a large number of reciprocal crosses attempted in various combinations, only few interspecific crosses produced fertile hybrids. Although interspecific hybridization is a substantially difficult breeding program, the use of exotic species for crossing with local diploids *(arboreum* and *herbaceum)* offers remarkable potential for transferring novel traits of feral species to cultivated cottons.

Some fertile hybrids and their colchiploids are maintained as ratoon plants in the field as a reserve gene bank for utilization in gene transfer programs. The introgression of foreign species germplasm has been utilized effectively in several cases to improve genetic properties of our local *hirsutum* cultivars (Appendix III).

Gossypium Species and Insect Resistance

Cotton cytogeneticists and entomologists are increasing their efforts to find insect resistance in cotton. Both cultivated cottons and their wild relatives are being screened and measured for various components of resistance to insects based on morphological characteristics of the plant by visual observations, quantitative measurements of size, number, fecundity, mortality and biological activity of insect population. Soomro (1984) has done a comprehensive review of breeding for insect resistance in a multi-disciplined approach to cotton breeding. A brief account of major insect pests damaging cotton in Pakistan (Shafi, 1993) is also given here.

Pink Bollworm

The pest is native of the sub-continent, first recorded in 1894 in the united Punjab. It is the most destructive and insidious pest to control. It is distributed in all the cotton growing areas of Pakistan. The pest is active from April to October and is more common in humid areas. It passes through 4-6 generations in a year. It infests squares and bolls. The damage sometimes may be as high as 60%. Pink bollworm over-winters as larva diapausing in debris or in double seed. There is no potential alternate host plant in Pakistan. Its population can be considerably controlled by destroying the leftover bolls and double seeds where it diapauses during the off-season.

Spotted/Spiny Bollworm

These two species of spotted bollworm came into prominence in 1905. The caterpillars bore into young shoots, killing the growing plant. Larvae also feed on flower buds and green bolls. *E. insulana* is more common in severe climate while *vittella* thrives well in moderate climatic conditions. The pest passes through 6-8 generations in a year and remains active throughout the year. The peak activity is from July to September. The damage may be as high as 40%. Besides cotton, it also attacks *Abutilon indicum, Althea rosea, Hibiscus esculentus, H. rosasinensis, Corchorus* spp., *Grewia asiatica* and *Bombax matabaricum.*

Indian Cotton Jassid

According to Haq (1973), the pest first came into prominence as a serious cotton pest in 1913. Both adults and nymphs suck sap from the underside of the leaves. In a severe attack, the leaves dry up and fall off. The pest is active throughout the year. Peak activity is, however, in July and August. It is a major pest of cotton and okra and gets more serious in humid weather conditions. Damage to non-hairy varieties is to the extent of devastation. Besides cotton, it also feeds on brinjals, potato, beans, cucurbits, tomato, sunflower and hollyhock. There are about 11 generations in a year.

Sweet Potato Whitefly

According to Haq (1973), the pest was first noticed in the Punjab during 1915. It is polyphagous in feeding habit with about 12 generations a year. The attack is pronounced in fields which receive restricted irrigation. The pest sucks the sap of the leaves thus debilitating the plants. Besides this, the excretions make the lint sticky. The sooty moulds developing on the excretions blacken the lint. The peak activity of the pest is from July to September.

Bemisia tabaci has been identified as a vector of the cotton leaf curl virus disease (CLCV) belonging to the geminivirus group (Mansoor et al., 1993). Characterization studies based on the sequencing of coat protein, replicate and intergenic region of cloned CLCV showed that its nature is different from other geminiviruses. Two new sets of universal primers have been designed and used to differentiate CLCV from other geminiviruses found in Pakistan. (Annual Report of the National Institute of Biotechnology and Genetic Engineering, 1994).

American Bollworm

It is distributed throughout Pakistan and is a major pest of many crops including cotton. Other major host plants are gram, maize and sorghum. Alternate host plants include tobacco, tomato, many legumes and vegetables. The pest is a voracious feeder of flower buds and cotton bolls. The damage is sometimes colossal in unfavorable weather conditions. The population varies from year to year. The pest is active from February to October and passes through 6 to 8 generations in a year. Winter is passed in pupal stage in the soil or among plant debris.

Variability in *Gossypium* Species

The genetic variability in *Gossypium* species, as far as insect resistance is concerned, can be assessed through some morphological characters developed in plants. Morphological changes include hairiness of leaf (pilosity, dense pubescence, smoothness or glabrous, etc) size and shape of the leaf (small and okra), nectarilessness, high/low gossypol glands and modification in the bracts (small size and frego bract) which are related to insects directly or indirectly.

Hairiness

Jassid *(Amrasca devastans* Dist.) has been considered an injurious pest of cotton in Pakistan. It is active throughout the summer season with peak infestation in July and August especially in high humidity when the crop is at peak vegetative growth. Heavy infestations cause leaf shedding followed by the loss of flower-buds and bolls. When the attack is severe during boll formation, the quality of fiber is also affected (Afzal et al.,1943). Ahmad, et al., 1985 reported that season-long infestation of jassids caused significant crop loss and one jassid per leaf was the critical threshold for practical control purpose in the field.

Growing hairy varieties has been reported to have eliminated jassid as a major pest in tropical Africa (Reed, 1974). In Sudan, *barbadense* strains were developed carrying resistance to *E. libyca.* Selection for jassid resistance has also been successful in Australia.

Indigenous *arboreum* and *herbaceum* species collected from Mekran (Mirza and Ahmad, 1981) are highly resistant to jassids. From wild species germplasm grown at Multan, evaluation for insect resistance revealed that *G. tomentosum* 2 (AD)4 and *G. armourianum* D2-1 had high resistance to jassids which could be utilized in interspecific hybridization for transferring resistance. Parnell et al. (1949) considered the relationship of hairiness and jassid resistance to be one of the direct causes and effect, and not due to any genetic linkage between hairiness and some other factor conferring resistance. They concluded that lengthy hairs with increased density were associated with jassid resistance. Joshi and Rao (1959) cited that the presence of hairs does not necessarily confer resistance, but the toughness of leaf veins was the real source of resistance. Tidke and Sane (1962) concluded that the thickness of the leaf lamina is more determinant of resistance than the number of hairs on veins or lamina, length of hairs and angle of their insertion on the surface. Hairy varieties confer resistance to these sucking insects and sometimes to boll weevil (Stephens and Lee, 1961; Hunter et al., 1965; Niles, 1980) and to pink bollworm (Smith, 1975); but they intensify the problem of trash in lint, especially in machine harvested cotton.

Pilose (Dense Pubescence)

A pilose (dense pubescence, designated as H_2) character of *G. tomentosum* when transferred to *G. hirsutum*, imparts resistance to sucking insects like jassids, black thrips, yellow thrips and whitefly. Lee (1982) reported that H_2 gene, conditioning the degree of pilosity, is pleiotropic for short and coarse fibers.

Hybrid lines developed at Multan, from crosses of *hirsutum* x *tomentosum* from Hawaii, have considerable degrees of dense pubescence allowing a scarce jassid population but no damage to the leaves. Strain Cyto 12/91 has a ginning outturn of 33.7%, staple length 27.5 mm, micronaire 4.2 ug/inch and Pressley strength of 100 thousand lbs/sq inch. This is a new genetic source for breeding jassid resistant cotton. (Annual Research Report of CRI, Multan, PCCC, 1994).

Glabrous (Smoothness)

Smoothness (glabrous character) is reported to be resistant to cotton leaf worm *(Alabama argillacea)* and is useful in suppressing the *Heliothis* population, resulting in a 40-60% decrease in egg deposition on the leaves (Lukefahr et al., 1971). It is also reported to be resistant to boll weevil (Lukefahr et al., 1965). This character reduces Predatomoscelis *(Psulu senatus)* population by 5% when averaged over three years, two sites, genetic background and sampling dates (Ha, 1987).

Glabrous is controlled by a single dominant gene, D_2, (Meyer, 1957) which has been transferred to commercial cotton from a cross of *hirsutum* with *armourianum* following a backcross program beginning with a hexaploid hybrid (Mirza et al., 1992)

Stable lines readily crossible with this hexaploid have been developed and one strain, Cyto 2/91, finally selected has small, glabrous leaves less attractive to bollworms and leaf curl virus. In addition, Cyto 2/91 is early maturing, fits well in cotton-wheat-cotton rotation, sheds foliage parts at harvest and gives easier picking. (Annual Research Report of CRI, Multan, PCCC, 1994).

D_2 smoothness is responsible for removing hairs from upland cotton. This is a valuable trait in cotton, especially mechanically picked cotton because it reduces the amount of trash contaminating the lint. It also eliminates the incidence of bysinosis. These varieties are, however, susceptible to jassids and tarnished plant bugs *(Lygus)*, as reported by Meredith and Schuster (1979) and Jenkins (1982).

Nectariless

Both foliar and involucral nectaries are absent in *Gossypium gossypiodes and G. tomentosum.* Two recessive nectariless genes from the latter species were transferred to *G. hirsutum* and *G. barbadense* (Meyer and Meyer, 1961). Nectariless cottons do not provide an important food source for *Helicoverpa* adults, thus reducing their longevity and fecundity. Nectariless cottons also reduce population of cotton leaf worm, cabbage looper, tobacco bud worms and plant bugs without affecting predator population (Meyer, 1974). The material synthesized from *G. hirsutum* x *G. tomentosum* back crossed with *hirsutum* at Multan in 1980 is nectariless, moderately hairy and less attractive to insects. These lines which have a range of economic characters are shown below:

Table 2. Nectariless Strains with Economic Characters at CRI, Multan

Material	G.O.T. %	Fiber Properties		
		Staple Length	Fineness (ug/inch)	Strength (tppsi)
B 19/89	36.5	31.7 mm	3.9	100.2
B 1/91	36.5	29.0 mm	4.2	100.8
Cyto 11/91	36.0	29.7 mm	3.8	100.7

Nectariless and glabrous genotypes, however, suffer significant losses in yield and earliness components due to heavy infestation of cotton fleahopper (Lidell et al., 1986) which is, fortunately, not prevalent in Pakistan.

Modification in Bracteoles/Leaves

Modified flower bracteoles of *G. thurberi* and *G. hirsutum* race marie-galante offer resistance to pink bollworm (Anonymous, 1968) while frego bracts cause non-preference to boll weevil (Reddy and Weaver, 1975). Angelini (1959) reported that pink bollworm oviposition was reduced in *G. herbaceum* race *acerifolium* and *G. armourianum* having deciduous bracts. Khalifa (1977) reported that genotypes homozygous for frego bract, nectariless flower and high gossypol have significantly lower egg deposition of pink bollworm.

Table 3. High Gossypol Strains and their Fiber Properties Developed at CRI Multan

Strain	Origin	G.O.T %	Fiber Characters: Staple Length (mm)	Fineness (ug/inch)	Strength (tppsi)
Cyto HG-2/87	Back crosses of *G. hirsutum* with (*arboreum* x *herbaceum*) x 2 (*arboreum* x *sturtianum*)	35.0	25.9	4.3	94.3
Cyto HG-4/87	Back crosses of *G. hirsutum* with 2 (*arboreum* x *herbaceum*) 2 (*arboreum* x *sturtianum*)	34.0	26.7	3.7	95.3
Cyto HG-8/87	Back crosses of *G. hirsutum* 2 (*arboreum* x *herbaceum*) 2 (*arboreum* x *nandewarense*)	31.5	27.2	4.0	90.9
Cyto HG-9/87	Back crosses of *G. hirsutum* with 2 (*thurberi* x *davidsonii*) x 2 (*arboreum* x *australe*)	30.0	28.5	3.4	92.8

Some hexaploids developed from *hirsutum* x *armourianum* at Multan had deciduous bracts. This hybrid had very low infestation of bollworms as compared with their sibs with non-deciduous bracts.

Okra leaf has been reported to contribute significant resistance to pink bollworm damage (Wilson and George, 1982). Wilson (1986) showed that okra leaf breeding stocks, in certain backgrounds, yielded as much or more lint than normal leaf cultivars, in addition to resistance to pink bollworm. A high yielding variety Siokra (okra leaves) has been introduced in Australia (Thomson, 1985). This character has not been found to be of much advantage either for yield or insect resistance over normal leaf commercial varieties in Pakistan.

Chemical Changes

Chemical changes in the cotton plant include high gossypol, glandlessness and high tannins in various plant parts which may attract or repel various insects infesting it.

High Gossypol

Gossypium spp. is characterized by the presence of darkly pigmented lysigenous glands that occur throughout the plant, including the embryo of the seed. Strains selected for high gossypol in squares (up to 2.05% dry weight) were resistant to *Heliothis* and pink bollworm (Lukefahr et al., 1966; Lee, 1976). The initial source of high gossypol was a wild cotton endemic to Socorro Island. Dilday and Shaver (1976a,b) identified the range of genetic diversity of high gossypol types. Several primitive stocks of *G. hirsutum* possessed relatively high levels of gossypol in flowerbuds and better than the Socorro Island stock. Lukefahr (1975) reported that high bud gossypol in combination with glabrousness suppresses larval population up to 50-60 percent. Some other plant constituents, besides gossypol, such as terpenoids (heliocide and hemigossyplore) also carry antibiotic effects on *Heliothis* but gossypol has been found to be the most active biologically (Lukerfahr, 1977).

Boll weevils were smaller than average or required a longer than average development period when fed on diets containing powder from the squares of wild diploid species *G. thurberi, G. davidsonii, G. arboreum, G. herbaceum* and SA-13 strain of *G. hirsutum* indicating antibiotic type of resistance (Reddy and Weaver, 1975).

A number of interspecific crosses have been developed at Multan (Table 3) with a high level of gossypol on the entire plant body. These have proved highly resistant to sucking as well as bollworm complex. Agronomic and economic properties of these lines/strains are being improved to make them competitive with the commercial cultivars. This is a new source of genetic resistance for many insects, the level of which is being assessed in association with the entomologist at the Central Cotton Research Institute of PCCC at Multan.

Glandlessness

McMichael (1960) recovered a Mutant gl_2 gl_3 from a cross between a cultivated and a primitive race of *G. hirsutum* that eliminates glands from aerial plant parts as well as seeds.

Table 4. New Strains Developed for CLCV and Insect Resistance

Strain	G.O.T %	Fiber Characters: Staple Length (mm)	Fineness (ug/inch)	Strength (tppsi)
Cyto 7/91	42.6	28.0	4.1	110.0
Cyto 8/91	40.7	30.4	4.3	108.5

Appendix I
List of *Gossypium* Species - 1995

Sr. No.	Species/(Genomes)	Chromosome Size	Distribution
I. Asian (n=13)			
1.	*G. herbaceum* Al	Large	Asian-old world cultigen
2.	*G. arboreum* A2	Large	Asian-old world cultigen
II. African (n=13)			
I	*G. anomalum* (B 1)	Medium	Northern and Southern Africa
2.	*G. triphyllum* (B2)	Medium	Southern Africa
3.	*G. barbosanum* (B3)	Medium	Cape Verde Island
4.	*G. capitis viridis* B4	Medium	Cape Verde Island
5	*G. sensarense* (3?)	Medium	Africa
6.	*G. trifurcatum* (B?)	Medium	Africa
III. Australian (n=13)			
1.	*G. sturtianum* (CI)	Large	Central Western Australia
2.	*G. nandewarense* (C I -n)	Large	Eastern Australia
3.	*G. robinsonii* (C2)	Large	Western Australia
4.	*G. australe* (C3)	Large	Northern Australia
5.	*G. castulatum* (C5)	Large	North Western Australia
6.	*G. populifolium* (C6)	Large	North Western Australia
7.	*G. cunninghamii* (C7)	Large	Northern Australia
8.	*G. pulchellum* (C8)	Large	North West Australia
9.	*G. nelsonii* (C9)	Large	North Australia
10.	*G. pilosum* (C?)	Large	Western Australia
IV. American (n=13)			
I.	*G. thurberi* (DI)	Small	Sonora, Mexico/Arizona
2.	*G. armourianum* (D2-1)	Small	Baja California, Mexico
3.	*G. harknessii* (D2-2)	Small	Baja California, Mexico
4.	G. *klotzschianum* (D3-k)	Small	Galapagos Island
5.	*G. davidsonii* (D3-d)	Small	Sonora, Baja California, Mexico
6.	*G. aridum* (D4)	Small	West-Central Mexico
7.	*G. raimondii* (D5)	Small	West-Central Peru
8	*G. gossypioides* (D6)	Small	Oaxaca, Mexico
9.	*G. lobatum* (D7)	Small	Michoacan, Mexico
10.	*G. laxum* (D8)	Small	Guerrero, Mexico
11.	*G. trilobium* (D9)	Small	West Central Mexico
12.	*G. turneri* (D?)	Small	Baja California
13.	*G. schewendimanii* (D?)	Small	South America
V. Arabian/African (n=13)			
1.	*G. stocksii* (El)	Large	Arabian/Pakistan
2.	*G. somalense* (E2)	Large	North-Eastern Africa
3.	*G. areysianum* (E3)	Large	Arabian peninsula
4.	*G. incanum*	Large	Arabian peninsula
5.	*G. briccheitii* (E?)	Large	Africa
6.	*G. benadirense* (E?)	Large	Africa
VI. Redesignation (n=13)			
1.	*G. longicalyx* (F1)	Large	North-Eastern Africa
2.	*G. bickii* (GI)	Small-medium	North-Central Australia
VII. New World - Polyhybrids (n=26)			
1.	*G. hirsutum* (AD) 1	13 Large + 13 small	New World cultigen
2.	*G. barbadense* (AD)2	13 Large + 13 small	New World cultigen
3.	*G. tomentosum* (AD)3	13 Large + 13 small	Hawaiian Islands
4.	*G. mustelinum* (AD)4	13 Large + 13 small	North-Eastern Brazil
5.	*G. lanceolatum* (AD)?	13 Large + 13 small	Mexico
6.	*G. darwinii* (AD)?	13 Large + 13 small	Galapagos Island
VIII. New Additions*			
1.	*G. exiguum*		
2.	*G. nobile*		
3.	*G. rotundifolium*		

*** Fryxell et al. (1992), yet to be designated**

Glands are absent in some Australian species viz., *G. sturtianum, G. nandewarense, G. australe* and *G. bickii*. Glandless cottons produced through transgressive segregation, were previously outside the range of variation of the cultivated cottons. Tetraploid of *G. arboreum* x *G. australe* has glandless seed and glanded plants. The 4th and 5th backcross with *G. hirsutum* has yielded material resistant to CLCV and variable in resistance to insects. Similar material from a tetraploid of *arboreum* x *sturtianum* is also available which is a new genetic source for insect resistance. Though shy in bearing, these strains have excellent fiber characteristics as given in Table 4.

It may not be out of place to mention that Meredith (1972) released a glandless seeded cotton in USA which did not differ significantly in yield and fiber properties from their contemporary varieties but was variable in resistance to insects and tolerance to bollworm complex (Jenkins, 1982).

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Appendix II
Distinguishing Characters of *Gossypium* Species

G. herbaceum	Style relatively short, buried in anthers, capsule more or less round, storm proof, drought/heat tolerant.
G. arboreum	Petal color white (also yellow with petal spot), capsule glands sunken, resistant to bacterial blight, rust and sucking insects.
G. anomalum	Gossypol glands raised on fruit, narrowly ligulate bract, capsule glandular (1 mm diam) and raised, resistant to bacterial blight, rust and drought.
G. triphyllum	Weak sub-shrub, leaves trifololiate, petal spot covering more than half, corolla narrowly funnelform when fully open, capsule densely pubescent, fiber loosely appressed to seed, drought tolerant.
G. barbosanum	Drought tolerant.
G. capitis viridis	Gossypol glands raised on fruit, capsule glands raised.
G. sturtianum	Waxy epidermis, gossypol glands raised on fruit, gossypol glands absent from embryo but present on plant parts, petal color mauve, capsule glands raised,, seed 5 mm long, fibrous root, less vulnerable to root rot, cold tolerant.
G. nandewarense	Glanded plant, glandless seed, cold tolerant.
G. robinsonii	Waxy epidermis, foliar nectaries bright red, leaves deeply dissected, fiber color grayish, cold tolerant.
G. australe	Foliar nectaries bright red, calyx glands (gossypol) few, gossypol glands absent from embryo but present on plant parts, filiform bract, capsule densely pubescent, after opening of capsule the inner suture line bears long fragile hairs, seed 4-5 mm long, straight fiber, cold tolerant.
G. nelsonii	Long suture hairs, straight fiber, cold tolerant.
G. pilosum	Involucellar nectary absent, involucel bract reflexed, cold tolerant.
G. populifolium	Involucellar nectary absent, involucel bract reflexed, petal color white, capsule glands sunken, procumbent perennial, petal glands absent, gossypol glands sunken on fruit, lacks seed fuzz, cold tolerant.
G. pulchellum	Involucellar nectary absent, petal color white, cold tolerant.
G. cunninghamii	Petal glands very few, cold tolerant.
G. thurberi	Leaves deeply dissected, narrowly ligulate bract, petal color pale, small petal spot, corolla fully rotate, long suture hairs present, lacks seed fiber, high fiber strength and fineness, resistant to pink bollworm.
G. armourianum	A double palisade layer, deciduous bracts, smoothness (D_2-genes), style long, after dehiscence of capsule the inner suture line bears long fragile hairs, capsule glands sunken, seed 8-10 mm long, fiber brown.
G. harknessii	A double palisade layer, gossypol glands sunken on fruit, deciduous bracts, corolla widely flared when open, style long, long sture hair present, capsule glands sunken, seed 8-10 mm long, fiber tightly appressed to seed, fiber color grayish, resistant to spidermite, cytoplasmic male sterility.
G. klotzschianum	No petal spot, long suture hairs present, seed 5 to 6 mm long with sparse inconspicuous fibers, carries genes for fiber strength, heat tolerant.
G. davidsonii	Foliaceous bracts, long suture hairs present, lacks seed fiber, carries genes for fiber strength.
G. aridum	Fully arborescent, calyx glands small, inconspicuous triangular scaly bracts, petal color lavender or rose, corolla narrowly funnel form when fully open, fiber brown.
G. raimondii	Foliaceous bracts, fiber loosely appressed to seed, fiber brown, rust resistant, furnished D-chromosomes for tetraploid cotton.
G. gossypioides	Foliar nectaries absent, involucellar nectaries absent, foliaceous bracts, petal color lavender or rose, petal spot covers more than half, capsule glandular, fiber color greyish, less attracted to insects.
G. lobatum	Fully arborescent, calyx glands small and few, gossypol glands raised on fruit, inconspicuous triangular scaly bracts, petal spot covering more than half, capsule elongated and glandular, glands raised, fiber color greyish.
G. laxum	Fully arborescent, inconspicuous triangular scaly bracts, petal spot covering more than half.

Appendix II (Continued)

G. trilobum	Foliaceous bracts, corolla fully rotate, capsule oblong, long suture hairs present.
G. turneri	A double palisade layer, deciduous bracts, petals bright yellow, corolla widely flared when open, long suture hairs present.
G. stocksii	Procumbent, perennial, drought resistant.
G somalense	Foliaceous bracts, corolla narrowly funnelform when open, long suture hairs present, heat/drought resistant.
G. areysianum	Petal glands (gossypol) absent, narrowly ligulate bract, heat/drought resistant.
G. incanum	Petal glands very few, heat/drought resistant.
G. longcalyx	Scandent shrub, involucellar nectary absent, petal bright yellow, no petal spot, fiber color greyish, immune to reniform nematodes.
G. bickii	Weak sub-shrub, gossypol glands absent from embryo but present on plant parts, filiform bract, petal color white (certain strains of *G. bickii*), the inner suture lines bear long hairs, capsule glandular and glands raised, fiber tightly appressed to seed, fine and strong fiber, cold tolerant, prolific flowering.
G. hirsutum	Foliaceous bracts, style relatively short, buried in anthers, commercial crop.
G. barbadense	Gossypol glands sunken on fruit, foliaceous bracts, capsule elongated, excellent fiber length, strength and fineness, resistant to bacterial blight and spider mite.
G. tomentosum	Foliar nectaries absent, involucellar nectaries absent, petal brightly yellow, no petal spot, style long, fiber brown, tomentose character, escapes sucking insects, heat resistant.
G. mustelinum	Capsule glands raised, latent fiber properties.
G. lanceolatum	Leaves deeply dissected, gossypol glands sunken on fruit, capsule more or less round.
G. darwinii	Style long, capsule glands sunken.

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Appendix III
Gossypium Species Hybrids Synthesized at CRI Multan of PCCC

Sr. No.	Hybrids	Characters
A) Diploids and Tetraploids		
1.	*G. herbaceum* A1 x *G. anomalum* B1	Heat/drought tolerant.
2.	*G. arboreum* A2 x *G. herbaceum* Al	Resistant to sucking insects.
3.	2 (*G. arboreum* A2 x *G. herbaceum* A1)	Heat/drought resistant to sucking insects.
4.	*G. arboreum* A2 x *G. anomalum* B1	Drought/heat resistant, resistant to sucking insects, bacterial blight.
5.	2 (*G. arboreum* A2 x *G. anomalum* B1)	Resistant to drought/heat, sucking insects and bacterial blight.
6.	*G. arboreum* A2 x *G. barbosanum* B3	Resistant to sucking insects.
7.	*G. arboreum* A2 x *G. sturtianum* C1	Cold tolerant.
8.	2 (*G. arboreum* A2 x *G. sturtianum* C1)	Cold tolerant, fine and strong fiber.
9.	*G. arboreum* A2 x *G. nandewarense* C1-n	Cold tolerant.
10.	2 (*G. arboreum* A2 x G. *nandewarense* Cl-n)	Cold tolerant, fine and strong fiber.
11.	*G. arboreum* A2 x *G. australe* C3	Cold tolerant.
12.	2 (*G. arboreum* A2 x *G. australe* C3)	Cold tolerant, fine and strong fiber.
13.	*G. arboreum* A2 x *G. thurberi* D1	Drought resistant.
14.	2 (*G. arboreum* A2 x *G. thurberi* D1)	Fine and strong fibre, drought resistant.
15.	*G. arboreum* A2 x *G. stocksii* E1	Drought resistant.
16.	2 (*G. arboreum* A2 x *G. stocksii* E1)	Resistant to sucking insects.
17.	*G. stocksii* E1 x *G. arboreum* A2	Drought resistant.
18.	2 (*G. stocksii* E1 x *G. arboreum* A2)	Drought/sucking insect resistant.
19.	*G. arboreum* A2 x *G. somalense* E2	Drought/sucking insect resistant.
20.	2 (*G. arboreum* A2 x *G. somalense* E2)	Drought resistant.
21.	*G. arboreum* A2 x *G. bickii* G1	Cold tolerant.
22.	2 (*G. arboreum*)	Resistant to sucking insects.
23.	2 (*G. arboreum*) x *G. hirsutum*	Resistant to sucking insects.
24.	2 (*G. arboreum*) x *G. barbadense*	Resistant to sucking insects.
25.	*G. anomalum* B1 x *G. australe* C3	Heat/drought tolerant, resistant to sucking insects.
26.	*G. anomalum* B1 x *G. davidsonii* D3-d	Heat/drought tolerant, resistant to sucking insects.
27.	*G. anomalum* B1 x *G. stocksii* D1	Heat/drought tolerant, resistant to sucking insects.
28.	*G. thurberi* D1 x *G. anomalum* B1	Heat/drought tolerant, resistant to sucking insects.
29.	*G. thurberi* D1 x *G. davidsonii* D3-d	Heat/drought tolerant, resistant to sucking insects.
30.	2 (*G. thurberi* D1 x *G. davidsonii* D3-d)	Heat/drought tolerant, resistant to sucking insects.
31.	*G. thurberi* D1 x *G. somalense* E2	Heat/drought tolerant, resistant to sucking insects.
32.	*G. davidsonii* D3-d x *G. somalense* E2	Heat/drought tolerant, resistant to sucking insects.
33.	*G. somalense* E2 x *G. stocksii* E1	Heat/drought tolerant, resistant to sucking insects.
34.	*G. hirsutum* x *G. tomentosum*	Dense pubescence, nectariless.
B) Triploid, Hexaploid and Octoploid		
35.	*G. hirsutum* x *G. anomalum*	Resistant to sucking insects.
36.	2 (*G. hirsutum* x *G. anomalum*)	Resistant to sucking insects, fine and strong fiber.
37.	*G. hirsutum* x *G. sturtianum*	Cold tolerant.
38.	2 (*G. hirsutum* x *G. sturtianum*)	Fine and strong fiber, cold tolerant.
39.	*G. hirsutum* x *G. nandewarense*	Cold tolerant.
40.	*G. hirsutum* x *G. armourianum*	Smooth leaves, caducous bracts.
41.	2 (*G. hirsutum* x *G. armourianum*)	Smooth leaves with fine and strong fiber.
42.	*G. hirsutum* x *G. bickii*	Cold tolerant.
43.	*G. barbadense* x *G. nandewarense*	Cold tolerant.
44.	2 (*G. hirsutum)*	Tolerant to sucking pests.

C) Tri and Tetra Species Hybrids

Back crosses of the following species hybrids (4-6) generations with *G. hirsutum*.

Sr. No.	Hybrids	Characters
1.	2 (G. *arboreum* x *G. herbaceum*)	Very early (strain Cyto-8/87), heat tolerant (strain Cyto-10/91)
2.	2 (*G. arboreum* x *G. herbaceum*) x *G. barbadense*	Heat/drought tolerant.
3.	2 (*G. arboreum* x *G. anomalum)*	Resistant to CLCV (Cyto-9/91), bacterial blight resistant (cyto-1-3/88)
4.	2 (*G. arboreum* x *G. anomalum*) x *G. barbadense*	Tolerant to sucking insects.
5.	*G. barbadense* x 2 (*G. arboreum* x *G. anomalum*) x *G. hirsutum*	Tolerant to sucking insects.
6.	2 (*G. arboreum* x *G. sturtianum*)	Glandless plant and seed (Cyto-8/91)
7.	2 (*G. arboreum* x *G. australe*)	Resistant to CLCV, glandless seed and plant (Cyto-7/91).
8.	2 (*G. arboreum* x *G. stocksii*)	Tolerant to sucking insects.
9.	2 (*G. stocksii* x *G. arboreum*)	Tolerant to sucking insects, drought resistant.
10.	2 (*G. hirsutum* x *G. armourianum*) x *G. hirsutum*	Early maturing, short duration, prone to shed some parts of leaves at harvest, tolerant to CLCV and *Heliothis* (Cyto-2/91).

Appendix III (Continued)

11.	*G. hirsutum* x (*G. hirsutum* x *tomentosum*)	Dense pubescent, resistant to sucking insects (Cyto-12/91), nectariless, reduces bollworms population (Cyto- 11/91).
12.	2 (*G. arboreum* x *G. thurberi*) x *G. hirsutum*	Resistant to sucking insects.
13.	2 (*G. arboreum* x *G. thurberi*) x *G. barbadense*	Resistant to sucking insects.
14.	2 (*G. arboreum* x *G. anomalum*) x 2 (*G. arboreum* x *G. australe*)	Tough leaf, insect resistant, (Cyto-786/86), High G.O.T., insect tolerant (Cyto-876/86).
15.	2 (*G. arboreum* x *G. herbaceum*) x 2 (*G. arboreum* x *G. australe*)	Cyto-2/87, high gossypol, repels insects.
16.	2 (*G. arboreum* x *G. herbaceum*) x 2 (*G. arboreum* x *G. sturtianum*)	Cyto-4/87, high gossypol, repels insects.
17.	2 (*G. arboreum* x *G. herbaceum*) x 2 (*G. arboreum* x *G. nandewarense*)	Cyto-8/87, high gossypol, repels insects.
18.	2 (*G. thurberi* x *G. davidsonii*) x 2 (*G. arboreum* x *G. australe)*	Cyto-9/87, high gossypol, repels insects.

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