dropped to 543 kg/ha in 1992/93 due to the cotton leaf curl epidemic. The disease is caused by geminiviruses carried by whitefly. No immediate remedy was available against the disease, and Pakistan suffered heavy economic losses. The National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan, the Cotton Research Institute, Faisalabad, Pakistan, the University of Arizona, Tucson, USA, and the John Innes Centre, Norwich, United Kingdom, undertook a joint project to characterize the geminiviruses and develop genotypes resistant to the leaf curl disease. The five-year project is finished, and the third article in this issue of *THE ICAC RECORDER* summarizes the findings. The project was successful, and the average cotton yield for Pakistan in 2002/03 was 625 kg/ha.

The IV Brazilian Cotton Congress will be held in Goiânia, Goiás, September 15-18, 2003. The theme of the congress will be "Cotton: A Market in Evolution." The congress is held every two years. The IV Congress will have conferences, workshops and short courses on all aspects of production, from cotton in family agriculture to agricultural politics for cotton cultivation, the textile industry, and the mechanism of the futures market. More information on the congress can be requested at:

Av. 87 No. 662 - Setor Sul - Goiânia, Goiás 74093-300, Brazil
Phone: 55-62-5410163
Email: 4cba@cultura.com.br
Website: http://www.4cba.com.br

The 9th Meeting of the Latin American Association for Cotton Research and Development (ALIDA) will be held during the IV Brazilian Cotton Congress and hosted by the National Cotton Research Center (CNPA) of the Brazilian Agriculture and Livestock Research Corporation (EMBRAPA). CNPA also hosted the 3rd ALIDA meeting in August of 1991. ICAC is sponsoring the meeting. Delegates from countries in the region are expected to attend. Mr. Alderi Emidio de Araujo, Manager of Research and Development, CNPA, coordinator of the meeting, can be contacted at:

Rua Oswaldo Cruz 1143 Centenario
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VIP Cotton: A New Type of Transgenic Cotton

The Bt gene contained in Bollgard (Cry1Ac) is most effective against tobacco budworm *Heliothis virescens* and cotton bollworm *Helicoverpa zea*, among all lepidopterans controlled by the Cry1Ac protein. However, most cotton fields around the world are attacked not only by these two species, but also by a variety of other lepidopterans, including *Spodoptera* species, such as fall armyworm and beet armyworm. Also, there is a need to avoid the problem of development of resistance to the Cry1Ac toxin. On a long-term basis, the objectives of broad-spectrum insect control and resistance management can only be achieved by exploring and utilizing new generations of transgenes significantly different from Cry1Ac. While Monsanto has come up with a second generation Bt gene in Bollgard II expressing Cry2Ab, the Syngenta Group Company has developed an entirely new generation of the Bt gene with a broad spectrum effect on insects threatening cotton production.

Soil bacterium *Bacillus thuringiensis* (Bt), used by Monsanto and Syngenta to impart in-built resistance in cotton, is a naturally occurring bacterium found worldwide. Different Bt strains produce different Cry proteins and there are hundreds of known Bt strains. Researchers all over the world have already identified over sixty types of Cry proteins that could be used to control a wide variety of pests, but most Cry proteins are specific in their action and control only a limited number of pest species.

The use of Cry proteins is not new in cotton. Cry proteins have been used for over thirty years in liquid and granular formulations of Bt insecticides. Since October 1995, when transgenic cotton having the Bt gene imbedded into the cotton genome was approved for commercial production in the USA for the first time, the technology has changed the usefulness of the Bt bacterium. The Bt gene in the embedded form has overcome the following limitations faced during commercial use of Bt as an insecticide formulation:

- The Bt insecticide must be sprayed so that all plant parts eaten by the target insects are covered by a minimum quantity of the Bt insecticide. If a plant or part of a plant is missed during the Bt foliar application, the target insects will survive on those plants/parts.
- The Bt toxin is rapidly degraded by ultraviolet light, heat, high leaf pH or desiccation. The toxin can be degraded before it is actually consumed by the target insects.
- A specific dose of the insecticide is required to kill insects. Insects must eat enough of the treated plant part to accumulate a lethal dose.
- Experience shows that Cry proteins are less toxic to older larvae.
- Bt insecticides must be sprayed, which is an additional expenditure and also demands that all precautions/requirements for chemical insecticide applications be followed, including equipment, uniform, complete coverage, drift, etc. The Bt gene within the plant changes the toxin delivery system to a more effective method.

VIP Technology

VIP (vegetative insecticidal protein) cotton is an alternate technology to Bollgard or Bollgard II insect control. The difference is that VIP cotton contains a protein that controls target pests through a novel mode of action. Transgenic varieties, either Bollgard or Bollgard II, utilize proteins from the *Bacillus thuringiensis* bacterium known as d-endotoxins. Bt d-endotoxins were discovered in the early 1900s and have been used in many insect control applications other than cotton. In 1994, Syngenta Group Company discovered VIP, an exotoxin derived from the same soil bacterium *Bacillus thuringiensis*. As an exotoxin, VIP is structurally, functionally and biochemically different than Bt d-endotoxins. VIP is expressed in the entire cotton plant, including the floral parts, to provide protection against target species. When VIP cotton is ingested, the protein causes larvae to stop feeding and soon die. According to Mascarenhas et al (2003), VIP technology differs from Bollgard and Bollgard II technology in the following respects:

- VIP is a protein that is secreted by *Bacillus thuringiensis* as it grows, and thus it is classified as an exotoxin. Bollgard and Bollgard II genes are found during the sporulation phase and thus are classified as delta-endotoxins.
- Cry1Ac and Cry2Ab are found in a crystalline phase and require solubilization before they can be activated by midgut proteases. On the contrary, VIP is already in a soluble state and readily available.
- Both technologies, Bollgard/Bollgard II and VIP, target the midgut receptors in target species.
- Structurally, both types of proteins are different from each other.

VIP Effects on the Midgut Cells

It is known that ingestion of endotoxins cause swelling and disruption of the midgut epithelial cells by osmotic lysis in the target insects. The following four factors play an important role for any insecticidal protein to be effective against its targets; they also determine the insect host range:

- Presence of specific binding sites on susceptible cells
- Insertion of the bound toxin into the membranes
- Solubilization of the crystal protein
- Processing of midgut proteases

Ingestion of the insecticidal protein and target binding sites, where the protein will be bonded, are the most important factors without which the biology of insecticidal proteins fails to work. How quickly the insecticidal protein is solubilized into the target cells/receptors will determine the duration of the effects of the insecticidal protein on the target insect. If the protein is easily solubilized, the effects on target insects will become visible faster in the form of slower feeding and slower movements. If the protein has been ingested but it is sitting there until it is properly absorbed into the epithelial cells, the insects will continue to feed and damage the crop.

The VIP gene shows insecticidal activity against a variety of lepidopterans and exhibits severe bioactivity towards *Agrotis ipsilon* (black cutworm), *Spodoptera frugiperda* (fall armyworm) and *Spodoptera exigua* (beet armyworm). A detailed study on the effect of VIP3A on the midgut epithelium cells of susceptible insects was published by Yu et al (1997). They determined the VIP mode of action and examined the protein's effects on target cells in susceptible and non-susceptible insects. They used two highly-susceptible insects and one non-susceptible insect in the same order, i.e., black cutworm and fall armyworm, and European corn borer *(Ostrinia nubilalis)*, respectively. Studies were conducted on the histopathology of the insects' midgut upon ingestion of VIP3A and in vivo binding of VIP3A to target cells. The second and third instars larvae of the three lepidopterans were used in the study. Larvae were reared on an artificial medium without VIP3A and with VIP3A in various doses.

Feeding and Gut Clearance

The second instar larvae of black cutworm and fall armyworm were monitored following the ingestion of a diet containing VIP3A, from the time of initial administration of the toxin until larval death. For this purpose, larvae were placed on small diet cubes with a surface area of about 1 cm^2, which had been coated with different amounts of VIP3 mixed with blue food coloring. Feeding behavior and gut clearance activities were monitored by checking the deposition of blue frass by light microscopy. The quantitative measure of gut clearance was obtained by counting the colored frass in different treatments. The larvae given a control diet (no VIP3) showed active feeding followed by uninterrupted gut clearance. But the addition of VIP3 to the diet showed a significant effect on the feeding behavior of black cutworm and fall armyworm. The larvae kept on feeding on-and-off for 10-20 minutes at low protein doses of 4 ng/cm^2 of diet. No mortality was recorded at 4 ng/cm^2, even after 48 hours. The presence of blue color in the guts indicated feeding, but the clearance of the gut contents was dramatically affected as judged by the amount of frass. The larvae suffered gut paralysis upon ingestion at a concentration of 40 ng/cm^2 of VIP3, and no frass was seen, indicating an almost complete lack of gut clearance. 50% of the larvae died after 48 hours at 40 ng/cm^2. When the dose was increased to over 40 ng/cm^2, the larvae stopped feeding after only a few bites, with no frass and a mortality rate reaching close to 100%. Black cutworm and fall armyworm, both susceptible to the VIP3 toxin, showed similar behavior to various doses of the toxin, but the European corn borer did not show any changes in feeding behavior, even after the VIP3 toxin dose was increased to over 40 ng/cm^2.

Stability of VIP3A in Midgut and Insecticidal Activity

Fresh gut juices were extracted from the third and fourth instars larvae of the three insects by dissecting them and extracting their gut contents by pipette. Gut juices were centrifuged and protein concentration was measured in the gut juices. Pro-

teolytic reactions were also performed using different amounts of VIP3A containing supernatants and gut juices. The results showed that when equal amounts of VIP3A were incubated with black cutworm, fall armyworm and European corn borer gut fluids, four major VIP3A proteolysis products were identified. However, whole insect and midgut tissue extracts from both susceptible insects, probed with the same purified anti-VIP3A antibody, revealed no background bands. The relationship between the proteolytic processing and insecticidal activity of VIP3A was studied by Yu et al (1997) from the bioassays conducted, following the incubation of VIP3A with gut fluids isolated from all three insects. VIP3A processed by black cutworm and fall armyworm gut fluids was active against the black cutworm and fall armyworm. VIP3A processed by the European corn borer was found active against black cutworm and fall armyworm. None of the VIP3A processed forms showed acute activity against the European corn borer, a non-susceptible insect.

Histopathological Studies

The second and third instar larvae were used for histopathological studies. The larvae were fed an artificial diet containing either no or 100-200 ng of VIP3A per cm^2 of diet cube. The larvae were killed after 24, 48 and 72 hours of exposure. The dead larvae, after specific processing including dehydration, were embedded in paraffin for longitudinal cross section. Analysis of black cutworm gut cross-section showed extensive damage to the midgut epithelium, indicating that midgut tissue is the primary site for the VIP3A action. No damage was found in the foregut and hindgut. Sections from the black cutworm and fall armyworm larvae, which had been fed for 24 hours on a diet containing VIP3A, showed that distal ends of the epithelial columnar cells had become distended and bulbous. The goblet cells indicated some morphological changes but did not show any damage. Damage to the epithelial columnar cells increased with the increase in the time from ingestion to sample taken. The goblet cells exhibited some damage after 48 hours, but cells still remained attached to the basement membrane. On the other hand, midgut cells from the untreated larvae showed no signs of damage and remained closely associated with each other. The European corn borer, fed on the diet containing VIP3A similar to the susceptible species, did not show any signs of tissue damage either.

The work done by Yu et al (1997) concluded that VIP3A showed its real impact on larvae after 48 to 72 hours, a little longer than Cry proteins, where the impact has been confirmed to be within 16-24 hours after ingestion of the toxin.

The insecticidal protein VIP3A(a) has been purified from the *Bacillus thuringiensis* strain AB88, and its homologue VIP3A(b) from the *Bacillus thuringiensis* strain AB424. A number of supernatants were collected and tested for insecticidal activity against lepidopterans and found to carry insecticidal activity against lepidopterans, but VIP3A was found to be very effective. Estruch et al (1996) have studied the release, expression and insecticidal activity spectrum of VIP3A. They detected VIP3A at growth stages before sporulation as early as 15 hours after culture initiation. The Cry proteins could not be detected until 36 hours after culture initiation. A high level of expression combined with the high stability of the protein, produced in large amounts in supernatants of sporulating cultures, makes it extremely suitable for use as an insecticidal product. The activity spectrum of VIP3A was determined in insect bioassays in which recombinant *E. coli* carrying VIP3A genes was fed to larvae. In these assays, cells carrying the VIP3A(a) or VIP3A(b) genes showed different degrees of larvicidal activity against *Agrotis ipsilon*, *Spodoptera frugiperda*, *Spodoptera exigua*, *Heliothis virescens* and *Helicoverpa zea*.

VIP3A scored complete mortality of larvae in the three major pests after six days of feeding on the protein. All larvae of black cutworm, fall armyworm and beet armyworm were killed after six days of exposure to VIP3A at 140 ng/cm^2. VIP3A protein also proved fatal for tobacco budworm and corn earworm larvae, but not all the larvae were killed. The results are an average of at least three trials with a minimum of twenty larvae per trial. The bacterial extract containing VIP3A did not show any activity against the European corn borer *Ostrinia nubilalis*.

According to Estruch et al (1996), Cry1A(b) and Cry1A(c) proteins possess insecticidal activity against the black cutworm with LC_{50S} of >80 µg and 18 µg of diet per ml respectively. VIP3A provided 100% mortality at 62 ng of diet per ml. This amount of protein is 260-fold lower than the amount of Cry1A proteins needed to achieve just 50% mortality, and it is similar to the levels of d-endotoxins used for insects susceptible to d-endotoxins.

Effect of VIP3A on Various Insects

Dose		Insect Mortality (%)					
µg Protein per cm2	ng VIP3A per cm2	Black Cutworm	Fall Armyworm	Beet Armyworm	Tobacco Budworm	Corn Earworm	European Corn Borer
15.0	28	82	44	65	19	20	12
3.8	70	96	78	95	25	22	10
7.5	140	100	100	98	35	25	8
15.0	280	100	100	100	45	35	10
22.5	420	100	100	100	75	50	7

Field Testing of VIP Technology

A number of trials were conducted in seven states in the USA during 2002/03 by the Syngenta Group Company and universities. Data are currently available only on a non-transgenic Coker variety and its transformed VIP form (Mascarenhas et al, 2003). Both varieties were treated equally, and no insecticides were applied for the control of lepidopterans, while all other insects were controlled as needed, but only with narrow spectrum insecticides. Data were recorded for percent infestation and percent damage in terminals, squares, flowers, bloom tags and bolls. At the end of the season, yield data were also taken to see the impact of infestation on the quantity of seedcotton harvested per plot.

The results revealed that the VIP variety is able to resist a number of lepidopterans and produce significantly higher yields over its respective non-transgenic variety. The results showed that the Heliothine complex has no preference for a variety with or without a transgene for oviposition. The average of all locations showed that the cumulative percent of terminals with at least one egg were 12.4 and 11.3% for VIP and Coker respectively. However, the VIP gene did not allow the hatched larvae to survive for a long time on the VIP variety, because terminal infestation was low in VIP compared to normal Coker. Terminal infestation ranged from 0 to 4.3% in VIP, compared to 1.4% to 34.5% in Coker. Square infestation ranged from nothing to 6% in VIP compared to 2.5% to 34% in Coker. The level of damage to flowers and bolls was much higher in Coker compared to the VIP variety. Percent boll infestation ranged from 0.4% to 3% in VIP plots compared to 3.1% to 41.5% in Coker. On average across the six locations, larvae were found 9.2 times more in Coker bolls than in VIP bolls. Cumulative percent damaged bolls ranged from 0.6% to 8.2% and 3.2% to 66.5% for VIP and Coker, respectively. Averaged across all locations, percent damaged bolls in VIP plots were 6.8 times lower than in Coker.

Beet armyworm and soybean looper populations were significantly reduced on VIP cotton. Beet armyworm larvae decreased by 89.3% to 100%, while soybean looper larvae decreased by 60-97% on the VIP variety compared to normal Coker. The VIP variety showed 3.2% damage by the cotton leaf roller compared to 48.7% damage on the Coker variety.

A lower infestation of terminals, squares, flowers and bolls in VIP contributed to productivity. Coker produced fewer first position bolls, which may have an effect on quality, but no quality data are available. However, the yield difference alone is significant, almost double in the VIP variety, which proves the usefulness of this new technology compared with insecticide use.

The table reveals that yield differences between the VIP variety and a non-transgenic Coker variety were lowest in both trials in Georgia. The Georgia trials lowered the difference in the average of all trials by 20%. According to Mascarenhas et al (2003), environmental conditions and a little insect pressure are responsible for equally good performance of Coker at Georgia locations.

Effect of VIP Gene on Seedcotton Yield

Location	Seedcotton Yield (kg/ha)	
	Coker	VIP Variety
Beasley, TX	1,569	4,725
Brooks, GA	3,516	4,067
Houston Co., AL	2,130	3,081
Jamesville, NC	1,834	6,242
Leland, MS	2,997	6,496
Newport, AR	3,966	5,283
Tift Co., GA	3,521	3,669
Waco, TX	1,134	5,461
Winnsboro, LA	2,409	3,405
Winnsboro, LA	3,227	5,528
Average	2,630	4,796

Plans for VIP Cotton

Although the new technology is currently called VIP, it could be offered under a different brand name when it is released for commercial use. VIP cotton is not currently registered or offered for sale or use in any country. The Syngenta Group Company has submitted VIP Cotton™ for registration with the U.S. Environmental Protection Agency, and Syngenta anticipates registration in time for commercial sale in the USA for the 2004/05 season.

If VIP technology is approved by the U.S. Environmental Protection Agency, it will be stacked with the existing herbicide glyphosate-resistant trait.

The technology has to be introduced through the accepted varieties if the required regulatory approval is received from the U.S. government. In addition to Syngenta, Delta and Pine Land Company is already testing VIP technology in potential cotton growing areas. Access to VIP technology in other countries will follow approval in the USA.

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Factors Affecting Adoption of Bt Cotton

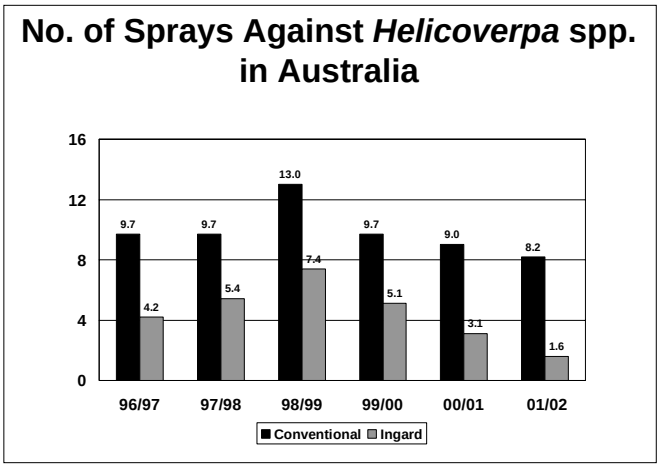
Apart from the controversy over whether or not biotechnology is based on environmentally sound science, there are many factors that determine whether or not to make use of genetically engineered products. This article looks into the issue beyond the acceptance of this technology as an innovative and successful alternative to corresponding conventional practices. It is known that two types of genetically engineered cotton are available for commercial use, and a third stacked gene form is also available. The three forms have been used on a commercial scale, although the transgenic cotton resistant to insects has become more popular on the basis of nine countries that have adopted transgenic cottons so far. This article is limited to the planting of Bt cotton that is applicable to all countries, compared to herbicide resistant or stacked gene forms, which are not officially approved in all countries that have adopted transgenic cotton varieties.

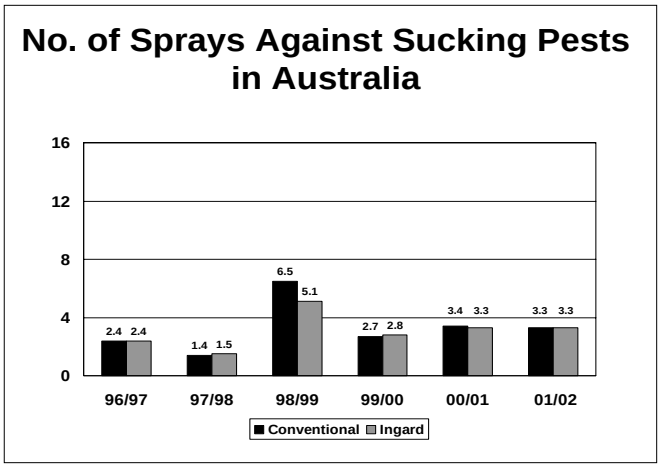
It is accepted that:

- The presence of Bt gene assures that a toxin will be found and it will kill target insects. Target insects, in the case of Bollgard and Bollgard II genes, are known and have been extensively discussed in many previous issues of the *ICAC RECORDER*. Even critics of the technology do not challenge the toxicity of Cry1Ac and Cry2Ab genes. It is recognized that once the Bollgard and/or Bollgard II genes are in cotton, target insects cannot survive and reach an economic threshold, thus saving the crop from losses.
- There is a fee for the Bt technology, and there is a cost for insecticides and spraying on a crop if Bt genes are not used. The Bt technology fee is known before planting, while the cost of insecticides vary depending on population growth of target pests during a season. The data on the technology fee for the Bt gene and the herbicide gene within the USA, and the technology fee for the Bt gene across countries, indicates that the technology fee is based on the benefits expected from the technology. The technology fee for the herbicide resistant genes (BXN or Roundup ready) is many times lower that the fee for the Bt gene, although the processes involved in inserting these genes into cotton are the same. Cotton growers have paid a different fee for the same Bt gene grown in different countries. Because the fee is based on economic benefits from the technology, the cost of the technology has to be kept lower than the cost of insecticides used to save the crop against target pests.
- It is also certain that reduced use of insecticides is beneficial for the environment. All pesticides carry risks to handle and apply, but insecticides are more dangerous under small-scale farming systems where most spraying is done with motorized, but manually carried, sprayers. Air drift inhalation and mishandling of insecticides and containers are hazardous and carry serious consequences. High volume insecticide-application methods also throw insecticides on the ground, which is one form of pollution. Many additional forms of pollution are unavoidable. The use of Bt cotton assures that fewer insecticides will be used, which is good for the environment.
- However, the presence of Bt genes does not mean that insecticides will not be required to control insects on the Bt crop. Insecticides still need to be used against non-target species of insects on the transgenic crop. Data from Australia for six years of commercial production with the Bt gene show that some insecticide applications are required against bollworm species not effectively controlled by Bollgard, and there is also a variation in the number of insecticide applications against sucking pests.
- The Bt gene does not guarantee that cotton yields will be higher than with a non-transgenic variety. When comparing yields between Bt and non-Bt varieties grown under similar agronomic practices other than insect control, yield variation will be determined by a single factor, which is how effectively insects were controlled on the non-Bt varieties. If insects are controlled effectively on non-Bt varieties, there will be no difference in yield.
- It is wrong to assume that the economics of growing Bt varieties are always better than for non-Bt varieties. A number of factors will determine if it is economical to grow Bt cotton. The cost of insecticides and insect pressure are the primary variables. There is always a variation in pest pressure from year to year, and under low pressure growers can save on insecticides without using the Bt gene.
- Planting Bt cotton does not eliminate the need to monitor the crop in the field and undertake scouting for insects. Agronomic requirements also do not change in Bt versus non-Bt varieties.

Insecticide Use in Australia on Bt vs. Non-Bt

Australia was one of the first countries to adopt commercial production of transgenic cotton. In Australia, average cotton yields are very high—the highest or the second highest in the world for over two decades—but the Australian cotton production system is characterized by high input use. Soils are rich in phosphorous and potassium, and only nitrogen is needed for a good harvest. Pre and post herbicides are used on 100% of the cotton area. Insecticides have been used extensively for many years, which resulted in the development of resistance. Cotton used to be sprayed over 13 times every season during the 1980s. But thanks to an insecticide resistance management program, the number of insecticide applications has come down by over 3 sprays per season. The Australian insecticide resistance management program is one of the few stories of successful resistance management in the world.





Mirids *Creontiades* spp., thrips, mites and aphids *Aphis gossypii* are common sucking insects but the major pests during the bollworm stage are *Helicoverpa armigera* and *Helicoverpa punctigera*. Work in Australia proved a strong correlation between *Helicoverpa* spp. eggs and larvae and yield, and convinced cotton growers to use insecticides intensively and avoid losses. The average number of sprays has come down to about 10 sprays per season, but as seen in the chart, the number of sprays in some years can still be as high as 15 per season, and most of these sprays are against *Helicoverpa* species. According to Bruce Pyke (2003), who presented the data in the chart above at the World Cotton Research Conference–3 held in Cape Town, South Africa from March 9-13, 2003, from 1996/97 to 2001/02, the number of insecticide sprays against *Helicoverpa* spp. on Bt cotton was 56% less than in conventional cotton. Planting of Bt cotton also affected the number of sprays against other pests as follows:

Pest	Change in No. of Sprays
Aphids	2% more
Mirids	10% more
Mites	9% less
Thrips	3% less

On average, three sprays are used against all kinds of sucking insects and mites. The total number of sprays made to control sucking pests did not change but the relative population of sucking pests did change thus necessitating enhanced control against certain pests while reducing the focus on others as their population has fallen over the years since 1996/97. According to Pyke (2003), production of Bt cotton in Australia has reduced the use of both selective and broad-spectrum insecticides, not only on Bt cotton, but also on conventional cotton. The total number of sprays in 2001/02 on conventional cotton was 10, compared to 15 in 1996/97, the beginning of cultivation of Bt cotton. In Australia, production of Bt cotton was limited to only 10% of the cotton area in the beginning, which was later capped at 30% of total cotton area. With the introduction of the second generation of the Bt gene as Bollgard II, there will be no limit on the area to be planted to transgenic Bt varieties from 2004/05. However, necessary refuge requirements are expected to be followed.

Technology Fee in Various Countries

There is a variation in the technology fee charged to growers in various countries. Cotton growers in Australia pay more than growers in any other country. Australian cotton growers used to pay a fee higher than US$104 (Australian $185) in the beginning. That has now come down to US$104. In India, the price of Bt seed also includes the cost of F_1 generation hybrid seed and is supposed to give a higher yield than straight varieties.

The technology fee in Australia was as high as US$137 (A$245) in 1996/97. The fee was lowered to US$118 (A$210) net with the resistance management plan (without the plan, it was still US$137). The fee was lowered to US$104 in 1998/99. The reasons for fixing a higher technology fee in one country over another are not obvious. It has been often assumed that if growers are saving more on insecticides by using Bt gene varieties, they will be willing to pay a higher technology price, but it does not seem to apply in the case of China (Mainland) where in the Yellow River Valley the number of sprays had surpassed the number in Australia when Bt cotton was adopted in China (Mainland). Whatever may be the basis for fixing the technology fee, it is not related to yield levels among countries. Yield differences are huge among countries, but differences in the technology fee are not as large.

The technology fee has to be paid up front without knowing whether target insects will be as serious as they are normally. A grower will benefit from growing Bt cotton if target pests are as serious as usual or more serious, but if target insects do not become that serious in any particular year, and only a limited number of sprays are enough to control them, a grower could be at a disadvantage in spending more on the technology fee than he would have spent on insecticides. Reports from India indicate that some farmers were hesitant to make a high initial investment without looking at crop conditions and pest damage. The technology fee has an impact on the adoption of Bt

cotton, which will be discussed in more detail after presenting the situation in Argentina.

Bt Cotton in Argentina

In Argentina, the National Institute of Agricultural Technology-INTA (Instituto Nacional de Tecnologia Agropecuaria) signed an agreement with Monsanto for the incorporation of a Bt gene into varieties developed by INTA. The government of Argentina approved Bt gene varieties for commercial production in June 1998 and they were planted starting in 1998/99. However, seed multiplication and distribution of Bt gene varieties was handled by Genética Mandiyú, a joint venture between Monsanto, Delta and Pine Land Company and Ciagro, a local company. Genética Mandiyú had the sole authority to market cotton varieties carrying Bt and herbicide resistant genes. The first herbicide resistant variety, Guazuncho 2000, was approved for commercial cultivation starting in 2002/03. For more details on the approval of Bt and other transgenic cotton varieties refer to the article titled "The Cotton Production System and Bt Cotton in Argentina," published in the June 2002 issue of the *ICAC RECORDER*.

Bt cotton area reached its self-imposed limit of 30% of the total area in Australia in five years after the adoption of Bt cotton in 1996/97. In South Africa and the USA, Bt cotton area reached over 70% of the total area in five years. China (Mainland) and Mexico planted at least 50% of the total area to transgenic varieties in less than five years since the initiation of Bt cotton commercial planting in these countries. Indonesia planted Bt cotton for the second year in 2002/03 while India planted Bt hybrids for the first time in 2002/03. So, the acceptance of Bt cotton and increases in area are yet to be seen in India and Indonesia. In the last five years, from 1998/99 to 2002/03, area under Bt cotton has not increased in Argentina.

Bt Cotton Area in Argentina

Year	% Bt Area
1998/99	0.8
1999/00	3.9
2000/01	6.1
2001/02	4.6
2002/03	8.0

In the USA, the total cotton area planted to transgenic varieties was 77%, but only 40% was under Bt varieties either pure Bt or Bt stacked with herbicide resistant genes. 2002/03 was the first year of the herbicide resistant gene in Australia and South Africa, so it is assumed that all the transgenic area was under Bt varieties. The situation with regard to the adoption of Bt cotton in Argentina is not only different but also needs to be analyzed as to why the Bt cotton area has not increased as in other countries.

The Impact of the Technology Fee

At the time of the introduction of Bt cotton in Argentina, the technology fee was fixed at US$106 plus 21% tax for a total fee of US$128/ha. The fee was reduced to US$90 ($25 for seed and $65 for technology) plus 21% tax, for a total of US$109/ha. Although the fee has been reduced, the table shows that the fee is still the highest in Argentina. Qaim and de Janvry (2002 and 2003) have also analyzed the situation and concluded that the high cost of Bt seed is affecting the adoption of Bt cotton in Argentina.

Australia, where the fee is lower than Argentina, can afford to pay a higher price for the technology because of more relief from insecticides and higher yields. The average number of sprays in Australia is more than double the number in Argentina. In Argentina, 50% of the cotton is sprayed less than five times per season, 35% of cotton area gets only two sprays and 2% of area is not sprayed at all. There is no non-sprayed area in Australia. Australia does not expect much variation in the number of sprays. The average number being 10 shows that the range could vary from 8-12 sprays per season. Thus, reduced savings on insecticides in Argentina do not encourage cotton growers to plant Bt cotton.

Insecticide use has been high prior to the adoption of Bt cotton in China (Mainland), so it is expected that farmers save more on insecticides by adopting Bt cotton. According to Russell (2003), farmers in China reduced insecticide use by about 60% in 2001/02, saving US$107/ha. Savings on insecticides will vary among various provinces in China (Mainland) depending on use. Savings on insecticides is not the only benefit: additional benefits include savings on labor to spray insecticides and also

Bt Cotton in Various Countries–2002/03

Country	First Year of Bt Cotton	Bt Area %	Approved Characters	Technology Fee for Bt in 2002/03
Argentina	1998/99	8	Bt + Herbicide	US$90 ($25 for seed & $65 for technology) + 21% tax = US$109/ha
Australia	1996/97	30	Bt + Herbicide	US$104/ha. Full compliance with resist. mgment plans gives US$17/ha-rebate
China (M)	1998/99	51	Bt (Local+Monsanto)	US$69/ha
India	2002/03	< 1	Bt (F_1 Hybrids)	US$106/ha (1,600 rupees for Bt and 450 rupees for refuge, for one acre)
Indonesia	2001/02	1-2	Bt	US$52/ha including the cost of planting seed
Mexico	1996/97	50	Bt + Herbicide	US$33-72/ha
South Africa	1998/99	74	Bt + Herbicide	US$70/ha (700 rands/bag of 25 kg for one hectare)
USA	1996/97	40	Bt + Herbicide	US$79/ha depending on variable seed drop rate

Technology Fee in Mexico 2002/03 (US$/ha)

Region	Bollgard	Stacked
Mexicali	55.6	110.8
Caborca	68.9	122.8
Sonora South	32.9	88.1
Chihuahua North	40.9	163.5
Chihuahua South	61.6	118.7
La Laguna	71.8	127.9

higher yields. This is one of the reasons that transgenic cotton area is increasing rapidly in China (Mainland).

The price of the technology seems higher in India. However, it is not only the fee for the Bt technology but also includes the price of hybrid seed, which is expensive compared to normal varieties, as it is supposed to give higher yields.

In Mexico, the technology fee is charged per bag containing 50 pounds of planting seed. The values given in the table below have been calculated from the price per bag, assuming that 50 kgs of seed are used to plant 1.6 hectares (four acres) of cotton. In Mexico, Roundup Ready is not licensed as a single product, but is registered as a stacked product with Bollgard. The stacked form includes the Bt gene and Roundup Ready genes together in the Delta and Pine Land varieties. In Mexico, the stacked package includes the license fee for Bt and 2.47 liters of herbicide, except in the region of Chihuahua North, where it includes the license fee and 3.7 liters of the herbicide.

The technology fee varies greatly among regions in Mexico. The highest fee of US$71.8 is one of the lowest among all countries. In general, the technology fee for the Bt gene is lower in Mexico compared to other countries, but the technology fee for the herbicide resistant gene including the herbicide to be used on the stacked varieties is comparatively higher as the price of the herbicide alone is approximately US$18/liter.

The technology fee in South Africa is almost equal to the fee in China (Mainland), yields are much lower in South Africa than in China (Mainland). In South Africa, Bt cotton has been adopted by the majority of small growers who have received the maximum benefit in terms of increases in yield compared to large growers. Small growers' higher increases in yield indicate that their chemical insect control operation was not good. The higher increases have convinced small growers to adopt Bt cotton in South Africa.

In the USA, where Bt cotton was planted on only 50% of the transgenic cotton area in the country, target pests are not serious pests throughout the USA. Tobacco budworm, the most sensitive insect to the Bt toxins, is not uniformly distributed throughout the cotton belt in the USA. Bt cotton was planted on less than 15% of the cotton area in Texas compared to the national average of 40% of area under Bt cotton in the USA in 2002/03. Yields are the lowest in Texas, and the tobacco budworm is not a serious pest, as it is in Alabama, Mississippi and other states where Bt cotton has been planted on over 60-70% of the total area. The states where target pests are not serious and yields are low get relief in the technology fee, which keeps them growing Bt cotton.

Economic Impact of Bt Cotton in Argentina

Qaim and de Janvry (2003) undertook an extensive analysis of Bt cotton adoption in Argentina and its effects on insecticide consumption, yield, insecticide resistance development and net income to technology adopters. In Argentina, *Heliothis virescens* and *Helicoverpa gelotopoeon* are usually the major insects among lepidopterans, damaging cotton every year. *Alabama argillacea*, *Pectinophora gossypiella* and *Spodoptera* spp. also occur on cotton. A number of other insects that are not controlled by the Bt gene also cause significant damage to cotton every year. Qaim and de Janvry surveyed 89 cotton growers who had grown Bt cotton for the second year and 210 farmers who did not grow Bt cotton. The surveyed cotton growers were divided into two groups: small growers owning less than 90 hectares and large growers owning over 90 hectares. Large growers who planted Bt cotton also planted conventional varieties and were interviewed for their practices on both cotton varieties. Large-scale growers produce 70% of the total production in the country. The survey was undertaken in the provinces of Chaco and Santiago del Estero that grow about 90% of cotton in the country. The studies were carried out for two years in 1999/00 and 2000/01.

The results showed that adopting farmers (who planted Bt cotton) reduced insecticide use by more than 50% on Bt fields compared to their conventional fields and that the reductions were in the highly toxic insecticides belonging to groups I and II as defined by the World Health Organization.

Average Number of Insecticide Sprays in Argentina

Category	No. of Sprays
Large grower Bt field	2.14
Large grower conventional fields with Bt users	4.52
Average of all large growers	4.75
All conventional fields (large + small)	3.74

The data do not show the need for spraying, but the actual number of sprays made by farmers. The data showed that small growers used insecticides less than large growers, which means that small growers suffer losses due to inadequate control of insects. The difference between the "average of all large growers" and "large grower conventional fields with Bt users" showed that Bt users who are supposed to be advanced in production practices use insecticides more wisely on non-Bt fields and thus save insecticides by 0.23 sprays/season. Less use of insecticides by small growers indicates brighter prospects for potential increases in yields with Bt cotton compared to large growers.

Bt Advantage Over Conventional Cotton in Argentina

Category	Conventional Production		
	All Farms	Large Farms	Small Farms
Reduction in insecticide use (kg/ha)	1.9	2.6	1.4
Insecticide savings (US$/ha)	20.4	28.3	15.2
Yield gain (kg/ha)	386.8	294.6	446.8
Gross benefit (US$/ha)	91.3	82.4	97.2

Qaim and de Janvry (2003) also concluded from their economic estimates that conventional cotton insecticide applications have to be doubled in order to achieve the same output per hectare as in fields with Bt varieties. They predicted a net gain in yields of 19% for large growers and 41% for small growers using Bt varieties. The durability of the technology was studied by simulating the development of resistance to the Bt toxin using physiology-based models of Bt cotton-pest interactions based on local agroecological and entomological data. The models showed that the development of resistance is unlikely if minimum refuge requirements are properly followed. However, the model does not suggest any long-term guarantee/sustainability of the Bt gene because the situation could change due to many factors, including technology's complex interactions with the environment.

Economic analysis of the data by Qaim and de Janvry (2003) showed that on average Bt adopters had a gross benefit of US$91.3, US$82.4 and US$97.2 over all conventional plots, conventional plots of large growers and conventional plots of small growers, respectively. The two important contributors to the gross benefits are increase in yield and savings on insecticides. However, the current technology fee in Argentina is US$109/ha, which is more than the gross benefit. This is why Bt cotton has not become popular in Argentina.

Conclusion

In Argentina, the high technology fee proportional to the gross economic benefit from the technology has limited Bt cotton area to less that 10% in the last five years. In the same period, Bt cotton area has significantly increased in other countries. The net benefit from the adoption of Bt technology is a crucial factor in determining whether or not to plant Bt cotton. In a country like India, where the cost of the planting seed, being hybrid, is already high, growers may be reluctant to make a high initial investment on the technology. The net economic benefit to Indian cotton growers from the technology will be the determining factor in how adoption proceeds in the largest cotton growing country of the world.

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Genome Characterization of Whitefly-transmitted Geminivirus of Cotton and Development of Virus-resistant Plants Through Genetic Engineering and Conventional Breeding

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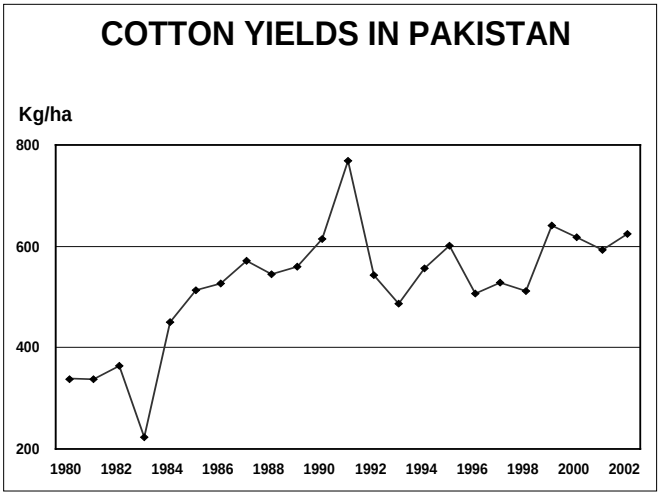
(This article is based on the findings of the CFC/ICAC07 project funded by the Common Fund for Commodities and local institutions in Pakistan, the UK and the USA. The five-year project was coordinated by the National Institute for Biotechnology and Genetic Engineering, Pakistan. The International Cotton Advisory Committee supervised the project.)

Pakistan is the fourth largest cotton producing country in the world with total production of 1.7 million tons in 2002/03. Cotton is grown on the average on three million hectares, and the average yield in 2002/03 was 625 kg per hectare. The government of Pakistan launched an intensive educational program called "Cotton Maximization Project" in both major cotton producing provinces of the country in the late 1970s. The primary objective of the project was to educate cotton growers in the project areas about cotton production technology. Educational activities were intensified in the project areas and extension workers were provided first-hand training by researchers. The project areas were moved every few years; however, at the end of every season, yields in the project areas were compared with adjoining non-project areas. Yield evaluation was undertaken by a neutral government agency as a contractual assignment. The program worked successfully for about 10 years, and consequently, cotton production more than doubled in Pakistan during the 1980s. The project motivated cotton growers throughout the country. Coupled with contributions from researchers through the development of heat tolerant varieties and insect identification and control strategies, the project increased cotton production to 2.2 million tons in 1991/92 and Pakistan became the third largest cotton producing country in the world. The average yield rose to 769 kg/ha, indicating that Pakistan has the potential to produce yields this high.



However, Pakistan enjoyed the status of being the third largest cotton production country in the world, after China (Mainland) and the USA, for only one year in 1991/92. The following year, cotton yields decreased to 543 kg/ha, and production dropped by 30% to 1.54 million tons due to the leaf curl virus disease. The government quickly realized the significance of the problem and started looking for solutions. The short-term approach was to get rid of varieties that were susceptible to the leaf curl disease. However, a long-term solution required in-depth scientific analysis of the causal organism (geminivirus) and its control.

Cotton is prone to many diseases and thus its production is constantly under threat. According to the International Cotton Advisory Committee, the epidemic of cotton leaf curl virus in Pakistan threatened to spread to other countries, particularly to China (Mainland) and India. Whitefly serves as a vector for the transfer of geminiviruses, and whitefly was already a serious pest in both countries. Researchers realized that it was impossible to eliminate whitefly as a pest on cotton, so the solution lay in the development of varieties that were immune to the virus responsible for causing the disease.

The cotton leaf curl virus (CLCuV) appeared as an epidemic in 1992/93 in Pakistan. The disease had been recorded in Pakistan many years before, but was never considered responsible for causing economic losses in yield. It was estimated that in 1992/93, production losses due to the CLCuV epidemic in Pakistan were around US$5 billion. The project was originated by researchers in the UK and the USA, and later joined by

1. National Institute for Biotechnology and Genetic Engineering, Pakistan
2. John Innes Centre, UK
3. University of Arizona, USA
4. Cotton Research Institute, Pakistan

Pakistan with funding from the Common Fund for Commodities (CFC). The following institutions worked on the project:

- National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan
- Cotton Research Institute (CRI), Faisalabad, Pakistan
- University of Arizona (UoA), Tucson, Arizona, USA
- John Innes Centre (JIC), Norwich, United Kingdom

The National Institute for Biotechnology and Genetic Engineering, Pakistan, served as the project executing agency. As the international commodity body on cotton, the ICAC supervised the project. The CFC provided a grant of US$1.55 million, with US$2.4 million contributed by the institutions mentioned above from local resources in the form of in-kind and co-funding contributions. The five-year project started in January 1996, and was extended for one year to December 2001. Related projects funded by FAO, the International Atomic Energy Agency, the Asian Development Bank and local projects in the three countries also contributed to the implementation of the project's activities. It has taken a team effort to combat the leaf curl virus disease.

The project had three major objectives:

- Characterization of the cotton leaf curl virus of the Old and New World (NIBGE/JIC/UoA)
- Development of transformation technology for model cotton (NIBGE, Pakistan).
- Development of virus-resistant commercial cotton varieties by conventional methods as well as by genetic engineering (NIBGE/CRI, Pakistan).

Among the three objectives, the last two were exclusively carried out by Pakistani components.

Characterization of the Virus

The cotton leaf curl disease is caused by geminiviruses, single stranded DNA (SSDNA) viruses with circular genomes that are grouped in the family Geminivirdae. Geminviruses have been divided into four genera based on their insect vectors and genome organization (mono, bipartite). The whitefly-transmitted geminivirus (genus Begomovirus) is the most import group of geminiviruses.

The cotton leaf curl disease in Pakistan turned out to be much more complex than initially thought. Many important discoveries have been made by the project (Mansoor S. et. al. 2003). The major achievement in molecular virology includes the establishment of routine purification of geminivirus particles from cotton plants.

The project determined that the cotton leaf curl disease is associated with several distinct monopartite begomoviruses showing homology to other begomoviruses from the region. Full-length clones of these viruses were systemically infectious to tobacco and cotton but were unable to develop typical disease symptoms, suggesting that unusual components are required to cause symptoms. The conclusion resulted in concerted efforts to identify additional components that resulted in the discovery of DNA 1 and DNA ß.

This project identified a third group of begomoviruses, namely monopartite begomoviruses that are associated with a satellite molecule (termed DNA ß, called DNA beta) to induce symptoms in some hosts. DNA ß was shown to require begomovirus for replication, spread in plants and insect transmission, behaving as a satellite. The identification of this molecule, as well as the satellite-like molecule DNA 1, has opened up a whole new area of geminivirus research. In fact, DNA 1 and DNA ß are the first examples of functional satellites in DNA viruses.

Four distinct begomoviruses cloned from infected cotton were found to be associated with a single species of DNA ß. Co-inoculation of any of distinct DNA A with DNA ß was sufficient to cause disease symptoms. Distinct begomoviruses associated with the disease are very often found in a multiple infection, but these results showed that a multiple infection is not necessary to develop disease symptoms.

Another phenomenon observed in the course of the project was the emergence of new begomoviruses by recombination of existing viruses and expansion in the host range of cotton leaf curl and other begomoviruses. Several natural hosts and other begomoviruses from the region were detected in several new host plants.

The project showed that the geographic range of diseases associated with DNA ß molecules covered all areas where begomoviruses are known, with the exception of the Americas. This suggests that DNA ß and begomovirus interaction may be an ancient one. These diseases cause losses in a number of important crops including cotton, peppers, okra, tobacco and tomato. Certainly the scale and number of diseases caused by begomovirus-DNA ß complexes are on the increase in this region.

Cotton Leaf Curl Virus—Sudan

Genomic-size begomoviral DNAs (2761 bp) were cloned and the sequences obtained for natural population of isolates exhibiting leaf curl and vein thickening symptoms in cotton, okra, and *Sida alba* from Gezira, and in okra from Shambat. Attempts to detect a B component were unsuccessful. Host range studies revealed that the CLCuV-SD wild type okra isolate was transmissible by the whitefly vector to okra (*M. parviflora)* and hollyhock, but not to cotton, whereas, the CLCuV-SD cotton isolate infected cotton and hollyhock, but not okra.

The four viral genomes were found to be less than 3% divergent, indicating the begomoviral component associated with symptomatic source plants is of the same virus (Idris and Brown, *in press).* Prospective satellite DNAs (1.3 kb) from cotton, okra and *Sida* spp. were amplified by PCR, cloned, and the sequences were determined. Phylogenetic analysis and sequence comparisons indicated the satellite DNAs were distinct from one another.

When full-length clones for the cotton leaf curl virus–Pakistan (CLCuPKV) and CLCuV-SD associated *Sida* spp. satDNA were co-inoculated to cotton, leaf curl disease symptoms were observed in cotton 10 days post-inoculation. This indicates that both CLCuV-PK and CLCuSDV-Sida clones are infectious in cotton when present together with CLCuV-SD associated satellite DNA from Sida. Secondly, the results provided preliminary proof of Koch's postulates for CLCuV from Sudan.

Cotton Leaf Crumple Virus—USA

The cotton leaf crumple virus (CLCrV), a begomovirus occurring in Arizona and California, USA, and Sonora, Mexico, was found to be a distinct bipartite begomovirus species. CLCrV-Sonora (CLCrV-Son) shares >97% nt sequence identity with the previously characterized isolate of CLCrV from Arizona (Brown et. al., 2001). Experimental and natural host range studies indicated that CLCrV had a relatively narrow host range within the Malvaceae and Fabaceae families. The genome of the Sonora isolates, designated CLCrV-Son, was cloned and completely sequenced. Cloned CLCrV-son DNA A and B components were infectious by biolistic inoculation to cotton and bean, and progeny virus was transmissible by the whitefly vector, *Bemisia tabaci*, thereby completing Koch's postulates for the first time. CLCrV-AZ DNA A shared the highest nucleotide (nt) sequence identity with the Bean calico mosaic virus (BCMoV), cabbage leaf curl virus (CaLCV) and the squash leaf curl virus (SLCV-R), at 76%, 76%, and 75%, respectively. The CLCrV DNA B component shared the highest nt sequence identity with the potato yellow mosaic virus (PYMV), tomato mottle virus (ToMoV), and the abutilon mosaic virus at 67%, 66%, and 66%, respectively. Collectively, these results provide intriguing evidence that CLCrV is a double recombinant, having sequences from two distinct species and forming a new prototype group among New World begomoviruses. An extensive analysis of coat protein gene sequence among a large number of geminivirus isolates resulted in the establishment of the Geminidetective web side: http:Gemini@biosci.Arizona.edu.

A molecular virology study resulted in the development of polyclonal anitsera against coat protein. A battery of six tests was developed at NIBGE to help national breeders to screen the cotton advance lines. In the related studies, alternate hosts of the cotton leaf curl virus have been identified. It was observed that there was an expansion in the host range of the virus and several non-Malvaceous plants including radish were found to be infected with CLCuV.

Development of *in-vitro* Transformation of Cotton

The cotton plant is non-responsive to tissue culture, and regeneration is restricted to only a few lines, i.e., Coker 312 and Siokra 1-3. Moreover, most of this information resides with the private sector. In such a scenario, NIBGE, Pakistan, made great efforts in the establishment of a routine system for the transformation of regenerable (Coker) varieties of cotton. Twenty-one cotton lines, including commercially approved varieties, promising breeding lines, and exotic varieties were selected for regeneration purposes.

Gossypium hirsutum L.
NIAB-78, S-12, MNH-93, Gohar-87, FH-87, FH-682, SLS-1, NIAb-26, BH-36, CIM-70, CIM-109, CIM-240, RH-1, B-557, A-1-85, A-18/87, AEM-52, Coker-312, Siokra 1-3, Siokra-324

Gossypium arboreum L.
Ravi

The callus produced on both the above media was sub-cultured on 40 different combinations of MSO, MSk(MS+KNO_3 1.9 g/l), BAP, IAA, NAA, kinetin and 2,4-D and zeatin hormones. No callus growth was observed on IAA and BAP combinations. In medium containing zeatin, callus remained green, dividing but no embryo was detected. Callus became brown and dead in the presence of Zeatin, IAA and Kinetin (NAA = Naphthalene acetic acid, IAA = Indole acetic acid, BAP = Benzyl amino purine, MS0 = Murashige and Skoog medium without hormones, MS (K) = MS medium with kinetin).

Response to embryogenesis was restricted to Coker and Siokra lines. Ninety percent of coker-312 seeds showed embryogenic callus. All other varieties showed no response. Screening of 22 genotypes substantiates the genotype specificity of the embryogenic response in cotton. Coker-312 exhibited a higher index of embryogenesis compared to all other varieties under the same set of treatments. Data from these experiments suggested that genetic components rather than culture components is the most critical factor in obtaining sufficient regeneration in cotton.

Response of Cotton Genotypes to Meristem Tip Culture

The results of the meristem shoot tip indicated that its size contributed significantly to the rate of plant formation. Mortality rate was 50% when meristems of less than 0.5 mm size were used for the formation of seedlings. The results of these experiments revealed that media containing 0.46 mM kinetin was better for shoot development whereas faster rooting was observed on media containing 2.68 mM NAA and 0.46mM kinetin. No intervarietal variability among 19 cultivars was observed. The project concluded that the methodology is simple and could replace the existing protocols for meristem tip culture of cotton.

The availability of a regeneration system and the establishment of transformation procedures (agrobacterium and biolistic gun method) enabled the project to develop virus resistant Coker plants. This resulted in the availability to cotton researchers all over the world of a source of genotypic immunity to the viruses. Concurrently, a transformation system of local elite cultivars of cotton based on the transformation of mature embryos by Biolistic and *Agrobacterium* was established and is now being undertaken as a routine.

Development of Virus Resistant Genotypes

The ultimate and most important objective of the project was to develop virus-resistant genotypes. This part of the work was carried out exclusively in Pakistan. To achieve this goal two strategies were adopted:

Conventional Breeding

Development of virus-resistant varieties through conventional breeding was an important objective of the project. This component was exclusively taken up by the Cotton Research Institute, Faisalabad, Pakistan, which is the largest monocrop institute of the Punjab government with five cotton research stations at various locations throughout the province. The Cotton Research Institute maintains a huge genetic stock of local and exotic germplasm of world collections. Breeders extensively searched the available germplasm for possible existing sources of CLCuV resistance. The project funding accelerated the pace of conventional breeding programs of the institute.

Efforts to identify sources of natural genetic resistance to the disease and their utilization for the development of new commercial cultivars have been extensive. Upland cotton, which is an allotetraploid (AADD), is cultivated on more than 95% of the total cotton area in Pakistan. Sources of genetic resistance identified so far fall into two main categories. In the first are two diploid cotton species of Asiatic origin, namely *G. arboreum L.* (AA) and *G. herbaceum L.* (AA), but these species have not been utilized in the development of new commercial varieties because of their diploid nature and inability to develop interspecific hybrids with *hirsutum*. The second category includes two genotypes belonging to *G. hirsutum*, namely LRA5166 and CP15/2. All breeders in both cotton growing provinces of the country have extensively used LRA5166 and CP15/2 in their hybridization programs.

In the first years of the project, the Cotton Research Institute screened 414 accessions—including 292 exotic and 122 local—of *Gossypium hirsutum*. L. for leaf curl virus resistance/susceptibility. Some genotypes that indicated at least some level of tolerance to the disease were selected for extensive testing over more than one year. The selected genotypes were planted in replicated forms at multiple locations and in known hot spots of the disease. A team of breeders visited experimental locations at various stages of crop development before advising breeders to use LRA5166 and CP15/2 in breeding programs.

Extensive crossing with exotic sources of resistance, evaluation of segregated generations and multi-location trials resulted in the development of four commercial varieties. In Pakistan, the varietal approval system is executed by an independent body, the Punjab Seed Council (PSC) in Punjab, and the Sindh Seed Council in Sindh, while the Federal Seed Certification & Registration Department (FSC&RD) is the supreme body for the approval and maintenance of seed quality in the country. The seed councils require three years of coded candidate varieties testing, coupled with agronomic requirements data in addition to yield and fiber quality performance data on any variety that is submitted for approval as a commercial variety. The candidate varieties are tested against candidate varieties developed by the Pakistan Central Cotton Committee, the Pakistan Atomic Energy Commission, universities, provincial governments (like the Cotton Research Institute) and private companies. Each institution has more than one team of cotton breeders, and each team competes against all others for approval of a variety. All candidate varieties are tested in one coded trial called the National Cotton Variety Trial at multiple locations within and across provinces.

The variety approval is not only highly competitive but also a requirement for release of any variety for commercial production. The project developed and released four varieties FH-900, FH 901, MNH 552 and MNH 554 for general cultivation in different zones in the Punjab province. Release of four varieties after formal approval and distribution of virus-resistant genotype to farmers in Pakistan is a significant achievement of the project. These commercial varieties now cover more than 20% of the cotton area in the country. Several other strains developed by the institute and NIBGE are in the pipeline.

Genetic Engineering for Virus Resistance

The development of Bt insect resistant cotton in the USA through genetic engineering at the start of the project prompted the project team to utilize this technology for the development of virus-resistant cotton. Pathogen- (virus) derived resistance—by putting some genomic component of virus into commercial cotton varieties—was the main objective. This ambitious objective was challenging because no commercial variety of any crop against Gemini (SS DNA) viruses had been developed in any part of the world through genetic engineering at that time. This project achieved another milestone by developing virus resistance in elite commercial varieties (S-12 and NIAB-78) by utilizing various genomic (C1, C2 and C3) components in-sense and anti-sense orientation. Evaluation of transgenic plants was conducted in the lab, in a containment facility and in net house in the field. Many elite lines showing partial, delayed or complete resistance are ready to be released after approval by the National Biosafety Committee.

Capacity Building

The project laid a strong infrastructure and expertise at NIBGE, Pakistan, to investigate any viral epidemic at the molecular level. Similarly, cotton transformation technology and genetic engineering were established in the public sector, which will help the country to undertake any other biotech program with surety and confidence. Another important aspect of the project is the appearance of many high quality articles in reputed journals. International linkages have also been developed allowing transfer of technology and expertise in cotton biotechnology. Human resource development was another important contribution as three Ph.D., nineteen M.Phil. and nine M.Sc. (Hon) research

theses on cotton biotechnology were accomplished during the project duration. This vital aspect resulted in developing a very strong and dynamic team capable of meeting any future challenges in cotton production.

The project published a detailed report on activities and achievements of the project in the form of Technical Report No. 22 of the Common Fund for Commodities. The 112-page report is available on the web at http://icac.org/icac/projects/commonfund/leaf/leafmain.html and free hard copies can be requested from the ICAC at publications@icac.org.

Conclusion

International funding/support from the Common Fund for Commodities/International Cotton Advisory Committee, in addition to the Asian Development Bank, the Food and Agriculture Organization, and well-focused aggressive research by researchers in the country with deep collaboration with teams at JIC, UK and UoA, USA, enabled Pakistan to control the leaf curl virus problem and regain production of more than 1.8 million tons during the last three years. Pakistan has gained the expertise and the experience to undertake any future challenge in cotton biotechnology. Participating institutions are indebted to the international organizations, local institutions and participating researchers for their financial, technical and moral support for the implementation of the project.

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